INTRODUCTION

*Klebsiella pneumoniae* was recognized in 1887, when Trevisan (1887) has described a bacterium from the lungs of a patient who had died of pneumonia. *K. pneumoniae* is a lactose-fermenting, facultative anaerobic, non-motile with a prominent capsule bacillus in the family *Enterobacteriaceae*. This opportunistic pathogen found in a variety of environmental sources such as soil, vegetation, water and industrial wastes including mucosal surfaces of mammals with common association of a broad range of infections *viz.* peritonitis, septicemia, pneumonia, urinary tract infections, and meningitis (Podschun and Ullmann, 1998). It is an important pulmonary pathogen for camels, horses, dogs, foals, sow and calves (Arora and Kalra, 1973; Boguta et al., 2002; Henton et al., 1994; Selbitz et al., 2002). In camels, *K. pneumoniae* reported...
from acute destructive bronchopneumonia and community-acquired bacterial pneumonia with increased tendency to develop abscess, cavitation and empyema (Arora and Kalra, 1973; Zubair et al., 2004; Kane et al., 2005; Abubakar et al., 2010).

*Klebsiella pneumoniae* strains exhibit many virulence factors such as capsular polysaccharides (CPS), lipopolysaccharide (LPS), iron-scavenging systems (siderophores), adhesins and hypermucoviscosity (Podschun and Ullmann, 1998) along with other virulence factors such as urease production, hemolysins, protein-tyrosine kinase, phosphotyrosine-protein phosphatase and heat-labile & heat-stable endotoxins (Dworkin et al., 2006). The capsule is an important virulence factor to govern pathogenesis, which protects the bacteria from phagocytosis and bactericidal effects of serum factors (Highsmith and Jarvis, 1985, Struve and Krogfelt, 2003). Sometime *K. pneumoniae* also exhibits large amounts of mucopolysaccharide web of capsular and extracapsular polysaccharides to produce more virulent hypermucoviscosity strain (Wiskur et al., 2008). *Klebsiella* is a non-hemolytic for human red blood cells but found to be hemolytic for rabbit blood agar and urease production of this organism has significant role in colonization and survivability in various systemic infections (Dworkin et al., 2006; Maroncle et al., 2006).

Camel (*Camelus dromedarius*) is a uniquely morphological & physiological adapted animal in desert ecosystem with good potential to thrive well on meager resources under extreme climatic conditions. It has important utilities in human society such as drought, farming, milking and many other important farming purposes but sometime certain sudden environmental variations make this animal susceptible to various infections (Kebede and Gelaye, 2010). In India, *Klebsiella pneumoniae* has been also reported from cases of “khurak” (local name of pneumonia) in camels. “Khurak” is an infectious respiratory disease characterized by alveolar serofibrinous exudation, capillary hyperaemia and microbial bronchopneumonia with respiratory distress, pyrexia, prolonged cough and septicemia in camels. It is a highly morbid, causing considerable loss of production and deaths especially in adult camels (Arora and Kalra, 1973; Sharma, 2012). *K. pneumoniae* found as commensal in healthy individual as well as pathogenic agent in diseased animals. So it is required to detect variations among *Klebsiella pneumoniae* strains obtained from healthy and diseased individuals. Thus this study has designed to compare *Klebsiella pneumoniae* isolates obtained from pneumatic and healthy camels on the basis of primary and secondary biochemical characters and virulence properties.

**MATERIALS AND METHODS**

**Sample Collection and Identification**

A total of 163 severe nasal discharge samples from pneumatic (96) and healthy (67) camels were collected. The pneumatic and healthy camels were determined on the basis of clinical symptoms viz. the camel with severe nasal discharge, respiratory distress, depression and pyrexia up to 41°C temperature in early stages. After a week or so, the temperature tends to drop down to normal, but cough persists for a long time was considered as pneumatic camel. While the camels, which were not show any clinical and medication history since last six month considered as healthy. Samples of pneumatic camels has been collected from clinical complex of college of veterinary and animal science, Bikaner (Rajasthan) and samples of healthy camel has been from surrounding villages of Bikaner area. All samples were collected without any discrimination of age, sex and breed. The samples were collected directly from nasal cavity aseptically with the help of sterile cotton swabs. And proceeded on selective medium Simmon’s citrate agar with 1% inositol (SCAI) (Van Kregten et al., 1984) and single yellow dome shaped colonies were further checked for genotypic confirmation with 16S-23S rDNA internal transcribed spacer (ITS) region based specific primers (Liu et al., 2008). For genotypic confirmation genomic DNA was used as template and isolation was carried out by method of Chen and Kuo (1993). All amplified PCR products were analyzed by 8% polyacrylamide gel electrophoresis (PAGE).

**Phenotypic Characterization and Detection of Virulence Traits**

Genotypically confirmed 65 isolates were included, out of which 47 from pneumatic and 18 isolates were from healthy camels. All were subjected to various primary and secondary biochemical reactions such as catalase, oxidase and motility then checked for lactose fermenter mucoid colonies on MacConkey agar (MCA) and nonmetallic dark colored mucoid growth on EMB agar further secondary biochemical
characters such as IMViC pattern, growth on TSI, gelatin liquefaction, aesculin hydrolysis, nitrate reduction, malonate utilization, lysine decarboxylation and ONPG (o-Nitrophenyl-β-d Galactopyranoside) tests were conducted (Quinn et al., 1994 and Garcia and Isenberg, 2007). For phenotypic detection of virulence traits, Maneval’s capsular staining method was used for capsular detection, haemolysin production was observed on sheep blood agar, urease production was tested by change in color of media from yellow to red observed for seven days in urease broth as described in literature and virulent hypermucoviscosity trait checked by string test (Wiskur et al., 2008).

Figure 1: Klebsiella pneumoniae showing Yellow, dome shaped mucoid colonies on SCAI

RESULTS

Only 65 samples (47 were from pneumonic and 18 from healthy camels) showed typical yellow dome shaped colonies (Figure 1) on Simon’s citrate agar with 1% inositol (SCAI) from 163 nasal discharge samples of pneumonic and healthy camels and further these 65 isolates were confirmed with 16S-23S rDNA internal transcribed spacer (ITS) region based specific primers showing species-specific amplicon of 130 bp (Figure 2). In the present study, without any variation all isolates showed pink lactose fermenter mucoid colonies on MacConkey agar (MCA). Non-metallic dark colored mucoid growth on EMB agar, non-hemolytic grey-white, mucoid colonies on sheep blood agar (Figure 3). And mucoviscosity observed in all other growth medium with large capsule. All isolates showed typical IMViC pattern (−+++ ) and other biochemical characters (Table 1) as described in literature, except for six isolates from pneumonic camels that were found ability to produce indole. 100% isolates from pneumonic camels were urease producing and most of isolates of pneumonic camels were urease producers within first three days of observation while out of 18 isolates from healthy camels, three (16.66%) were urease negative and rest of the 15 isolates showed positive results after three days of incubation (Table 2). Only 25 (53.19%) isolates from pneumonic camels showed existence of virulent hypermucoviscosity trait (Figure 4) while none of the K. pneumoniae isolates from healthy camel exhibited hypermucoviscosity.

Figure 2: Klebsiella pneumoniae isolates showing typical 130bp size product with specific primers on 8% polyacrylamide gel

Figure 3: Klebsiella pneumoniae showing non-hemolytic grey-white, mucoid colonies on sheep blood agar
Table 1: Secondary biochemical tests of *Klebsiella pneumoniae* isolates from pneumonic and healthy camels

| S. No. | Test                  | Reaction                                      |
|--------|-----------------------|-----------------------------------------------|
| 1      | Growth on 10°C        | Negative                                      |
| 2      | Growth on 44.5°C      | Positive                                      |
| 3      | Growth on SCAI        | Yellow mucoid dome shaped colonies            |
| 4      | Growth on EMB agar    | Dark mucoid non-metallic colonies             |
| 5      | Growth on BCP agar    | Yellow mucoid colonies                        |
| 6      | Growth on TSI agar    | Acid/Acid/No H₂S (Y/Y/-)                     |
| 7      | Gelatin Liquefaction  | Negative                                      |
| 8      | Aesculin Hydrolysis   | Positive                                      |
| 9      | Nitrate Reduction     | Positive                                      |
| 10     | Malonate Utilization  | Positive                                      |
| 11     | Arginine Hydrolysis   | Negative                                      |
| 12     | Lysine Decarboxylation| Positive                                      |
| 13     | Phenylalanine Deamination | Negative                          |
| 14     | ONPG (o-Nitrophenyl-β-d Galactopyranoside) | Positive |

DISCUSSION

In the present study, we have examined the primary and secondary biochemical characters and virulence traits of *Klebsiella pneumoniae* isolates obtained from severe deep nasal discharge of pneumonic and healthy camels. Samples which were showing yellow dome shaped colonies on Simmon's citrate agar with 1% inositol (SCAI) also found confirmed with *K. pneumoniae* species specific primers. Similar to present study, Sharma et al. (2013) has also reported specificity of these species specific primers for detection of *K. pneumoniae* from acute respiratory tract infections of camels. In addition, Liu et al. (2008) was also detected this organism with same primers and similar conditions from infant formula food sources. It proves genotypic specificity of this method for *K. pneumoniae* detection not only from food sources but also from animal origin. It also indicates the good discriminative capacity of SCAI agar therefore SCAI agar can be used as a selective medium for isolation of *K. pneumoniae*. Van Kregten et al. (1984) had also reported selectivity of SCAI agar for *K. pneumoniae* with similar results, who developed a medium based on the presence of two carbon sources, citrate and myo-inositol (Dworkin et al., 2006).

In agreement with present study, Smith and Ngui-Yen (1980) were also found non hemolytic *K. pneumoniae* while Albesa et al. (1980) reported hemolytic *K. pneumoniae* in rabbit blood agar. In support of present study, indole production in *K. pneumoniae* has been also reported by Brown and Seidler (1973), Rennie and Duncan (1974) and Edwards and Ewing (1972) with 28%, 16% and 6% frequency respectively. None of the significant difference was observed in hemolytic pattern of *K. pneumoniae* isolates obtained from pneumonic and healthy camels in present study but variations were observed in indole production. This ability may contribute in virulence and antibiotic resistance since indole producing ability can act as a signalling molecule to regulate the expression of adhesion and biofilm-promoting factors (Martino et al., 2003; Nishida et al., 1978). Other secondary biochemical characters in present study were found similar as described earlier literature (Eguchi et al., 1987; Rennie and Duncan, 1974). These similar biochemical characters may explain through previous studies, who reported similar biochemical properties of *K. pneumoniae*. 

Figure 4: *Klebsiella pneumoniae* showing virulent hypermucoviscosity trait (string test)
Table 2: Urease production in *Klebsiella pneumoniae* isolates from pneumonic and healthy camels

| Observation day | Pneumonic camels | Healthy camels |
|----------------|------------------|----------------|
|                | Positive isolates | Negative isolates | Positive isolates | Negative isolates |
| First          | 37 (78.72%)       | -               | 15 (83.33%)      | 3 (16.66%)        |
| Second         | 4 (8.51%)         | -               | -                | -                |
| Third          | 3 (6.38%)         |                 | -                | -                |
| Fourth         | 1 (2.12%)         | 4 (22.22%)      | 7 (38.55%)       | 3 (16.66%)        |
| Fifth          | 2 (4.25%)         |                 | 3 (16.66%)       |                 |
| Sixth          | 1 (5.55%)         |                 | 1 (5.55%)        |                 |
| Seventh        | 1 (5.55%)         |                 | 1 (5.55%)        |                 |
| Total          | 47 (100.0%)       | -               | 15 (83.33%)      | 3 (16.66%)        |

*pneumoniae* from various sources without significant variations (Bouvet et al., 1989; Munoz et al., 2007). Thus it may also conclude that source of bacterial isolation not too much affects primary and secondary biochemical reaction of *K. pneumoniae* isolates.

In accordance to present study, Mobley and Warren (1996) and Wilson and Miles (1975) also reported that clinical isolates of *K. pneumoniae* were 100% urease producing. And present investigation also found that *K. pneumoniae* isolates from pneumonic camels were faster urease producing as compared to isolates from healthy camels. Urease production enables the bacteria to grow in acidic environment (Mobley and Warren, 1996). Thus it has significant role in colonization, survival and pathogenesis of several bacterial species including *K. pneumoniae* (Burne and Chen, 2000; Maroncle et al., 2006).

In the present study, we found some differences regarding indole production, urease production and virulent hypermucoviscosity trait of isolates from pneumonic and healthy camels. It may indicates the differences in virulence and suggest further genotypic characterization of isolates to find genotypic mechanisms of virulence of *Klebsiella pneumoniae* to make proper diseases diagnosis and prevention.

**ACKNOWLEDGMENTS**

We acknowledge the support and facilities provide by Head of department of veterinary microbiology and biotechnology, Dean of college of veterinary and animal science, Bikaner and Dean of Post Graduate Institute of Veterinary Education and Research- Jaipur for this study.

**REFERENCES**

- Abubakar MS, Fatihu MY, Ibrahim NDG, Oladele SB, Abubakar MB (2010). Camel pneumonia in Nigeria: Epidemiology and bacterial flora in normal and diseased lung. African. J. Microbiol. Res. 4(23): 2479-2483.
- Albesa I, Eraso AJ, Frigerio CI, Lubetkin AM (1980). Hospital outbreak in a care unit for infants, due to
- Arora RG, Kalra DS (1973). A note on isolation of Klebsiella pneumoniae and Diplococci from cases of bronchopneumonia in camels. Ind. J. Anim. sci. 43(12): 1095-1096.

- Boguta L, Gradzki Z, Borges E, Maruin F, Kodjo A, Wlniarczyk S (2002). Bacterial Flora in Foals with Upper Respiratory Tract Infections in Poland. J. Vet. Med. 49(6): 294-297. http://dx.doi.org/10.1046/j.1439-0450.2002.00570.x

- Bouvet OMM, Lenormand P, Grimont PAD (1989). Taxonomy diversity of the D-glucose oxidation pathway in the Enterobacteriaceae. Int. J. Syst. Bacteriol. 39(1): 63-67. http://dx.doi.org/10.1099/00207713-39-1-61

- Brown C, Seidler RJ (1973). Potential pathogens in the environment: Klebsiella pneumoniae, a taxonomic and ecological enigma. Appl. Microbiol. 25(6): 900-904.

- Burne RA, Chen YY (2000). Bacterial ureases in infectious diseases. Microbes. Infect. 2(5): 533-542. http://dx.doi.org/10.1086/315786

- Chen WP, Kuo TT (1993). A simple and rapid method for the preparation of Gram-negative bacterial genomic DNA. Nucleic Acids Res. 21 (9): 2260. http://dx.doi.org/10.1159/000237775

- Dworkin M, Falkow S, Rosenberg E, Schleifer K, Stackebrandt E (2006). The Prokaryotes: A Handbook on the Biology of Bacteria, 3rd Ed. Springer Science+Business Media, New York, USA. Pp. 159-196.

- Edwards PR (1928). The relation of encapsulated bacilli found in metritis in mares to encapsulated bacilli from human sources. J. Bacteriol. 15(4): 245-266.

- Edwards PR, Ewing WH (1972). Identification of Enterobacteriaceae. 3rd Ed. Burgess Publishing Co., Minneapolis. Pp. 291.

- Eguchi M, Yokomizo Y, Kuniyasu, C (1987). Biochemical characteristics of Klebsiella pneumoniae derived from horses. Jpn. J. Vet. Sci. 49(2): 279-283. http://dx.doi.org/10.1292/jvms1939.49.279

- Garcia LS, Isenberg HD (2007). Clinical Microbiology Procedures Handbook. 2nd Ed. American Society for Microbiology. Washington.

- Hentton MM, Coetzter JA, Thomson GR, Tustin RC (1994). Klebsiella sp. infections, In: Infectious Diseases of Livestock. (Eds.) Oxford University Press, Cape Town, Oxford, New York. Pp. 1080-1084.

- Highsmith AK, Jarvis WR (1985). Klebsiella pneumoniae: selected virulence factors that contribute to pathogenicity. Infect. Control. 6(2): 75-77.

- Kane Y, Kadja MC, Bada-Alamedji R, Bezeid OE, Akakpo JA, Kabore Y (2005). Lung lesions and bacteria of the One-Humped camel (Camelus dromedarius) at Nouakchott Slaughter house in Mauritania. Revue d Elevage et de Med. Vet. de. Pays Tropic. 58(3): 145-150.

- Kebede F, Gelaye E (2010). Studies on major respiratory diseases of Camel (Camelus dromedarius) in Northeastern Ethiopia. African J. Microbiol. Res. 4(14): 1560-1564.

- Lin YT, Jeng YY, Chen TL, Fung CP (2010). Bacteremic community-acquired pneumonia due to Klebsiella pneumoniae. Clinical and microbiological characteristics in Taiwan, 2001-2008. BMC Infect. Dis. 10: 307. http://dx.doi.org/10.1186/1471-2334-10-307

- Liu Y, Liu C, Zheng W, Zhang X, Yu J, Gao Q, Hou Y, Huang X (2008). PCR detection of Klebsiella pneumoniae in infant formula based on 16S–23S internal transcribed spacer. Int. J. Food Microbiol. 125(3): 230-235. http://dx.doi.org/10.1016/j.iflfoodmicro.2008.03.005

- Maroncle N, Rich C, Forestier C (2006). The role of Klebsiella pneumoniae urease in intestinal colonization and resistance to gastrointestinal stress. Res. Microbiol. 157(2): 184-193. http://dx.doi.org/10.1016/j.resmic.2005.06.006

- Martino PD, Fursy R, Bret L, Sundararaju B, Phillips RS (2003). Indole can act as an extracellular signal to regulate biofilm formation of Escherichia coli and other indole producing bacteria. Can. J. Microbiol. 49(7): 443-449. http://dx.doi.org/10.1139/w03-056

- Mobley HLT, Warren JW (1996). Urinary tract infections: molecular pathogenesis and clinical management. American Society for Microbiology. Washington. Pp. 295-312.

- Munoz MA, Welcome FL, Schukken YH, Zadoks RN (2007). Molecular epidemiology of two Klebsiella pneumoniae mastitis outbreaks on a dairy farm in New York State. J. Clin. Microbiol. 45(12): 3964-3971. http://dx.doi.org/10.1128/JCM.00795-07

- Nishida M, Asano H, Kamimura T, Yokota Y (1978). Differential susceptibility of indole-positive and -negative strains of Klebsiella pneumoniae to cefazolin, chloramphenicol and tetracycline. Chemotherapy. 24(3): 154-60. http://dx.doi.org/10.1159/000237775

- Podschan R, Ullmann U (1998). Klebsiella spp. as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors. Clin. Microbiol. Rev. 11(4): 589-603.

- Quinn PJ, Carter ME, Markey BK, Carter GR (1994). Clinical Veterinary Microbiology. Wolfe Publishing, Mosby-Year Book Europe Ltd. Lynton House. 7-12. Tavistock Square, London WCH 9LB, England. Pp. 209-236.

- Rennie RP, Duncan IBR (1974). Combined Advances in Animal and Veterinary Sciences.
biochemical and serological typing of clinical isolates of *Klebsiella*. Appl. Microbiol. Biotechnol. 28(4): 534–539.

- Selbitz HJ, Rolle M, Mayr A (2002). Medical Microbiology, Infectious and Infectious Diseases 7th Ed. Enke Verlag Stuttgart: 481–482.

- Sharma SK (2012). Phenotypic and Genotypic Characterization of *Klebsiella pneumoniae* isolated from pneumonic camels (*Camelus dromedarius*). M.V.Sc. thesis submitted to RAJUVAS, pp. 68, 2012.

- Sharma SK, Kataria AK, Shringi, BN, Nathawat P, Bhati T, Mohammed N (2013). Detection of hypermucoviscous *Klebsiella pneumoniae* in camel (*Camelus dromedarius*) during an outbreak of acute respiratory tract infection. J. Camel Practic. Res. 20(2): 139–143.

- Simoons-Smit AM, Verweiji-van Vught, AMJJ, MacLaren DM (1986). The role of K antigens as virulence factors in *Klebsiella*. J. Med. Microbiol. 21(2): 133–137. http://dx.doi.org/10.1099/00222615-21-2-133.

- Smith JA, Ngui-Yen JH (1980). Augmentation of clostridial partial haemolysis by some bacterial species. Can. J. Microbiol. 26(7): 839–843. http://dx.doi.org/10.1139/m80-144.

- Struve C, Krogfelt KA (2003). Role of capsule in *Klebsiella pneumoniae* virulence: lack of correlation between in vitro and in vivo studies. FEMS Microbiol. Lett. 218(1): 149–154. http://dx.doi.org/10.1111/j.1574-6968.2003.tb11511.x.

- Trevisan V (1887). Sul micrococco della rabia e sulla possibilita di riconoscere durante il periodo d’incubazione, dall'esame del sangue della persona morsicata, se ha contratto l'infezione rabbica. R. C. Ist Lombardo (Ser. II) 20: 88–105.

- Van Kregten E, Westerdaal NAC, Willers JMN (1984). New, simple medium for selective recovery of *Klebsiella pneumoniae* and *Klebsiella oxytoca* from human feces. J. Clin. Microbiol. 20(5): 936–941.

- Vila A, Cassata A, Pagella H, Amadio C, Yeh KM, Chang FY, Siu LK (2011). Appearance of *Klebsiella Pneumoniae* Liver Abscess Syndrome in Argentina: Case Report and Review of Molecular Mechanisms of Pathogenesis. Open Microbiol. J. 5: 107–113. http://dx.doi.org/10.2174/1874285801105010107.

- Whitehouse CA, Keirstead N, Taylor J, Reinhardt JL, Beierschmitt A (2010). Prevalence of Hypermucoid *Klebsiella pneumoniae* among Wild caught and Captive Vervet Monkeys (*Chlorocebus aethiops sabaenus*) on the Island of St. Kitts. J. Wild. Dis. 46(3): 971–976. http://dx.doi.org/10.7589/0090-3558-46.3.971.

- Wilson GS, Miles A (1975). Topley and Wilson's principles of bacteriology, virology and immunity, E. Arnold, London. Pp. 781–783.

- Wiskur BJ, Hunt JJ, Callegan MC (2008). Hypermucoviscosity as a virulence factor in experimental *Klebsiella pneumoniae* Endophthalmitis. Invest. Ophthalmol. Vis. Sci. 49: 4931–4938. http://dx.doi.org/10.1167/iovs.08-2276.

- Yu WL, Ko WC, Cheng KC, Lee HC, Ke DS, Lee CC, Fung CP, Chuang YC (2006) Association between rmpA and magA genes and clinical syndromes caused by *Klebsiella pneumoniae* in Taiwan. Clin. Infect. Dis. 42(10): 1351–1358.

- Zubair R, Khan AMZ, Sabri MA (2004). Pathology of Camel Lungs. J. Camel Sci. 1: 103–106.