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Full Length Research Paper

Molecular characterization and genetic variability studies of clinical isolates of UTI pathogens

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***Klebsiella pneumoniae* and *Enterobacter* sp. are emerging as major health problems world-wide. UTI is a significant problem in children, as well as adults and requires a large scale study at regular intervals in order to identify organisms from time to time and recommend prompt treatment to reduce UTI related morbidity and mortality in humans. In the present study, organisms were isolated from urine samples of patients suspected for UTI. The isolated organisms were identified based on biochemical and molecular analyses and were characterized. They were identified as *K. pneumoniae* SSN, *K. pneumoniae* KSS and *Enterobacter* sp. SCSS. Also genetic variations between the two *Klebsiella* sp. were studied by virtual digestion using different restriction enzymes.**

Key words: UTI, *Klebsiella pneumoniae*, *Enterobacter* sp., 16S rRNA, biochemical characterization.

INTRODUCTION

Urinary tract infection is a common infection among human population. It occurs frequently in women when compared to men, but in men the symptoms are more severe and protracted, Abdulhadi et al. (2008). UTIs are normally caused by the bacteria belonging to the family Enterobacteriaceae which includes *Escherichia coli, Klebsiella pneumoniae, Enterobacter* sp., *Serratia* sp., *Proteus vulgaris* etc. These bacterial strains are said to be crucial in the development of nosocomial infections because of their adhesive properties, antibiotic resistance and involvement in colonization or infection (Livrelli et al., 1996).

Urinary tract obstruction is one of the main causes of UTI which produces the favourable environment for the growth of urinary tract pathogens. UTI can be identified by the presence of significant quantity of microorganisms in the urine along with signs and symptoms of infection. Urinary tract obstruction is also the important cause of bacteremia due to Gram negative organisms.

UTI are commonly treated by a short course of antibiotics. The widespread and irregular use of antibiotics has resulted in the development of resistant strains of bacteria, which makes the treatment of these organisms difficult. Organisms encoding multiple antibiotic resistance genes are becoming increasingly prevalent in the recent past. These problems have forced the scientific community to look out for new antibiotics and drugs that act on these organisms in a different way than the earlier antibiotics.

Hence the present study was focused to isolate, identify and characterize three UTI pathogens from suspected patients. The identification of the organisms was carried out using biochemical and molecular analyses such as sequencing of16s r RNA region of the DNA. Ribosomal RNA gene sequences have been extremely useful in defining bacterial relationships, especially in identifying environmental or non-cultured

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Abbreviation: UTI, Urinary tract infection.

isolates (Julio et al., 2004).

MATERIALS AND METHODS

Isolation of bacterial strains

Bacterial strains were isolated from the midstream urine samples of patients suspected for UTI at Scudder Laboratory and Vivek Diagnostic Laboratory, Nagercoil, Southern India. The organisms were isolated from the urine samples and the pure culture was maintained in nutrient agar slants for further studies. The samples were analyzed at the Department of Microbiology, Noorul Islam College of Arts and Science, Kumaracoil, Kanyakumari District Tamil Nadu, India.

Biochemical characterization of the organisms

Gram staining was performed for the three clinical isolates using the method put forth by Christian Gram (Cappuccino and Sherman, 1996). Biochemical tests namely Indole production, Methyl Red, Voges-Praskauer test, citrate utilization test, gelatin hydrolysis test, triple sugar iron agar test, H_2S production test, carbohydrate fermentation test and urease test were performed using standard protocols (Cappuccino and Sherman, 1996). The results of the tests were used for the identification of the isolated strains according to Bergey's Manual of Systematic Bacteriology (Holt et al., 1994).

16S rDNA sequencing

Genomic DNA was isolated from the three bacterial isolates and 16S rRNA region of the DNA was amplified using universal 16S rRNA primers in thermal cycler. The PCR reaction conditions were, initial denaturation for 5 min at 94°C, denaturation for 30 s at 94°C, annealing for 30 s at 55°C, extension at 72°C for 2 min and final extension at 72°C for 15 min. The PCR amplified products were then run on agarose gel, eluted, purified and sequenced.

BLAST analysis

The 16S rDNA sequences of the three isolates were subjected to BLAST analysis (Altschul et al., 1990) using NCBI BLAST tool at www.ncbi.nlm.nih.gov.

Virtual digestion

Virtual digestion of the two *K. pneumoniae* 16S rDNA regions was done using NEB cutter tool available in the World Wide Web (WWW). Sixty eight single cutter restriction enzymes available at NEB were used in the virtual digestion process.

GenBank submission

The three 16S rRNA gene sequences were submitted to GenBank database using the BankIt sequence submission tool and accession numbers were obtained.

RESULTS AND DISCUSSION

Urinary tract infection is one of the most common types of infectious diseases encountered in the practice of medicine today. In females, UTI is more common than men because of the short urethra and hormonal imbalance (Valiquette, 2001).

Bacterial cultures were isolated from urine samples of patients suspected for UTI in and around Nagercoil township and the three bacterial isolates were named as SSN, KSS and SCSS. The three isolates were found to be Gram negative *Bacilli*. The isolates SSN and KSS were found to be non-motile, while SCSS was found to be motile.

The other biochemical tests revealed the two bacterial isolates KSS and SSN as *K. pneumonia* (Table 1). Further 16S rRNA gene sequencing of the two strains was carried out as it is a valid method for identification and taxonomical purposes for *Klebsiella* as stated by Boye and Hansen (2003) and Guo et al. (2008).

The bacterial isolate SCSS also showed similar biochemical results but with difference in motility. This points the identity of the pathogen towards the genus *Enterobacter* (Table 1). *K. pneumoniae* is normally distinguished from *Enterobacter* sp. only by the presence of a thicker capsule and its lack of motility (Hans, 2004). Hence the more reliable and valuable sequence analysis of 16S rDNA was performed to confirm the identity of the third bacterial strain SCSS (Wang et al., 2006).

Sequencing of the 16Sr RNA region of the Genomic DNA of the three bacterial isolates revealed that the isolate SSN has 1422 Base pairs (bp), isolate KSS 1414 bp and SCSS 1424 bp. In the present investigation, the BLAST analysis of the 16s rRNA region of the DNA sequences of the two bacterial isolates revealed 99% similarity to *K. pneumoniae* and uncultured *Klebsiella* sp (Tables 2 and 3) and thus the isolates KSS and SSN were confirmed as *K. pneumoniae.* The bacterial isolate SCSS revealed 99% similarity to *Enterobacter* sp. based on the E-value in BLAST analysis and was therefore confirmed as *Enterobacter* sp. (Table 4). Madhavi (2003) reported that *K. pneumoniae* and *E. coli* are very common in UTIs among persons under treatment in Nellore district, Andhra Pradesh .

The *K. pneumonia* isolates KSS and SSN were subjected to virtual digestion using single cutter restriction enzymes available in NEB cutter tools. It revealed variation in their digestion pattern. Both the sequences showed restriction sites for enzyme like StuI, ApoI, EcoRI, BaeI etc however, variation was observed in the digestion patterns of the enzymes AgeI, Bsu36I, SmlI, BglI, BpuEI and RsrII (Figures 1 and 2). The variation in restriction profile may be due to mutation in the DNA sequences and this variation in turn shall result in antigenic variants of the species that may aid the bacterial strains to evade antibiotic activity. Sundar and Nelson (2006) reported that variation in genetic make-up of M-protein of *Streptococcus pyogenes* could lead to antigenic drift. Sreedevi (2008), performed RFLP profile of genomic DNA of wild and virulent strains of *Salmonella typhimurium* and reported that the virulent strain had more restriction sites than the wild strain.

All the three sequences were submitted to primary DNA database GenBank with all annotations using BankIt

Table 1. Biochemical tests of clinical isolates of UTI bacteria.

Sl. No.	Biochemical test	Isolate SSN	Isolate KSS	Isolate SCSS
1	Motility	-	-	+
2	Mannitol	+	+	+
3	Indole	-	-	-
4	Methyl red	-	-	-
5	Voges- Proskauer	+	+	+
6	Citrate utilization	+	+	+
7	Gelatin hydrolysis	-	-	-
8	Triple sugar iron	+	+	+
9	H_2S production	-	-	-
10	Urease	+	+	+
11	Gas in glucose	+	+	+
12	Acid in lactose	+	+	+

+ indicates present - indicates absent.

Table 2. BLAST analysis of 16S rRNA sequence of *Enterobacter* sp. (SCSS).

Sequences producing significant alignments				
Accession	**Description**	**Max score**	**E value**	**Max ident (%)**
GQ418145.1	Uncultured *Enterobacter* sp. clone F4aug.7 16S ribosomal RNA gene, partial sequence	2591	0.0	99
GQ418139.1	Uncultured *Enterobacter* sp. clone F4aug.1 16S ribosomal RNA gene, partial sequence	2591	0.0	99
GQ418123.1	Uncultured *Enterobacter* sp. clone F4jun.36 16S ribosomal RNA gene, partial sequence	2591	0.0	99
GQ418119.1	Uncultured *Enterobacter* sp. clone F4jun.32 16S ribosomal RNA gene, partial sequence	2591	0.0	99
GQ418079.1	Uncultured *Enterobacter* sp. clone F4apr.38 16S ribosomal RNA gene, partial sequence	2591	0.0	99
GQ416035.1	Uncultured *Enterobacter* sp. clone F5feb.66 16S ribosomal RNA gene, partial sequence	2591	0.0	99
FJ868806.1	*Enterobacter* sp. BSRA2 16S ribosomal RNA gene, partial sequence	2591	0.0	99
EF551364.1	*Enterobacter* cloacae strain Rs-35 16S ribosomal RNA gene, partial sequence	2591	0.0	99
GQ418160.1	Uncultured *Enterobacter* sp. clone F4aug.22 16S ribosomal RNA gene, partial sequence	2586	0.0	99
GQ418153.1	Uncultured *Enterobacter* sp. clone F4aug.15 16S ribosomal RNA gene, partial sequence	2586	0.0	99
GQ418143.1	Uncultured *Enterobacter* sp. clone F4aug.5 16S ribosomal RNA gene, partial sequence	2586	0.0	99
GQ418120.1	Uncultured *Enterobacter* sp. clone F4jun.33 16S ribosomal RNA gene, partial sequence	2586	0.0	99
GQ418084.1	Uncultured *Enterobacter* sp. clone F4apr.43 16S ribosomal RNA gene, partial sequence	2586	0.0	99
GQ416034.1	Uncultured *Enterobacter* sp. clone F5feb.65 16S ribosomal RNA gene, partial sequence	2586	0.0	99
GQ416033.1	Uncultured *Enterobacter* sp. clone F5feb.64 16S ribosomal RNA gene, partial sequence	2586	0.0	99

online submission tool. The accession number of the clinical isolates *Enterobacter* sp. SCSS, *K. pneumonia* KSS and *K. pneumoniae* SSN were HM0078911, HM0078912 and HM0078913, respectively.

Table 3. BLAST analysis of 16S rRNA sequence of *K. pneumoniae* (KSS).

Sequences producing significant alignments				
Accession	**Description**	**Max score**	**E value**	**Max ident (%)**
GQ416323.1	Uncultured *Klebsiella* sp. clone F7feb.67 16S ribosomal RNA gene, partial sequence	2586	0.0	99
GQ416322.1	Uncultured *Klebsiella* sp. clone F7feb.66 16S ribosomal RNA gene, partial sequence	2586	0.0	99
GQ416229.1	Uncultured *Klebsiella* sp. clone F5oct.25 16S ribosomal RNA gene, partial sequence	2586	0.0	99
GQ416141.1	Uncultured *Klebsiella* sp. clone F5jun.28 16S ribosomal RNA gene, partial sequence	2586	0.0	99
EF509874.1	Uncultured bacterium clone P4D7-616 16S ribosomal RNA gene, partial sequence	2586	0.0	99
EF509786.1	Uncultured bacterium clone P4D7-584 16S ribosomal RNA gene, partial sequence	2586	0.0	99
EF509013.1	Uncultured bacterium clone P3D5-483 16S ribosomal RNA gene, partial sequence	2586	0.0	99
GQ158250.1	Uncultured bacterium clone 16slp112-1a06.w2k 16S ribosomal RNA gene, partial sequence	2580	0.0	99
GQ158249.1	Uncultured bacterium clone 16slp112-1a01.p1k 16S ribosomal RNA gene, partial sequence	2580	0.0	99
GQ158247.1	Uncultured bacterium clone 16slp112-2a01.p1k 16S ribosomal RNA gene, partial sequence	2580	0.0	99
GQ158246.1	Uncultured bacterium clone 16slp112-1a05.q1k 16S ribosomal RNA gene, partial sequence	2580	0.0	99
GQ158235.1	Uncultured bacterium clone 16slp112-1b01.w2k 16S ribosomal RNA gene, partial sequence	2580	0.0	99
GQ418030.1	Uncultured *Klebsiella* sp. clone F4mar.11 16S ribosomal RNA gene, partial sequence	2580	0.0	99
GQ417042.1	Uncultured *Klebsiella* sp. clone F1Sfeb.16 16S ribosomal RNA gene, partial sequence	2580	0.0	99
GQ417041.1	Uncultured *Klebsiella* sp. clone F1Sfeb.15 16S ribosomal RNA gene, partial sequence	2580	0.0	99

Table 4. BLAST analysis of 16S rRNA sequence of *K. pneumoniae* (SSN).

Sequences producing significant alignments				
Accession	**Description**	**Max score**	**E value**	**Max ident (%)**
CP000647.1	*Klebsiella* pneumoniae subsp. pneumoniae MGH 78578, complete sequence	2601	0.0	99
EF509884.1	Uncultured bacterium clone P4D7-592 16S ribosomal RNA gene, partial sequence	2601	0.0	99
GU374008.1	*Klebsiella* sp. enrichment culture clone SRC_DSA23 16S ribosomal RNA gene, partial sequence	2595	0.0	99
GU373992.1	*Klebsiella* sp. enrichment culture clone SRC_DSA5 16S ribosomal RNA gene, partial sequence	2595	0.0	99
GU373991.1	*Klebsiella* sp. enrichment culture clone SRC_DSA7 16S ribosomal RNA gene, partial sequence	2595	0.0	99
GQ418035.1	Uncultured *Klebsiella* sp. clone F4mar.16 16S ribosomal RNA gene, partial sequence	2595	0.0	99
GQ418031.1	Uncultured *Klebsiella* sp. clone F4mar.12 16S ribosomal RNA gene, partial sequence	2595	0.0	99
GQ416323.1	Uncultured *Klebsiella* sp. clone F7feb.67 16S ribosomal RNA gene, partial sequence	2595	0.0	99

Table 4. Contd.

GQ416322.1	Uncultured *Klebsiella* sp. clone F7feb.66 16S ribosomal RNA gene, partial sequence	2595	0.0	99
GQ416229.1	Uncultured *Klebsiella* sp. clone F5oct.25 16S ribosomal RNA gene, partial sequence	2595	0.0	99
GQ416141.1	Uncultured *Klebsiella* sp. clone F5jun.28 16S ribosomal RNA gene, partial sequence	2595	0.0	99
GQ415955.1	Uncultured *Klebsiella* sp. clone F4oct.39 16S ribosomal RNA gene, partial sequence	2595	0.0	99
GQ415952.1	Uncultured *Klebsiella* sp. clone F4oct.36 16S ribosomal RNA gene, partial sequence	2595	0.0	99
GQ415943.1	Uncultured *Klebsiella* sp. clone F4oct.27 16S ribosomal RNA gene, partial sequence	2595	0.0	99
EF509891.1	Uncultured bacterium clone P4D7-395 16S ribosomal RNA gene, partial sequence	2595	0.0	99

Figure 1. Virtual digestion of 16S rDNA sequence of *Klebsiella pneumoniae* (SSN).

Figure 2. Virtual digestion of 16S rDNA sequence of *Klebsiella pneumoniae* (KSS).

The present study clearly signifies that *K. pneumoniae* and *Enterobacter* sp. are two important pathogens involved in urinary tract infections in Nagercoil Township. This is in context with Langley et al. (2001) who reported the causative agents of UTI as *E. coli, Klebsiella, Enterobacter* and *Enterococcus*. The findings confirm the possibility of strain variants in *Klebsiella* sp., which warrants further studies on antigenic determinants, identification of conserved regions and common drug targets in the virulent regions of the bacterium.

REFERENCES

Abdulhadi SK, Yashua AH, Uba A (2008). Organisms causing urinary tract infection in paedriatic patients at Murtala Muhammed Specialist Hospital, Kano, Nigeria, Int. J. Biomed. Hlth. Sci., 4: 165-167.

Altschul SF, Gish W, Myers EW, Lipman DJ (1990). Basic local alignment search tool, J. Mol. Biol., 215: 403-410.

Boye K, Hansen DS (2003). Sequencing of 16S rDNA of *Klebsiella,* taxonomic relations within the genus and to other Enterobacteriaceae, Int. J. Med. Microbiol., 292: 495-503.

Cappuccino JG, Sherman N (1996). Microbiology- A laboratory Manual, Benjamin Cummins, New York,

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994). Bergey's Manual of Determinative Bacteriology, 9th Ed. Baltimore, Md. Williams and Wilkins,

Guo XL, Wang DC, Zhang YM, Wang XM, Zhang Y, Zuo Y, Zhang DM, Kan B, Wei L, Gao Y (2008). Isolation, identification and 16S rDNA phylogenetic analysis of *Klebsiella pneumoniae* from diarrhea specimens, J. Clin. Microbiol., 29: 1225-1229.

Hans GS (2004). General Microbiology, Cambridge University, 7th Ed., p. 313.

Julio M, Lucia M, Monica R, Jesus S, Esperanza M (2004). How are gene sequence analyses modifying bacterial taxonomy. The case of *Klebsiella,* Int. Microbiol., 7: 261-268.

Langley JM, Hanakowski M, Leblanc JC (2001). Unique epidemiology of nosocomial urinary tract infection in children. Am. J. Infect. Control, 2: 94-98.

Madhavi LV (2003). Screening of urinary tract pathogens, M.Sc., Thesis, Bharathidasan University, Tiruchirapalli India,

Sreedevi S (2008). RFLP profiling of wild and mutant strains of *Salmonella typhimurium,* M. Phil. Thesis, Annamalai University, Tamil Nadu, India,

Sundar SK, Nelson R (2006). Sequences and structural analysis of *Streptococcus pyogenes* M protein, Ind. J. Microbiol., 46(3): 223-228.

Vale RL, Christophe DA, Patrick DM, Arette D, Christiane F (1996). Adhesive properties and antibiotic resistance of *Klebsiella pneumoniae* and *Serratia* clinical isolates involved in nosocomial infections, J. Clin. Microbiol., 34: 1963-1969.

Valiquette L (2001). Urinary tract infections in women. Can. J. Urol., 8(1): 6-12.

Wang YY, Li HR, Jia SF, Wu ZJ, Guo BH (2006). Analysis of bacterial diversity of kefir grains by denaturing gradient gel electrophoresis and 16S rDNA sequencing. Int. Microbiol., 81: 310-313.