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Full Length Research Paper

Detection and genotyping of rhinovirus from exacerbated asthmatic patients in Baghdad, Iraq

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Asthma is a chronic disease with multiple environmental and genetic causes. Determining the role of rhinovirus in asthma incidence and exacerbation could improve the controlling measures for this disease. This study aims to detect and genotype human rhinovirus (HRV) in asthmatic patients in Iraqi population. A total of 45 patients with asthma participated in this study. Viral RNA was extracted from nasopharyngeal swabs (NPS) and cDNA was created using reverse transcriptase-polymerase chain reaction (PCR). Specific primers for HRV were used with two rounds nested-PCR to amplify the 5'-noncoding region of the viral genome. PCR products of the second round nested-PCR underwent direct sequencing and the resultant sequences were aligned with reference sequences in GenBank. MEGA 5 software was used to construct phylogenetic tree between eight successfully sequenced isolates and eight reference isolates. Alignment of viral sequence revealed highly genetic diversity between these sequences and the reference isolates. Phylogenetic tree showed that five isolates belong to Human Rhinovirus- A (HRV-A), while three isolate belong to Human Rhinovirus-C (HRV-C). The HRV-C was detected and genotyped for the first time in Iraq. The results of the current study suggest the significant role of HRV infection among patients with exacerbated asthma in Iraq.

Key words: Human rhinovirus, genotyping, asthma exacerbation.

INTRODUCTION

Asthma is a very common chronic condition affecting all age classes especially children. Approximately, 235 million people are affected with asthma worldwide, with an unusual death figure of 250000 (WHO, 2011). Not

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only is the disease considered a big public health problem, but also the burden economic cost is very high. World Health Organization (WHO) estimated that the economic cost of asthma exceeds the cumulative cost of human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) and tuberculosis (WHO, 2004, 2011).

Asthma exacerbation may be caused by both genetic and environmental factors. Infectious agents are among the most important environmental factors that influence asthma exacerbation (Guilbert and Deulnger, 2010). Despite the fact that the respiratory system is a target for large numbers of bacteria, fungi, and even parasites, viruses seem to have more important role in asthma than other microorganisms. HRV has been noted as pathogens of the common cold for over 50 years; however, recent advances in viral molecular diagnostics have brought the attention for more significant role of these viruses in respiratory diseases (Arakawa et al., 2012; Moore et al., 2013).

HRVs are a group of single strands RNA positive sense, non-enveloped viruses, that are protected by an icosahedral proteineous capsid. They belong to the genus *Enterovirus* and Picornaviridae family (Pallansch and Roos, 2007). The viral RNA composes of about 7.2 kb and consists of a single gene that produces 2 proteins (Simmonds et al., 2010). The single open reading frame (ORF) has three regions: the first region (P1) encodes for the structural proteins, while the other two regions (P2 and P3) encode for the non-structural proteins (Jacobs et al., 2013). In the 5'-non-coding region (5'-NCR), there is located an internal ribosomal entry site (IREs) which is necessary for translation (Langereis et al., 2014).

HRVs were originally classified into two species (A and B). Due to the highly sensitive molecule methods, a novel species (HRV-C) was identified and designed (Lamson et al., 2006). The new species has genomic organization similar to the other two species; however, several distinct differences gave it a new classification. More than 50 different types of HRV-C have been identified on the basis of 13% nucleotide differences in VP1 encoding gene (Bochkov and Gern, 2012). To the best of the authors' knowledge, there is no previous study that investigated the most prevalent species of these viruses among Iraqi population. Thus, the current study aims to determine HRVs species among asthmatic patients.

METHODOLOGY

Study population

A total of 45 asthmatic adult patients with exacerbation were included in the study. Age range was from 16 to 48 years, 18 (40%) males and 27 (60%) females. These patients were attending Al Zahra'a Consultative Center for Allergy and Asthma, and Consultative Clinic for Chest and Respiratory Diseases/Baghdad, from January to April 2015. The diagnosis of asthma was performed by a specialist physician. The exclusion criteria were any history of respiratory illness like chronic obstructive pulmonary disease (COPD), tuberculosis, pneumonia, or bronchitis, and any other comorbid illness such as diabetes mellitus, hypertension, or pregnant females. Informed consent was obtained from all participants and ethical approval was obtained from the ethical committee of Colleges of Medicine, Al-Nahrain University.

Nasopharyngeal swabs

Nasopharyngeal swabs (NPS) were collected using the flexible flocked swab with personal protective equipment. The swab was gently entered along the base of one nostril (straight back, not upwards) and continued along the floor of the nasal passage until it reached the nasopharynx. After 2 to 3 times rotation, the swab was held in place for 5 s. A suitable tube with cover was used to keep the swab. The excess length of swab was broken off at the score mark to permit capping of the tube and transported with ice packs to the laboratory for processing. Time duration from swab collection to laboratory processing did not exceed an hour in any case.

Viral RNA extraction and cDNA synthesis

A ready kit (QIAampMinElute Virus Spin Kit, Germany) was used for RNA extraction from NPSs. The kit combines the selective binding properties of a silica-based membrane with flexible elution volumes of between 20 and 150 µl. The instruction manual of the company was followed precisely, with an eventual elution volume of 50 µl. Another ready kit (iNtRON Power cDNA Synthesis Kit/Korea) was used for reverse transcriptase-polymerase chain reaction (RT-PCR) according to the manufacturer's instructions.

HRV genotyping

Resultant cDNA samples were prepared to perform the genotyping study. Semi-nested PCR was used for the amplification of 5'-noncoding region of HRV genome. The primer pair for the first round was forward: P1-1 5'-CAAGCACTTCTGTYWCCCC and reverse: P3-1 5'-ACGGACACCCAAAGTAG. The PCR conditions were as follows: after an initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 45 s and elongation at 72°C for 1 min. These cycles were followed by final elongation at 72°C for 7 min. This round gives an amplicon with 390 bp which belongs to the 5'-NCR of all species of HRV.

In the second round, the same forward primer was used while three types of primers were simultaneously used as reverse primers. These were P2-1: TTAGCCACATTCAGGGGC, P2-2: TTAGCCACATTCAGGAGCC and P2-3: TTAGCCGCATTCAGGGG. This variation in primers was to ensure the amplification of all three species of HRV. The same above cycling conditions were applied except for annealing temperature which was 61°C instead of 58°C.

Out of 45 NPAs, only nine gave PCR product in the first and second round semi-nested PCR. These products were directly sequenced by Sandor lifescience Pvt. Ltd/India, using the ABI Big Dye Terminator v.3.0. Products are resolved by electrophoresis on an ABI 3730xl capillary sequencer.

The resultant sequences were undergone BLAST in National Center for Biotechnology Information (NCBI). Accordingly, eight close sequences were chosen to represent the three species of HRV. Furthermore, the sequences and chosen reference sequences were aligned together using Bioedit software. To identify the species of each isolate, a phylogenetic tree with 1000 bootstrap replicates was constructed using MEGA5 software.

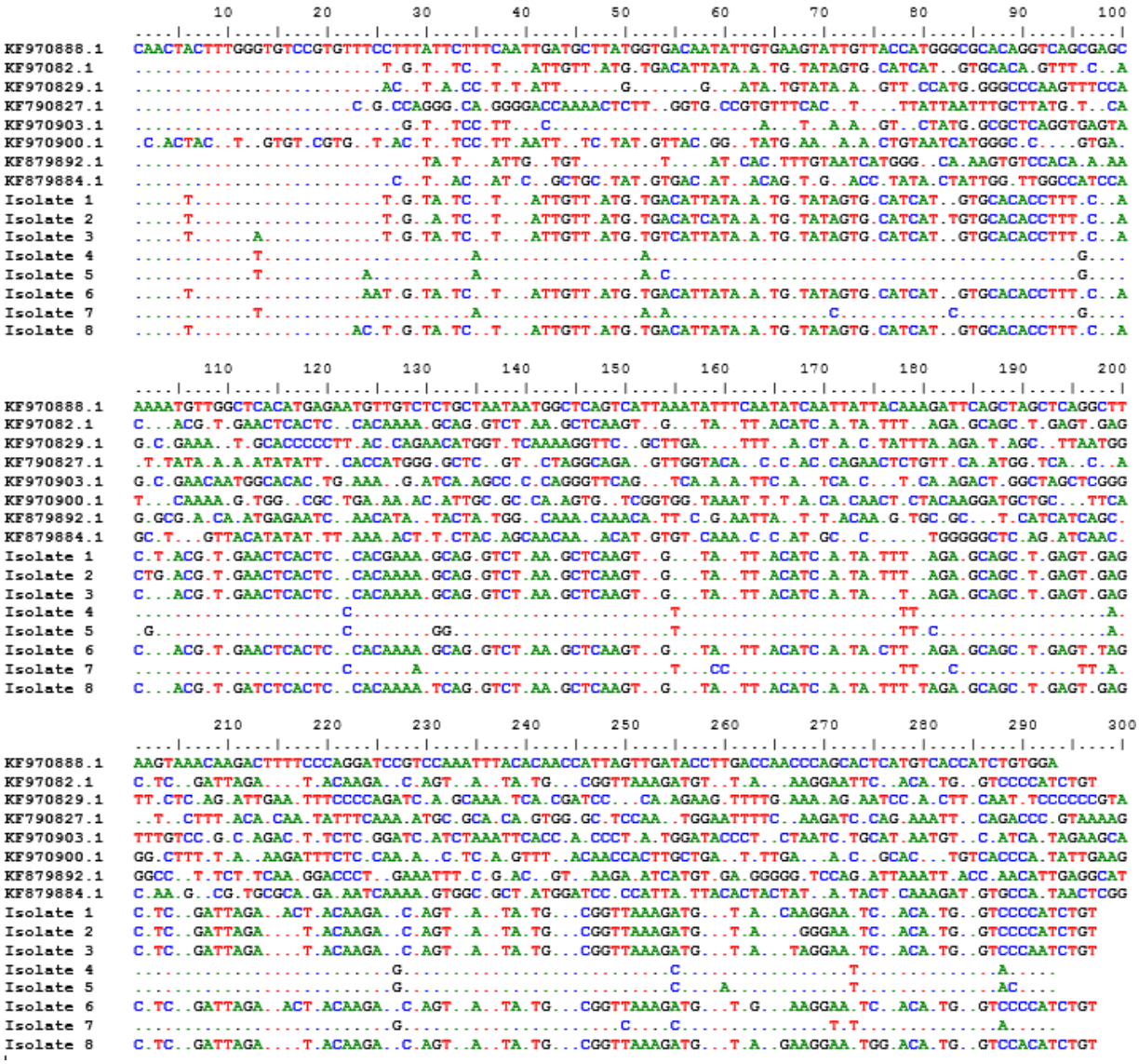

Figure 1. Alignment of the eight isolates with reference sequences from National Center for Biotechnology Information using Bioedit software.

RESULTS

Human Rhinovirus detection and genotyping

Viral RNA was successfully extracted from 9 out of 45 NPSs (20%). cDNA was created from extracted RNA. The resultant cDNA was amplified by semi-nested PCR using specific primers for the first round and second round PCR. Both rounds ended with successful amplification of the target gene.

Sequence alignment

From the nine positive samples for HRV, only eight were successfully sequenced. These sequences underwent BLAST in NCBI web page from which eight similar sequences were chosen for the alignment and for construction of phylogenetic tree with the isolates of the current study. The accession numbers of reference sequences were KF97082.1, KF970829.1 and KF970827.1 representing HRV-A, KF879892.1 and KF879884.1 representing HRV-B and KF970888.1; KF970903.1 and KF970900.1 representing HRV-C. Figure 1 shows the alignment results using BioEdit soft. It is obvious from the figure the high diversity among the isolates. A preliminary reading for this alignment reveals that the isolates number 4, 5 and 7 were very close to the reference sequence KF970888.1 (HRV-C), while the isolates numbers 1, 2, 3, 6 and 8 were very close to the

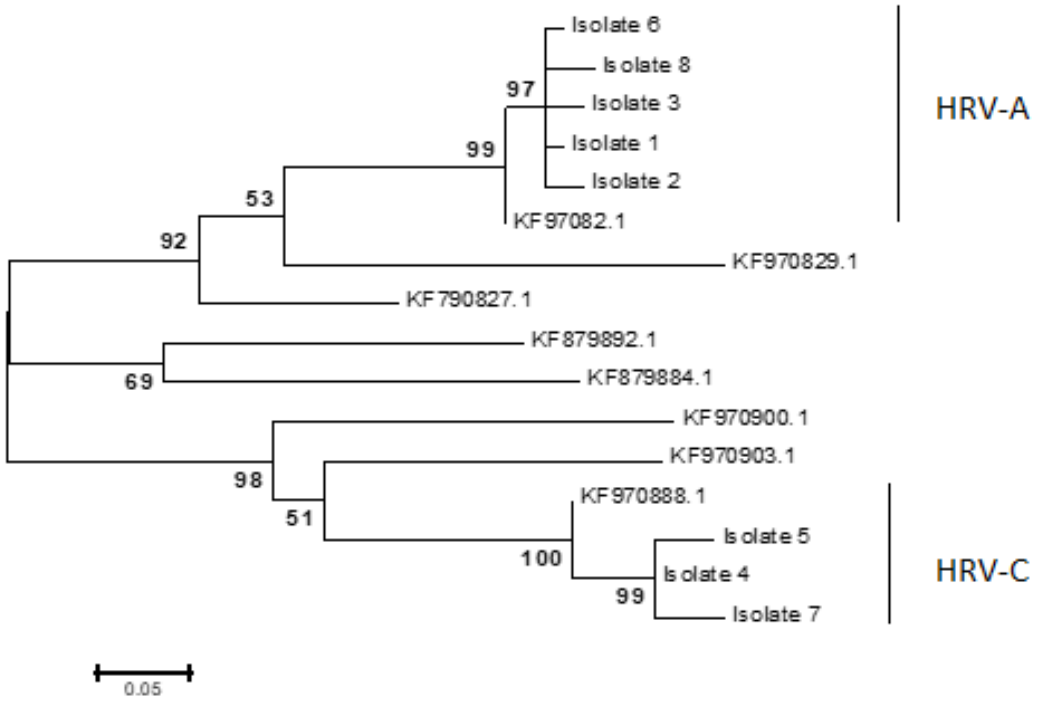

Figure 2. Phylogenetic tree based on 5'-non coding region constructed using maximum likelihood method with MEGA 5 software.

reference sequence KF97082.1 (HRV-A), although there are some substitutions in several nucleotides.

Figure 2 shows the phylogenetic tree for the 8 isolates and the reference sequences. The results of this phylogenetic analysis absolutely show that the isolates 1, 2, 3, 6 and 8 belong to HRV-A, while the isolates 4, 5 and 7 belong to HRV-C.

DISCUSSION

The current study revealed that 9 (20%) of the exacerbated asthmatic patients out of 45 were infected with HRV. Approximately close results were recorded in different parts of the world. In Hong Kong, Susanna et al. (2009) used RT-PCR for detection of HRV in asthmatic patients. They found that 220 NPS out of 1200 (18.3%) were positive for HRV. In China, Sun et al. (2016) reported that 14.7% of 709 hospitalized asthmatic patients have HRV. Very large percentage, however, was reported by some studies even with 60% in case of exacerbated asthma (Johnston et al., 2007). The difference in the percentage of HRV between the current study and the aforementioned studies may be partly explained by sample size, which becomes more representative when a high number of patients are involved, and based on the seasons at which the studies are performed. In this regard, studies conducted in cold and humid season have greater opportunity to find the virus as compared to that conducted in hot and dry seasons (Johnston et al., 2005; Leotte et al., 2017).

To the best of the authors' knowledge, this is the first study in Iraq regarding detection of HRV-C by molecular method. In fact, two species of HRV were detected among asthmatic patients. Among the 9 positive asthmatic patients, HRV-A was detected in 5 samples (55.5%) and HRV-C in 3 samples (33.3%), while the sequence in one sample could not be detected.

Most previous studies in this regard reported high prevalence of HRV-A and HRV-C with low prevalence of HRV-B in asthmatic patients. Lau et al. (2009), in Hong Kong, found that 21 out of 26 (81%) of HRV- positive nasopharyngeal aspirates (NPAs) belong to HRV-C and only 5 (19%) belong to HRV-A; while HRV-B was not detected. The first report from the Middle East (Jordan) pointed out that 26% of HRV- positive NPAs from asthmatic patients were related to HRV-C (Miller et al., 2009a). In Hong Kong, HRV-A, HRV-C and HRV-B represented 50, 41 and 8% respectively (Lau et al., 2009). However, this percentage outbalanced towards HRV-C which accounts for 50% of HRV- samples in the United State (Miller et al., 2009b). Equal or more percentages for HRV-C were reported in many other countries (Lamson et al., 2006; Renwick et al., 2007; Briese et al., 2008).

In fact, such disparities are expected depending on many factors, the most important of which is age of the patients. HRV-C is usually more associated with children

with asthma. Lau et al. (2009) demonstrated that wheezing episodes were more frequent in children infected with HRV-C than either HRV-A or HRV-B. Most studies recorded higher prevalence of HRV-C than HRV-A which were conducted on hospitalized patients suffering from asthma episode; while in the current study, samples were taken from out-patients. On the other hand, most adults with HRV-C had underlying diseases. Majority of the patients in the current study are adults, thus relatively low prevalence of HRV-C occurred. This tendency of HRV-C is not fully understood. It may be related to the immune status of individual. The second factor is the severity of asthma episode. HRV-C is more common in asthma exacerbation (Liao et al., 2016).

5'-Noncoding region and VP4/VP2 region are the most commonly used target for genotyping of HRV-C with 5'-NCR is more sensitive (Kiang et al., 2008; Han et al., 2009). Sequencing of short amplicon of this region (270 to 290 bp) is usually enough for strain typing (Lee et al., 2007). The analysis of alignment of these sequences revealed that the highly conserved HRV sequence is present only in 5'-NCR. In the current study, universal primers were used to anneal this highly conserved motif and PCR products were directly sequenced and aligned with the closest serotypes according to the GenBank database. The results of this alignment showed the high diversity among the different strains. In fact, such diversities were frequently reported. Daleno et al. (2013) found that nucleotide variability between HRV-A and HRV-C may reach 37%. The variability is not only found between different species, but also within strains that belong to the same species. Arakawa et al. (2012) showed that HRV-C genomes have more than 30% divergence. Furthermore, McIntyre et al. (2010) suggested that there are approximately 30 genotypes belonging to HRV-C based on diversity in nuclear acid sequence. However, Lee et al. (2007), in a previous study, reported 94 to 100% identity among HRV-C strains.

In phylogenetic analysis, the 8 isolates of HRVs were clustered into 2 different clusters; one near the strains which belong to HRV-A and the other cluster near the strains which belong to HRV-C. The relatively high numbers of bootstrap in the nodes indicate that these strains belong to the corresponding neighboring reference sequences.

HRV does not cause cytopathic effect, and the limited epithelial distribution is not correlated with the severity of exacerbated asthma. These facts suggest alternative mechanism(s) by which the virus induces asthma exacerbation. The current hypothesis assumes that HRV causes the release of epithelial cells of broad spectrum of chemokine's such as CXCL, CCL5 and CXCL10, which attract the inflammatory cells to the airways (Johnston et al., 2005). Edwards et al. (2012) suggested the pre-request of previous sensitization in the respiratory epithelial cells in order for HRV to cause exacerbation. Sensitization promotes Th2 cells and HRV stimulate these cells to release different cytokines and chemokines. These in turn attract airways more to Th2 cells which secrete three important cytokines: IL-5, IL-4 and IL-13. IL-5 promotes eosinophilia which results in increase in eosinophil cationic protein (ECP) and transforming growth factor β (TGF-β) and eventual inflammation of airway smooth muscle. IL-4 and IL-13 cause antibody class switching to IgE which binds mast cells. The cross linking of allergen on mast cell-bound IgE causes mast cell degranulation and release of profound mediators such as histamine, prostaglandin and leukotrienes. These mediators cause bronchoconstriction and further airway inflammation (Reuter et al., 2010). These data indicate the significant role of rhinovirus in asthma, and it seems that the vast majority of HRV species in Iraqi patients with asthma are HRV-A and HRV-C.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Urinary tract infection among children and adolescents of Garhwal Region of Uttarakhand, India

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Urinary tract comprises of kidney, ureter, urethra and genital organ and under normal circumstances these organs are sterile. Inflammation of these organ and parts may indicate the possibility of Urinary Tract Infection (UTI). It may be due to the colonization of wide range of bacteria either from normal microflora that is *Escherichia coli* or from other Gram negative or Gram positive bacteria. The present study was carried out to determine the prevalence of urinary tract infection in children and adolescents of hilly areas of Garhwal region of Uttarakhand, India. The sample collection was done from April 2013 to December 2013 from the Out Patient Department (OPD) and In Patient Department (IPD) of Hemvati Nandan Bahuguna Base Hospital, Srinagar Garhwal at an average height of 560 m (1837 ft) above sea level at foothills of Himalayas. Clean voided mid-stream urine samples were collected in sterile universal containers from 76 children of age group 0 to 10 years and 129 adolescents of age group 11 to 20 years. Bacterial counts greater than or equal to $1x10^5$ CFU/mL in culture plates were taken as positive, which was an indication of UTI as introduced by Kass. Colony morphological characteristics were used for identification of bacterial isolation followed by Gram's staining and biochemical tests. Our study showed a high prevalence of *E. coli* as the most dominant bacteria causing UTI in children and adolescents of hilly Garhwal region of Uttarakhand.

Keywords: Urinary tract infection (UTI), coagulase negative staphylococcus (CoNS), outdoor patient department (OPD), indoor patient department (IPD).

INTRODUCTION

Urinary tract infection (UTI) is one of the commonest conditions encountered by medical practitioners (Najar et al., 2009) and women (Fry, 1969; Royal College, 1995; Car, 2006; Nicolle et al., 2006). It was estimated that 60% of all women reported usually have UTI at least once in their lifetime (Foxman et al., 2000; Foxman, 2002; Al-Badr and Al-Shaikh, 2013). Worldwide, about 150 million people (Brumbaugh and Mobley, 2012; Stamm and Norrby, 2001) are diagnosed with UTI each year, costing in excess of 6 billion dollars (Gonzalez and Schaeffer,

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1999). Among both outpatients and inpatients, *Escherichia coli* is the most common isolate, accounting for 75 to 90% of uncomplicated UTI isolates (Junuzovic et al., 2014; Uzunović, 2009; Gupta et al., 2001). *Staphylococcus saprophyticus*, *Klebsiella* sp., *Proteus* sp., *Enterococcus* sp. and *Enterobacter* sp. are organisms less commonly isolated from outpatients (Hooton, 2012; Smith, 2002). It is the common practice of medical practitioner and health officer to prescribe broad spectrum antibiotics on the empirical basis before the final bacteriology that is, antibiotic sensitivity results become available. Therefore, it is essential to have area-specific monitoring studies to document the microorganisms causing UTI and their antimicrobial susceptibility is important for helping the selection of an effective empirical treatment (Smith and Coast, 2002). UTIs are often treated with different broad-spectrum antibiotics when commonest or narrow spectrum antibiotics activity may be appropriate because of concerns about infection with resistant organisms (Daoud and Afif, 2011). Fluoroquinolones are preferred as initial agents for empiric therapy of UTI in area where resistance is likely to be of concern (Schaeffer, 2002; Biswas et al., 2006). This is because they have high bacteriological and clinical cure rates, as well as low rates of resistance, among most common uropathogens (Goldstein, 2000; Gupta et al., 2002). Kulkarni et al. (2017) analyzed 1000 urine samples from a Tertiary Care Hospital of North Eastern Karnataka in which 395 cases were culture-positive for *Escherichia coli.* These isolates were also tested for antibiotic susceptibility by disk diffusion method. Majority of *E. coli* isolates are multi drug resistance (MDR) and show resistance to most commonly used antibiotic which was used to treat UTI.

The resistance pattern of community acquired UTI pathogens has not been studied extensively (Goldstein, 2000). The extensive uses of antimicrobial agents have invariably resulted in the development of antibiotic resistance, which, in recent years, has become a major problem worldwide (Kumar et al., 2006). The etiology of UTI and the antibiotic resistance of uropathogens have been changing over the past years, both in a community and nosocomial or hospital acquired infection (Manges et al., 2006; Kahan et al., 2006). However, there is need for study on etiology and resistance pattern of community acquired UTIs in India. This retrospective study was done to compare the frequency and drug resistance pattern in uropathogens isolated from patients with UTIs in Garhwal region, India. The diagnosis and treatment of UTI in children have been considered to be particularly important due to both short term and long term sequelae. UTI is one of the causes of serious bacterial illness in infants requiring hospital admission and has been associated with significant morbidity (Doern and Richardson, 2016). UTI has also been thought to associate with the development of renal scarring and later to renal failure, hypertension, and pre-eclampsia (Byington et al., 2003).

MATERIALS AND METHODS

Study population

Urine samples were collected from a total of 205 patients in which 76 children and 129 adolescents between the ages of 0 to 20 years, 0 to10 years children and 11 to 20 years (adolescents) - during the period of April 2013–December 2013. All these persons were IPD and OPD patients visiting the H.N.B. Base Hospital, Srikot Ganganali, Srinagar Garhwal, Uttarakhand. For the collection of urine from patients the following exclusion criteria were used: 1. sample in non-sterile container; 2. samples of patients on antibiotics were excluded from the study.

Sterilization of media and materials

The media used for the experiments were nutrient agar (NA), MacConkey agar (MCA), blood agar (BA) and Muller Hinton agar. All medium were purchased from Hi Media laboratory Ltd. All glassware and media was sterilized by autoclaving at 121 lbs for 15 min before setting an experiment.

Urine sample collection

Clean catch mid-stream urine specimens were obtained from patients in sterile universal containers which were given to their parents/attendant for collections and transported to the laboratory immediately for urine analysis.

Isolation of uropathogens from urine samples

Ten-fold serial dilutions were made by transferring 1.0 ml of the sample in 9.0 ml of sterile normal saline (0.85% NaCl); 1 ml was then added into molten nutrient agar in Petri dishes and rotated gently to mix. The contents were allowed to set and the plates were then incubated at 37°C for 24 h. Bacterial colonies appearing on the plates after the incubation period were enumerated to determine urine samples with significant bacteriuria. A loop full of each urine sample was also streaked on MacConkey agar and blood agar plate for the isolation of the bacteria present in the urine. After incubation, plates with growth were selected, the colonies were isolated using an inoculating loop and subsequently sub-cultured on agar slants for use in further tests.

Bacterial identification

Bacterial isolates were identified and characterized on the basis of Grams stain followed by a microscopic examination, motility test and other conventional biochemical tests such as catalase, oxidase, IMViC, urease, H_2S production, TSI etc. (Chandra et al., 2016).

Antibiotic sensitivity test

Antimicrobial sensitivity testing was done by using the agar disc diffusion method as described by Bauer et al. (1966). The turbidity of the bacterial suspension was compared with 0.5 MacFarland's standard. The standardized bacterial suspension was then swab-inoculated onto Muller Hinton agar using sterile cotton swabs, and

Table 1. Organism isolated for urinary tract infection in children.

Organism isolated	Count value number (%)		
	Male	Female	Total
Sterile	19 (25)	17 (22.4)	36 (47.4)
Escherichia coli	10 (13.2).	9 (11.8)	19 (25.0)
Klebsiella spp.	1 (1.3)	3 (3.9)	4 (5.3)
Citrobacter spp.	1 (1.3)	1 (1.3)	2 (2.6)
Proteus mirabilis	0 (0)	1 (1.3)	1 (1.3)
Acinetobacter spp.	*1 (1.3)*	1 (1.3)	2 (2.6)
Staphylococcus aureus	0 (0)	2 (2.6)	2 (2.6)
CoNS	1 (1.3)	3 (3.9)	4 (5.2)
Enterococcus spp.	0 (0)	2 (2.6)	2 (2.6)
Candida albicans	1 (1.3)	1 (1.3)	2 (2.6)
Candida non-albicans	2 (2.6)	0 (2.6)	2 (2.6)
Total	36 (47.4)	40 (52.6)	76 (100)

Table 2. Organism isolated from urinary tract infection of adolescents.

Organism isolated	Count value number (%)		
	Male	Female	Total
Sterile	29 (22.5)	39 (30.2)	68 (52.7)
Escherichia coli	3 (2.3)	20 (15. 5)	23 (17.8)
Klebsiella spp.	1 (0.8)	3 (2.3)	4 (3.1)
Citrobacter spp.	1 (0.8)	0 (0)	1 (0.8)
Proteus mirabilis	2 (1.6)	0 (0)	2 (1.6)
Acinetobacter spp.	1 (0.8)	3 (2.3)	4 (3.1)
Staphylococcus aureus	1 (0.8)	5 (3.9)	6 (4.7)
CoNS	6 (4.7)	5 (3.9)	11 (8.6)
Enterococcus	2 (1.6)	1 (1.6)	3 (2.3)
Candida albicans	0 (0)	3 (0)	3 (2.3)
Candida non albicans	0 (0)	1 (0)	1 (0.8)
Candida spp.	0 (0)	(10)	1 (0.8)
Mixed growth	0 (0)	2 (0)	2 (1.6)
Total	46 (35.7)	83 (64.3)	129 (100)

then left to dry for 10 min before placing the antimicrobial sensitivity discs. Antibiotics impregnated discs of 6 mm in diameter were used for the test. Discs containing the following antibacterial agents were placed onto the agar surface and incubated for 18 to 24 h. Thefollowing antibiotics were used for antibiotic sensitivity testing ampicillin, amoxicilin-clavulanic acid, amikacin, cefazolin, cefotaxime, cefuroxime, chloramphenicol, co-trimoxazole, oxacillin/cephoxitin, ciprofloxacin, erythromycin, gentamicin, netilmicin, penicillin, tetracycline, piperacillin tobramycin nitrofurantoin aztreonam, cefoperazone- sulbactum, cefepime, clindamycin, meropenem, rifampicin, ticarcillin-clavulanic acid, linezolid, teicoplanin, piperacillin- tazobactam polymixin-B, azithromycin, colistin and vancomycin. All antibiotic used for antibiotic sensitivity were purchased from HiMedia laboratory Ltd, India. After incubation, the diameter of the zone of inhibition was measured and compared with a zone diameter interpretative chart from HiMedia to determine the sensitivity/resistance of the isolates to the antibiotics. The procedure is intended for *in vitro* susceptibility testing of common rapidly growing and certain fastidious bacterial pathogens (Colle et al., 1996).

RESULTS

The results obtained from data analysis are represented in the following tables. Table 1 shows the microorganism responsible for causing UTI in children. For the children of age group 0 to 10 years, the most prevalent bacteria responsible for urinary tract infection is *E. coli* followed by *Klebsiella* sp. and coagulase negative Staphylococcus (CoNS).

It was evident from Table 1 that the organism highly responsible for urinary tract infections in children is *E. coli*; it is a causative organism of UTI in 13.2% male and 11.8% female children, which is 25% approximate of all the isolates. The other pathogens which were isolated in the urine sample in children population 0 to 10 year(s) age group, are *Citrobacter* sp. (2.6%), *Candida albicans* (2.6%), *Candida non-albicans* (2.6%), *Acinetobacter* sp. (2.6%) and *Enterococcus* sp. (2.6%).

The *Klebsiella* spp. is responsible in 5.3% children population (1.3% male and 3.9% female) for causing urinary tract infections, which is highest after *E. coli*; CoNS is responsible for causing urinary tract infections in 5.2% (1.3% male, 3.9% female) while the *Citrobacter* sp. is responsible for causing urinary tract infections only in 1.3 and 2.6% children population.

Table 2 shows the organism isolation in adolescent population that is, 11 to 20 years (age group). The main responsible organisms for causing the urinary tract infection among adolescents population was found to be *E. coli.* It was evident from the above table that the organism highly responsible for urinary tract infections is *E. coli*, meaning that it is the main pathogen for UTI in 2.3% male and 15.5% female adolescents, which is approximately 17.8%. The other bacteria isolated in the samples responsible for causing the urinary tract infections in the adolescent population are *CoNS* (8.6%), *Staphylococcus aureus* (4.7%), *Klebsiella* sp. (3.1%), *Enterococcus* (2.3%) and *C. albicans* (2.3%).

The second highly responsible organism for causing UTI in adolescent population was found to be *CoNS* which caused infection in 8.6% population and third prevalent organism for causing urinary tract infections was found to be *S. aureus*, which is responsible for affecting 4.7% population.

The antibiotic sensitivity pattern in children and adolescents in Garhwal region is shown in Table 3. On the basis of antibiogram, it was observed that ciprofloxacin (19, 25%), co-trimoxazole (17, 22%), cefazolin (17, 22%), cefuroxime (17, 22%), amoxicillin-clavulanic acid (16, 21%), cefotaxime (16, 21%), ampicillin (15, 20%), gentamycin (13, 17%), ticarcillin-clavulanic acid (10, 13%), cefepime (9, 12%), and aztreonam (5, 7%) antibiotics are highly resistant in case of children, who are of 0 to 10 years age group. The drug

Table 3. Drug sensitivity pattern in children and adolescents for UTI isolates.

Drug	0-10 Years (children)			11-20 Years (adolescents)		
	R	S	NR	R	S	NR
Ampicillin	15	1	60	18	6	105
Amoxicillin-clavulanic acid	16	3	57	21	18	90
Amikacin	5	16	55	8	33	88
Cefazolin	17	2	57	19	9	100
Cefotaxime	16	6	54	24	8	97
Ceftazidime	3	0	73	6	2	121
Cefuroxime	17	2	57	17	7	105
Chloramphenicol	1	5	70	4	8	117
Co-trimoxazole	17	9	50	25	12	92
Oxacillin/Cephoxitin	1	1	74	1	1	127
Ciprofloxacin	19	6	51	26	15	88
Erythromycin	3	3	70	7	5	117
Gentamicin	13	17	46	20	20	89
Netilmicin	0	2	74	1	3	125
Penicillin	1	2	73	1	2	126
Tetracycline	1	0	75	0	0	129
Piperacillin	1	1	74	2	0	127
Tobramycin	0	2	74	0	0	129
Nitrofurantoin	2	22	52	7	24	98
Aztreonam	5	10	61	15	10	104
Cefoperazone- sulbactum	2	17	57	5	19	105
Cefepime	9	7	60	10	8	111
Clindamycin	2	4	70	2	7	120
Meropenem	2	18	56	4	18	107
Rifampicin	2	3	71	0	3	126
Ticarcillin-clavulanic acid	10	3	63	18	4	107
Linezolid	1	4	71	1	5	123
Teicoplanin	0	4	72	1	7	121
Piperacillin- tazobactam	4	18	54	5	20	104
Vancomycin	1	5	70	0	6	123
Azithromycin	1	2	73	0	1	128
Colistin	0	0	76	0	0	129
Polymixin-B	0	0	76	0	0	129

R-Resistance; S, sensitive; NR, No response.

which was found effective against uropathogens isolated from children was nitrofurantoin (22, 28.9%), meropenem (18, 23.68%), piperacillin- tazobactam (18, 23.68%), cefoperazone-sulbactum (17, 22%), gentamicin (17, 22%), amikacin (16, 21%), aztreonam (10, 13.15%) and co-trimoxazole (9, 12%).

As far as adolescent population is concerned, the adolescent population showed high resistance for the drugs ciprofloxacin (26, 20.15%), co-trimoxazole (25, 19.37%), cefotaxime (24, 18.60%), amoxicillin-clavulanic acid (21, 16.27%), gentamycin (20, 15.50%), cefazolin (19, 14.72%), ampicillin (18,13.95%), ticarcillin-clavulanic acid (18, 13.95%), cefuroxime (17, 13.17%), aztreonam (15, 11.62%) and cefepime (10, 77.51%). For the sensitivity for the drugs against uropathogens in the case of adolescent group, it was found that the adolescent population showed their sensitivity for the drugs amikacin (33, 25.58%), nitrofurantoin (24, 18.60%), piperacillin-tazobactam (20, 15.50%), gentamicin (20, 15.50%), cefoperazone- sulbactum (19, 14.72%), amoxicillin-clavulanic acid (18, 13.95%), meropenem (18, 13.95%), ciprofloxacin (15, 11.62%), co-trimoxazole (12, 9.30%), and aztreonam (10, 7.75%).

It was also observed that there are some drugs for which the adolescent population has shown their resistance as well as sensitivity, meaning that there are some common drugs for which the population has showed their resistance and at the same time they also

showed their sensitivity to the same drugs, applied against the urinary tract infection causing bacteria (Table 3).

DISCUSSION

The study was undertaken to determine the incidence of urinary tract infection in children and adolescents as well as to evaluate the bacterial agents involved in this UTI. Out of the 76 patients in children group that participated in this study, pathogens were isolated only in 40 (17 males and 23 females, 52.63%) urine samples while 36 (19 male and 17 females, 47.37%) urine samples were sterile and out of 129 patients in adolescent group that participated in this study, pathogens were isolated only from 61(17 males and 44 females, 47.28%) urine samples, while in 68 (29 male and 39 females, 52.71%) urine samples, no pathogen could be isolated.

A large number of microorganisms were isolated from female patients with high bacteria count. This study shows a higher incidence of urinary tract infection in females than males. In this study, 22.36% males and 30.26% of females had positive urine cultures in 0 to 10 years age group, while in 11 to 20 years age group, 17 males (13.17%) and 44 females (34.10%) had positive urine cultures. This is similar to those obtained by other studies. Anochie et al. (2001) reported a predominance of female patients in a study carried out to determine the influence of instruction on the method of urine collection and storage on the prevalence of urinary tract infection. Similar findings were reported by Olowu (1996). The higher incidence of urinary tract infections in females is a result of a variety of factors, such as the close proximity of the female urethral meatus to the anus (Lipsky, 1990). Short incomplete urethra and in coordinate voiding of urine in school girls often leads to infection of the urinary tract (Mond et al., 1970). Alterations in vaginal microflora also play a critical role in encouraging vaginal colonization with coliforms and this can lead to urinary tract infection (Hooton et al., 1997).

The pattern and frequency occurrence of the bacterial isolates found in this study are similar to those reported by other workers. Alausa and Onile (1984) reported in their study that *E. coli* were the most commonly isolated pathogen in significant bacteriuria. The findings of Bishara et al. (1997) in Israel agreed with this statement. They reported that *E. coli* were responsible for 52% of cases of urinary tract infection and *Klebsiella* spp. (14%) and *Enterococcus* spp. in 4%. A higher percentage of the organisms found in this study were isolated mainly from females. The pattern reported in this work is similar to that reported by Okafor et al. (1993) in which 20.7% of case of urinary tract infection was reported in males. The result of this study shows that 47% of the isolates were sensitive to amoxicillin, 33.3% to cotrimoxazole, 50% to nitrofurantoin 30.6% to colistin, 63.9% to gentamicin, 77.8% to ciprofloxacin and 97.1% to ofloxacin. Prevalence of high number of *E .coli* that is, 67% was also reported by Yilmaz et al. (2016) and resistance rates of *E. coli* to antimicrobial agents was for ampicillin was 66.9%, cefazolin, 30.9%, cefuroxime, 30.9%, ceftazidime, 14.9%, cefotaxime, 28%; cefepime, 12%; amoxicillin-clavulanic acid, 36.9%; trimethoprim-sulfamethoxazole (TMP-SXT), 20%; ciprofloxacin, 49.9%; amikacin, 0.3%; gentamycin, 24%; nitrofurantoin, 0.9%, and fosfomycin 4.3%. They reported no resistance to imipenem or meropenem and the frequency of ESBL-producing *E. coli* strains was 24%.

Sorlózano-Puerto et al. (2017) reported similar finding in which the most prevalent bacterial species was *E. coli* which account for 60.3% of isolated uropathogens, followed by *E. faecalis* (22.4%) and *Klebsiella* spp. (6.5%). The highest *E. coli* susceptibility rates (>90%) were for piperacillin-tazobactam, cefuroxime, cefotaxime, ceftazidime, imipenem, gentamicin, nitrofurantoin, and fosfomycin, and the lowest were for amoxicillin-clavulanic acid and cotrimoxazole. They also suggested that empiric treatment with amoxicillin-clavulanic acid, cotrimoxazole, cephalosporins, and gentamicin may be inadequate due to their limited activity against uropathogens. The antibiotic sensitivity test of this study shows that ciprofloxacin for 0 to 10 years (19, 25%) and 11 to 20 years (26, 20.15%) was the most resistant antibiotic in *in vitro* testing followed by co-trimoxazole for age 0 to 10 years (17, 22%) and age 11 to 20 years (25, 19.37%). A reduced sensitivity to nitrofurantoin was observed in this study as opposed to the findings of Goldraichi and Manfrori (2002), who reported a higher efficacy of the drug against *E. coli in vitro*. They reported sensitivity of *E. coli* to nitrofurantoin of 92, 95 and 94%, respectively over a three year period. Olowu and Oyetunji (2003) reported a 57.9% sensitivity of pathogens towards nitrofurantoin. The most sensitive antibiotic in this study was Amikacin in 11 to 20 years age group and nitrofurantoin for 0 to 10 years age group followed by nitrofurantoin for age 11 to 20 years and meropenem and piperacillin- tazobactam for age 0 to 10 years, respectively.

In conclusion, this study specifies the incidence of urinary tract infection in children and adolescents. It also highlighted the major bacterial agent involved in this condition. The pattern of isolates reported in this study is consistent with the usually reported pattern, with *E. coli* being the most common organism isolated in cases of urinary tract infection. This was followed by *Klebsiella* spp., *P. mirabilis*, *E. faecalis* and *P. aeruginosa*. This study shows a high level of resistance to ciprofloxacin (19, 25%), co-trimoxazole (17, 22%), cefazolin (17, 22%), cefuroxime (17, 22%), amoxicillin-clavulanic acid (16,21%), cefotaxime (16, 21%), ampicillin (15,20%), gentamicin (13, 17%), ticarcillin-clavulanic acid (10, 13%), cefepime (9, 12%), aztreonam (5, 7%) antibiotics in 0 to 10 years age group and ciprofloxacin (26, 20.15%), co-trimoxazole (25, 19.37%), cefotaxime (24,

18.60%), amoxicillin-clavulanic acid (21, 16.27%), gentamycin (20, 15.50%), cefazolin (19, 14.72%), ampicillin (18,13.95%), ticarcillin-clavulanic acid (18, 13.95%), cefuroxime (17, 13.17%), aztreonam (15, 11.62%), cefepime (10, 77.51%) in 11 to 20 years age group, as such, these antimicrobials may not be suitable for treating case of UTI in this locality.

However, a large proportion of the isolates were sensitive to nitrofurantoin (22, 28.9%), meropenem (18, 23.68%), piperacillin- tazobactam (18, 23.68%), cefoperazone-sulbactum (17, 22%), gentamycin (17, 22%), amikacin (16, 21%) and co-trimoxazole(9,12%) in 0 to 10 years age group and amikacin (33, 25.58%), nitrofurantoin (24, 18.60%), piperacillin-tazobactam (20, 5.50%), gentamicin (20, 15.50%), cefoperazone-sulbactam (19, 14.72%), amoxicillin-clavulanic acid (18, 13.95%), meropenem (18,13.95%), ciprofloxacin (15, 11.62%), co-trimoxazole (12, 9.30%), aztreonam (10, 7.75%) in 11 to 20 years age group, and should be considered as first line drugs for treating cases of urinary tract infection in this Garhwal region. Ciproflaxin and co-trimoxazole are however best avoided in both groups children as well as in adolescents.

Since urinary tract infection may be asymptomatic in most cases (as this study has shown), it is therefore suggested that routine screening of patients of Pyrexia of unknown origin (PUO) be done for urinary tract infection and the appropriate antimicrobials administered after antibiotic sensitivity tests have been carried out in order to prevent the cases becoming symptomatic later with resultant renal damage.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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