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Intra and Interspecies Variations among Environmental *Klebsiella* Isolates

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Abstract : Majority of the study of *Klebsiella* is restricted to clinical isolates and its occurrence in fresh water environment is sporadically reported. Biochemical and molecular diversity of 40 *Klebsiella* isolates, isolated from 180 water samples of river Narmada from eleven stations (Amarkantak to Dahej) during 2005-2006 was assessed by employing Biotyping, SDS-PAGE profiling, Randomly Amplified Polymorphic DNA (RAPD) analysis, and Amplified Ribosomal DNA Restriction Analysis (ARDRA). The Antibiogram analysis suggested that most of the isolates were resistant against Ampicillin, Amoxycillin, Tetracycline, Chloramphenicol and Trimethoprim and all isolates were found to be sensitive against Norflaxcin, Cefotaxime and Gentamycin. RAPD and ARDRA prevailed over Biotyping and Protein profiling for identification, and Intra and interspecies variations among environmental isolates of *Klebsiella* spp.

Key words : *Klebsiella*, Fresh water, Antibiogram, SDS-PAGE, Molecular typing.

Introduction

Klebsiella spp. are found in surface water, sewage, soil and on plants, and also on the mucosal surfaces of mammals such as humans, horses, or swine, which they colonize Jemeyey et.al. (2006). Clinically, the most important species are *Klebsiella pneumoniae* and *K. oxytoca*, while *K. ornithinolytica*, *K. terrigena*, and *K. planticola* are rarely isolated from human clinical specimens Podschun and Ullmann (1998). *K. planticola* and *K. terrigena* are considered to be environmental species, as reflected in their species designations. *K. pneumoniae* and *K. oxytoca* exhibit a high degree of genetic heterogeneity, as demonstrated by capsular typing (Orskov and Orskov, 1984), O-antigen variation, biotyping (Rennie and Duncan, 1974), protein electrophoretic profiling, multilocus enzyme electrophoresis, ribotyping, randomly amplified polymorphic DNA (RAPD) analysis (Wong et. al. 1993). The association between genetic variability and virulence and transmissibility of *Klebsiella* strains is not well understood, but there is clear evidence for differential behavior of *Klebsiella* strains.

In humans, *Klebsiella spp* causing infections are often multidrug resistant and an increasing proportion of strains produce extended-spectrum â-lactamases (ESBLs) enzymes, that confer resistance to penicillins (such as ampicillin or amoxicillin), to first generation cephalosporins, to newer cephalosporins like cefotaxime, ceftazidime, cefoxitin and ceftiofur, and to aztreonam Deshpande et. al. (2000). The prevalence of antimicrobial resistance in

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environmental *Klebsiella* isolates is poorly documented.

People from India and other developing countries use river water directly not only for drinking but also for various recreational purposes. Narmada is the fifth largest river of India and originates from Amarkantak (M.P.) and merges in the Arabian Sea at Bharuch (Gujarat). The diversity studies of *Klebsiella* are restricted to the clinical isolates and to our knowledge the present study is the first report on the diversity of *Klebsiella*, an immense clinically significant organism, in the river Narmada.

Material and Methods

Water samples were collected from eleven stations of river Narmada (viz. Amarkantak, Dindori, Mandla, Jabalpur, Narsinghpur, Hoshangabad, Omkareshwar, Koral, Neelkantheshwar, Ankaleshwar and Dahej), over one year period from July 2005 to June 2006. 500ml water samples, collected aseptically under ice-cold condition were brought to the Bacteriology lab, Dept. of Biological Sciences, Rani Durgavati University, Jabalpur and concentrated on 0.45 mm pore diameter filters, enriched in 1.5% peptone water (pH 7.0) and streaked on MacConkey agar medium (Himedia, India) for isolation of *Klebsiella* spp (APHA, 1985).

All the isolates were examined for the following biochemical characteristics; Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate, indole, oxidase, urease, MR, VP, gluconate, malonate, Acid from arabinose, dulcitol, glucose, lactose, maltose, mannitol, raffinose, rahmnose, sorbitol, sucrose and xylose following method given by MacFaddin (1980). The isolates were identified with the help of Bergey's Manual of Systematic Bacteriology (Krieng and Halt, 1984) and PIB (Probabilistic Identification of Bacteria) computer kit Bryant (2003), maintained in the Bacteriology lab, Department of Biological Sciences, R.D. University, Jabalpur (MP), India and were given BGCC (Bacterial Germplasm Collection Centre) numbers. All the cells (n = 40), were grown to the stationary phase in Luria-Bertani (LB) broth. These cultures were used as the source of template DNA for RAPD and ARDRA analysis. For RAPD and ARDRA the genomic DNA was extracted by following the method of Sambrook, et. al. (1989). For ARDRA, oligonucleotide primers were derived from conserved regions present at the edges of the l6S rDNA. The sequences of primers were 16S rRNA F-5'–TACCTTGTTACGACTTCGTCCCA–3' and 16S rRNA R–5'- AGTTTGATC CTGGCTCAG – 3'. 1 ml (around 10 ng DNA) of the template DNA was added to 49 ml aliquots of PCR mixture containing 5 ml of l0X buffer supplied with enzyme Taq polymerase, 2.5 ml of each forward and reverse primer from a 10 picomol stock (i.e.12.5 picomol of each primer), 2.5 ml of 10 mM (each) deoxynucleotide triphosphates and 0.5 ml of DNA polymerase (3 unit/ ml), and final volume was made 50 ml by adding sterile distilled water. After initial denaturation at 94°C for 5 min, the reaction mixture was run through 35 cycles of denaturation at 94°C for 1 min, annealing at 51°C for 1 min and extension at 72°C for 1 min, finally a 10 min extension period at 72°C was carried out. The 16S rRNA type was determined by digestion of the amplicons with *HindIII* A/ AGCTT, *EcoRI* (G/AATTC) (Bangalore Genei, India) and analysed by electrophoresis on 1.5% agarose gel with staining by ethidium bromide (0.5µg/ml). For RAPD, PCR amplification of isolated template DNA was carried out with a primer [5' – CAT TCG

ACC 3'] by following the method of Lopes et. al. (2005). The amplification was performed in an automated thermal cycler (Eppendroff master cycler gradient, Humburg, Germany). The reaction was performed in 25 ml reaction mixture containing 2.5 ml of 10X PCR buffer [500 mM KCl, 100 mM Tris (pH 8.3)], 25 mM $MgCl_2$], 3ml of template DNA, 2.0 ml primer, 0.3 ml of Taq DNA polymerase, 2.5 ml of 2.5 mM dNTP's (Bangalore Genei, India) . The volume was raised to 25 ml with sterile triple distilled water. A negative control was maintained containing all components except template DNA. After initial denaturation at 94°C for 5 min the reaction mixture was run through 45 cycle of denaturation at 94°C for 1 min, annealing at 36°C for 1 min and elongation/extension at 72°C for 2 min followed by a 10 min final extension period at 72°C and the expected size of the amplicons were ascertained by electrophoresis in 1.5% Agarose gel with an appropriate molecular size marker (1 Kb DNA ladder, Bangalore Genei, India). Protein profiling was obtained by following the method of Laemmli (1970), Disk diffusion assay was followed to assess the antibiotic resistance/ sensitivity pattern as described (CLSI, 2002). Using commercially available disks (Himedia, India): Streptomycin (S, 25mcg), Ampicillin (A, 30mcg), Amoxicillin (Am, 30mcg), Chloramphenicol (C, 30mcg), Norflaxcin (Nx, 10mcg), Tetracycline (T, 30mcg), Ciprofloxacin (Cf, 5mcg), Cefotaxime (Ce, 30mcg), Nalidixic acid (Na, 30mcg), Ceftazidime (Ca, 30mcg), Trimethoprim (Tr, 10mcg), Gentamycin (G, 10mcg). RAPD-PCR and Protein profiling bands were scored as either present (1) or absent (0). All binary data were entered and genetic distances were calculated through Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc), version 2.02 (Exeter software, New York) and calculating Euclidean distance and then assembling a dendrogram using "Unweighted pair group method using arithmetic - average clustering criterion.

Results and Discussion

The molecular diversity and evolution of these groups of aquatic bacteria deserve in depth investigation, in order to understand better, their ecological role and function in the aquatic environment. The results of the present study provide the systematic investigation of intra and interspecies diversity of *Klebsiella* spp from riverine environment, the river Narmada. In the present study, conventional cultural characteristics, morphological and biochemical tests in conjunction with SDS-PAGE, RAPD, and ARDRA were employed in order to identify isolates of *Klebsiella* from fresh water of river Narmada.

Isolates were first characterized by conventional methods using biochemical tests. Biochemical characterization, which involved a number of biochemical reactions important among them, were lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, as the decarboxylase activity is an important diagnostic tool in identifying *Klebsiella* spp. from clinical and environmental samples (Trevison, 1987). Five different biotypes arbitrarily designated B1 to B5 were distributed among the 40 *Klebsiella* spp isolated from river Narmada on the basis of biochemical characteristics (Table 1). *Klebsiella pneumoniae* isolates were grouped into three biotypes B1 (VP and Urease positive, MR negative), B4 (VP and Urease negative, Lysine positive) and B5 (Lysine positive and Indole negative). All *K. oxytoca* and *K. terrigena* were grouped into biotypes B2 (Malonate and VP negative) and

Table 1 : Source, phenotype, genotype and antibiotype of 40 *Klebsiella* spp isolated from river Narmada.

Isolate no.	Species	Sites of isolation	Biotyping	ARDRA pattern		Protein pattern	Antibiotype
				EcoRI	*HindIII*		
1	*K. pneumoniae*	Amarkantak	B1	AR1	BR1	Pr1	A1
2	*K. terrigena*	Amarkantak	B2	AR2	BR2	Pr2	A2
3	*K. terrigena*	Amarkantak	B2	AR2	BR2	Pr2	A3
4	*K. pneumoniae*	Dindori	B1	AR1	BR1	Pr1	A4
5	*K. pneumoniae*	Dindori	B1	AR1	BR1	Pr1	A5
6	*K. pneumoniae*	Dindori	B4	AR1	BR1	Pr1	A3
7	*K. pneumoniae*	Dindori	B1	AR1	BR1	Pr1	A1
8	*K. oxytoca*	Mandla	B1	AR1	BR1	Pr1	A2
9	*K. pneumoniae*	Mandla	B3	AR3	BR3	Pr1	A3
10	*K. pneumoniae*	Mandla	B5	AR1	BR1	Pr1	A3
11	*K. pneumoniae*	Mandla	B1	AR1	BR1	Pr1	A5
12	*K. pneumoniae*	Mandla	B4	AR1	BR1	Pr1	A4
13	*K. terrigena*	Mandla	B2	AR2	BR2	Pr2	A6
14	*K. terrigena*	Jabalpur	B2	AR2	BR2	Pr4	A6
15	*K. pneumoniae*	Jabalpur	B4	AR1	BR1	Pr1	A5
16	*K. pneumoniae*	Jabalpur	B1	AR1	BR1	Pr1	A2
17	*K. pneumoniae*	Jabalpur	B1	AR1	BR1	Pr1	A3
18	*K. pneumoniae*	Jabalpur	B5	AR1	BR1	Pr1	A6
19	*K. pneumoniae*	Jabalpur	B3	AR3	BR3	Pr1	A3
20	*K. pneumoniae*	Narsinghpur	B1	AR1	BR1	Pr1	A7
21	*K. pneumoniae*	Narsinghpur	B4	AR1	BR1	Pr7	A8
22	*K. oxytoca*	Narsinghpur	B5	AR1	BR1	Pr3	A3
23	*K. pneumoniae*	Hoshangabad	B3	AR3	BR3	Pr8	A3
24	*K. pneumoniae*	Hoshangabad	B5	AR1	BR1	Pr8	A9
25	*K. pneumoniae*	Hoshangabad	B1	AR1	BR1	Pr8	A3
26	*K. pneumoniae*	Hoshangabad	B4	AR1	BR1	Pr7	A1
27	*K. pneumoniae*	Hoshangabad	B4	AR1	BR1	Pr7	A1
28	*K. pneumoniae*	Omkareshwar	B1	AR1	BR1	Pr8	A10
29	*K. pneumoniae*	Omkareshwar	B5	AR1	BR1	Pr8	A2
30	*K. pneumoniae*	Omkareshwar	B1	AR1	BR1	Pr3	A3
31	*K. oxytoca*	Omkareshwar	B3	AR3	BR3	Pr5	A10
32	*K. pneumoniae*	Neelkantheshwar	B4	AR1	BR1	Pr3	A10
33	*K. pneumoniae*	Neelkantheshwar	B5	AR1	BR1	Pr3	A3
34	*K. pneumoniae*	Neelkantheshwar	B1	AR1	BR1	Pr3	A11
35	*K. pneumoniae*	Neelkantheshwar	B1	AR1	BR1	Pr3	A1
36	*K. oxytoca*	Neelkantheshwar	B3	AR3	BR3	Pr3	A5
37	*K. pneumoniae*	Ankaleshwar	B1	AR1	BR1	Pr3	A7
38	K. pneumoniae	Ankaleshwar	B4	AR1	BR1	Pr3	A12
39	K. oxytoca	Ankaleshwar	B1	AR1	BR1	Pr3	A12
40	*K. pneumoniae*	Ankaleshwar	B1	AR1	B R1	Pr6	A2

B3 (Indole and Lysine positive). The biotyping of *Klebsiella* had shown biochemical differentiation among various species by generating different biochemical profiles among *Klebsiella* isolated from the same environment. However, the biotyping have limitations of being time consuming, which could be unrealistic for the identification of genus *Klebsiella* during any outbreak (Alves, et. al. 2006).

Ribosomal RNA analysis has been extremely useful in defining relationships of *Klebsiella* spp (Boye and Hanson, 2003). In this study, the 16S rRNA gene of each of the 40 *Klebsiella* isolates was amplified and restricted with the enzymes *EcoRI* and *HindIII*. This resulted in three separate restriction patterns (assigned as AR1, AR2, AR3, and BR1, BR2, BR3, respectively) for each species. Each enzyme generated different number of bands ranging between 150-400 bp for each species (Fig. 1). ARDRA using *HindIII* appears to be a promising tool for identification of environmental *Klebsiella* spp. In confirmation with Brisse, and Verhoef (2001), ARDRA with *EcoRI* did not appear suitable for cluster identification in the present study, but its higher discriminatory power is useful to subdivide *HindIII* ARDRA groups.

RAPD has been used as a typing tool to discriminate *K. pneumoniae* strains in previous investigations (Lopes et. al. 2005). We found 23 RAPD types among 40 *Klebsiella* spp studied, which demonstrates the high discriminatory power of RAPD. There was a common band in all the cases in the molecular weight of 300 bp. However no species-specific band was observed. The number of bands ranged from 3-10 in each run. The high number of serotypes in this species (Orskov and Orskov, 1984) also could explain the relevant degree of genetic

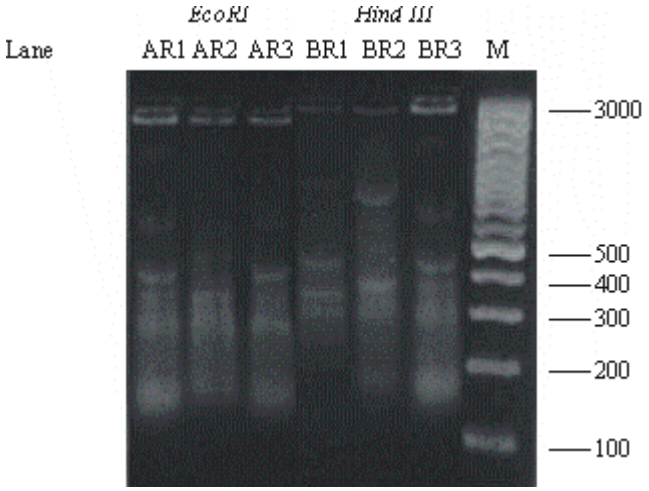

Fig. 1 : Representative 16S rRNA PCR-RFLP profiles obtained for *Klebsiella* isolates using restriction enzymes (*EcoRI* and *HindIII*). Lane M: DNA marker (100 bp Ladder). ARDRA types AR1, AR2 and AR3 correspond to *K. pneumoniae*, *K. oxytoca* and *K. terrigena* restricted by *EcoRI* respectively and ARDRA types BR1, BR2 and BR3 correspond to *K. pneumoniae*, *K. oxytoca* and *K. terrigena* restricted by *Hind III* respectively.

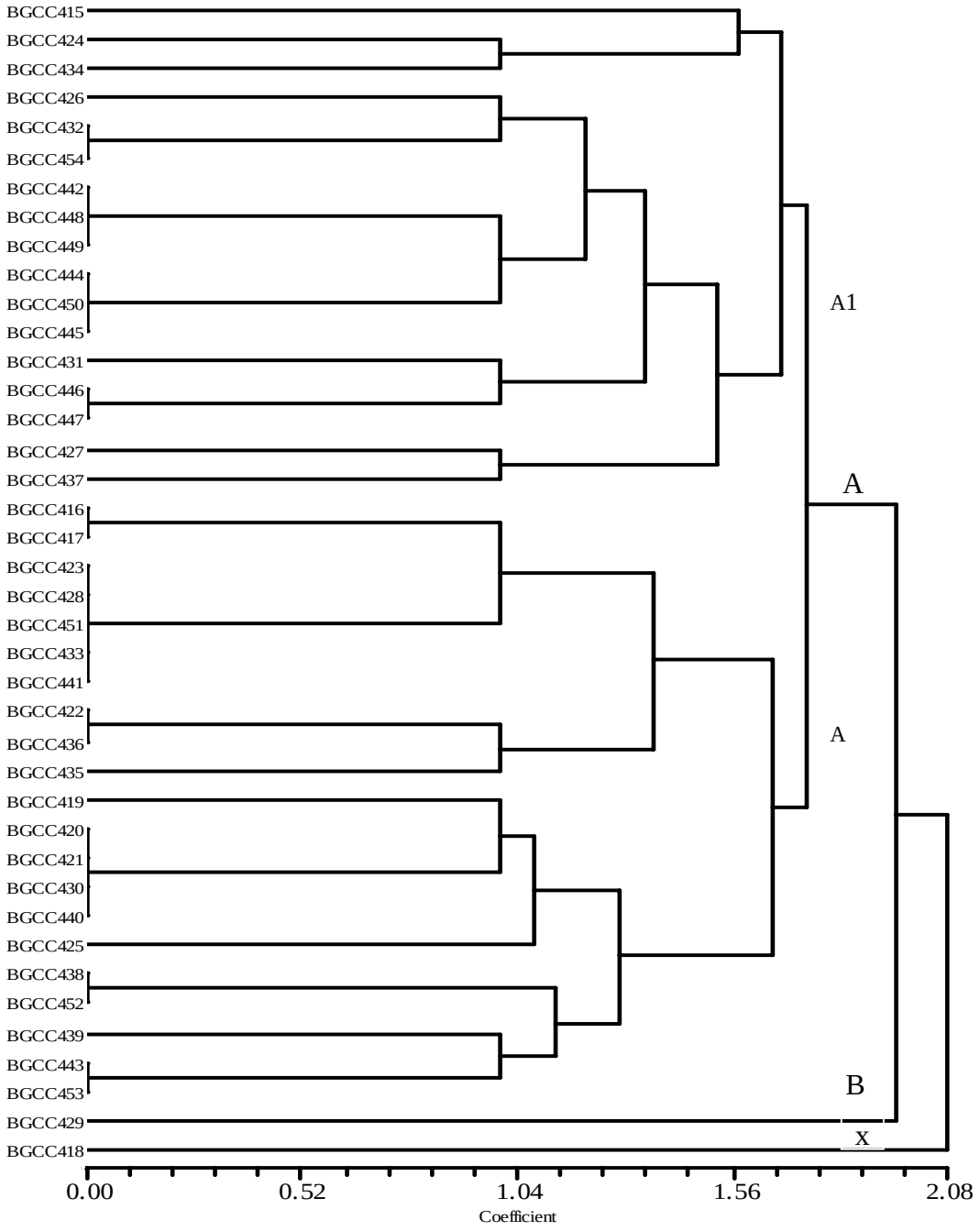

Fig. 2 : UPGMA Cluster Analysis of Klebsiella isolated from river Narmada on basis of RAPD.

diversity highlighted by RAPD analysis. The UPGMA cluster analysis showed 100% similarity at a coefficient of 2.08. At 1.97 coefficient, two clusters were formed (A and B). The cluster A was subdivided into two sub divisions (A1 and A2) at 1.73 coefficient level, consisting of 17 and 21 isolates respectively (Fig. 2).

Analysis of SDS-PAGE of total protein showed about 20 different bands, ranging in size from 25 kDa to 66.2 kDa. Depending upon sizes and number of electrophoretic bands the 40 isolates were grouped into 8 different patterns designated as Pr1 to Pr8 (Table 1). In the present study, out of 40 isolates, 16 isolates belonged to group PrI, 3 isolates belonged to group PrII, 10 isolates belonged to group PrIII, 3 and 5 isolates belonged to group PrVII and PrVIII, and only single isolate belonged to group PrIV, PrV and PrVI respectively. Protein analysis was found less efficient to differentiate *K. pneumoniae* strains than antibiotype, plasmid analysis and RAPD (Lopes, et. al. 2005).

The role of antibiotics in the management of human infections caused by *Klebsiella* has not been defined, although antimicrobial resistance could be an important problem for therapy directed against these organisms. *Klebsiella* isolates are naturally resistant to ampicillin, due to a constitutively expressed chromosomal class A beta lactamases (Haeggman, et. al. 2004). Resistance to Amoxycillin, Ceftazidime, Trimethoprim or Gentamicin compounds was nearly always associated with multidrug resistance is due to the acquisition of mobile elements carrying several resistance genes. The 40 *Klebsiella* spp isolated from river Narmada were grouped into 12 antibiotypes (designated A1 to A12) depending upon their resistance to different antimicrobial drugs (Table 2). It was found that most of the isolates (72.5%) were resistant to amoxycillin and 62.5% isolates were resistant against ampicillin. Sixteen isolates (44%) were resistant to three or more antibiotics. All *Klebsiella* isolates found to be sensitive against gentamycin, cefotaxime and norflaxcin antibiotics.

This study demonstrated the dynamic nature of the population structure and high level of diversity of *Klebsiella* in the river Narmada. Little is known about the ecology biochemistry and molecular properties of these organisms in fresh water environments (Podschum, et. al. 2001).

Since the river Narmada is a very significant source of fresh water for Central India sustaining millions of populace, and also used for recreational purposes, the occurrence of antibiotic resistant *Klebsiella* spp. raises a question regarding potential risk of human exposure; hence it's indispensable to monitor the river water recurrently to check the possibility of any epidemic. Moreover, many studies have been carried out almost exclusively with clinical isolates; while there have been few studies on the pathogenic aspects of environmental strains. Since the aquatic environment is implicated as the reservoir for these microorganisms, and consequently responsible for their transmission in humans, it is obvious that detailed studies on the pathogenic potential of the environmental *Klebsiella* strains will certainly contribute to understanding the virulence properties of these bacteria and to establish the importance of these organisms.

Form the present study we would conclude that RAPD and ARDRA clearly prevailed among the other typing methods (biotyping, protein analysis and antibiogram analysis) as a useful technique in distinguishing *Klebsiella* spp. There currently seems to be

Table 2 : Distribution of 40 *Klebsiella* spp according to antibiotypes isolated from river Narmada.

Strains [a]	Antibiotypes	Resistance phenotypes [b]
BGCC 415, 421, 440, 441, 449	A1	Na, Tr
BGCC 416, 422, 430, 443, 454	A2	Am, A, S, Cf
BGCC 417, 420, 423, 424, 431, 433, 436, 437, 439, 444, 447, 450	A3	Am, A
BGCC 418, 426	A4	Am, Na, Tr
BGCC 419, 425, 429	A5	Am, A, Na
BGCC 427, 428, 432	A6	Am, A, Tr
BGCC 434, 451	A7	C, T
BGCC 435	A8	Am, A, Na, Tr
BGCC 438	A9	A, Ca, C, Na, Tr
BGCC 442, 445, 446	A10	Na
BGCC 448	A11	Am A, Ca, Na
BGCC 452, 453	A12	Am

a paucity of information on the Taxonomy and evolution of *Klebsiella* species. Given their magnitude in human health and ecology, more studies need to be conducted to elucidate their genetic relatedness.

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References

Alves M. S., Dias R.C.S., Castro A. C. D., Riley L. W. and Moreira B. M., (2006): Identification of Clinical Isolates of Indole-Positive and Indole-Negative *Klebsiella* spp. *J. Clin. Microbiol.*, **44 (10)**, 3640-3646.

American Public Health Association (APHA) (1985): Standard methods for the examination of water and wastewater. 16th ed. American Public Health Association, Washiggton.

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

Brisse S. and Verhoef J. (2001): Phylogenetic diversity of *Klebsiella pneumoniae* and *Klebsiella oxytoca* Clinical isolates revealed by randomly amplified polymorphic DNA, *gyrA* and *parC* genes sequencing and automated ribotyping. *Int. J. Sys. Evolution. Microbiol*., **51**, 915- 924.

Bryant T.N. (2003): Probablistics identification of bacteria. PIB computer kit, Medical statistics and computing University of Southampton, Southampton General Hospital., Southampton, 5094 XYUX.

CLSI. (2002) Clinical Laboratory Standard Institute. *NCCC.*, **9**, 22: 1.

Deshpande L., Pfaller M.A. and Jones R.N. (2000): In vitro activity of ceffiofur tested against clinical isolates of *E. coli* and *Klebsiella pneumoniae* including extended-spectrum beta-lactamase producing strains. *Int. J. Antimicrob. Agents.*, **15**, 271-275.

Haeggman S., Lofolahl S., Paauw A., Verhoef J. and Brisse S. (2004): Diversity and evalution of the alas A chromosomal beta-lactamase gene in *Klebsiella pneumoniae*. Antimicrob. *Agents Chemother.*, **48**, 2400-2408.

Jemeyet A.W., Clements A., Farn, J.L., Wijburg O.L., MeGlinchey A., Spelman D.W., Pitt T.L., Kaufmann ME., Liolios L., Moloney M.B., Wesselingh S.L. and Strugnell R.A., (2006): Seroepidiemiology of *K. pneumoniae* in an Australian tertiary hospital and its implications for vaccine development. *J. Clin. Microbiol.*, **44(1)**, 102-107.

Krieg R.N. and Halt G. (1984): In: Bergey's Manual of Systematic Bacteriology, William and Wilkins Co. Baltimore., 1.

Laemmli U.K. (1970): Cleavage of structural proteins during assembly of head of bacteriophage T4. *Nature*., **227**, 680-685.

Lopes A. C. S., Rodrigues J.F. and Morais M.A. (2005): Molecular typing of *Klebsiella pneumoniae* isolates from public hospitals in Recife, Brazil. *Microbiol Res*., **160**, 37-46.

MacFaddin J.F. (1980): Biochemical tests for identification of Medical Bacteria, 2nd edt. Williams and Wilkins Co. Baltimore. USA., 1. pp. 7-318.

Orskov I. and Orskov, F., (1984): Serotyping of *Klebsiella*. In: Methods in Microbiology (Bergan, T., Ed.)., pp. 143-164.

Podschum R., Pietsch S., Holler C. and Ullmann U. (2001): Incidence of *Klebsiella* species in surface waters and their expression of virulence factors. *Appl. Environ. Microbiol.*, **67**, 3325-3327.

Podschun R. and Ullmann, U., (1998): *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin. Microbiol. Rev*., **11**, 589-603.

Rennie R.P. and Duncan I.B.R., (1974): Combined biochemical and serological typing of clinical isolates of *Klebsiella. Appl. Microbiol*., **28**, 534-539.

Sambrook J., Fritsch E.F. and Maniatus, T. (1989): Molecular cloning. IInd ed., Cord spring harbor lab. Press., 18.47 – 18.59.

Trevisan. (1887): *Klebsiella* spp. indentified up to date. Int. J. Syst. Bactriol., 30, 309 (AL); (742), (8085).

Wong N.A., Linton C.J, H. and Millar M.R., (1993) Randomly amplified polymorphic DNA typing: a useful tool for rapid epidemiological typing of *Klebsiella pneumoniae*. Epidemiol. Infect., **113**, 445-454.