DOI : 10.5958/2277-8934.2020.00025.9 *Vol 27 No 2, p 173-176*

Klebsiella oxytoca ISOLATED FROM NOSTRILS OF DROMEDARY CAMELS: RESISTANCE PATTERN AND *pehX* GENE BASED GENOTYPING

P.S. Rathore, S.K. Sharma, N. Arora[1] and P. Nathiya
Department of Veterinary Microbiology and Biotechnology,
Post Graduate Institute of Veterinary Education and Research (PGIVER), Rajasthan University of Veterinary and Animal Sciences (RAJUVAS) Jaipur, Rajasthan, India
[1]Veterinary Officer, Department of Animal Husbandry, Government of Rajasthan

ABSTRACT

The present study was aimed to characterise *Klebsiella oxytoca* obtained from nostrils of camels (*Camelus dromedarius*) with special reference to antibiotic resistance pattern. Total 68 samples of infected camels were examined and out of these, 33 (48.53%) were found to be positive for *K. oxytoca*, which were further confirmed phenotypically and genotyped by species specific primer based on *pehX* gene of 344bp amplicon size. The isolates were characterised for biochemical properties and analysed to evaluate the susceptibility against 26 antimicrobials. It was found that 100% isolates were resistant to eight antibiotics namely amoxycillin, ampicillin, bacitracin, clindamycin, oxacillin, rifampicin, sulfadiazine and vancomycin, while all isolates were completely sensitive to cefepime and imipenem. Variable resistance patterns were recorded for other studied antibiotics.

Key words: Camels, *Klebsiella oxytoca*, *pehX* gene, respiratory tract infections, superbug

K. oxytoca is ubiquitous in the environment and is an opportunistic pathogen that can be cultured from the skin, mucous membranes, oropharynx and intestines as well as a variety of tissues from β-clinically affected humans and animals (Stojowska and Krawczyk, 2016). In animals, Klebsiella are mostly associated with sepsis, urinary tract infections, meningitis and mastitis (Chander *et al,* 2011). Antibiotic resistance and virulence of *K. oxytoca* is poorly understood (Herzog *et al,* 2014; Darby *et al,* 2014).

K. oxytoca exhibits both natural resistance to penicillins and has acquired various chromosomal and plasmid associated genetic mechanisms of antibiotic resistance, thus hampering disease management and it also has the enzymes such as extended spectrum β-lactamases (ESBL) and carbapenemases that have a role in resistance to β-lactam antibiotics and other antibiotics (Fenosa *et al,* 2009). Thus, investigation undertaken in present study becomes important not only in terms of biochemical profile but also to analyse the antibiotic resistance pattern of *K. oxytoca*.

Materials and Methods

Sample collection

A total of 68 deep nasal swab samples were collected from nostrils of camels with nasal discharge, high temperature, loss of appetite and pneumonic clinical signs. Samples for bacteriology were placed in polythene bags and kept in flask containing ice and taken to the laboratory as early as possible (Cheesebrough, 2000).

Isolation and identification

All samples were subjected to cultivation on Simmon's citrate agar with 1% inositol (SCIA), MacConkey agar (MCA) and Eosin methylene blue (EMB) agar followed by various other biochemical tests (Van Kregten *et al,* 1984). The phenotypically identified isolates were further confirmed genotypically on the basis of *pehX* gene amplification with amplicon size of 344bp including forward primer (5′ GAT ACGGAG TAT GCC TTT ACG GTG -3′) and reverse primer (5′- TAG CCT TTA TCA AGC GGA TAC TGG -3′). The PCR reactions were carried out as described earlier by (Kovtunovych *et al,* 2003) using Promega (USA) gene amplification kit and thermocycler (Mastercycler® nexus gradient) conditions were: Initial denaturation step of 2 min at 95°C, followed by 30 amplification cycles (94°C, 20s; 59°C, 20s; 72°C, 30 s) and a final elongation step of 10 min at 72°C. The PCR products were separated in an 8% native Polyacrylamide gel electrophoresis PAGE

SEND REPRINT REQUEST TO S.K. SHARMA email: drsharmask01@hotmail.com

(Sambrook and Russel, 2001). The gel was analysed under UV light (Azure 300 Chemi docu system).

Investigation of antibiotic susceptibility pattern

Total 26 antibiotics (Hi-Media) of various groups were examined for antibiotic susceptibility pattern of confirmed *Klebsiella oxytoca* isolates by disc diffusion method as per technique described by Kirby-Bauer method (Bauer *et al,* 1966) by using Mueller-Hinton agar. The log phase cultures were spread evenly on Meuller Hinton agar plates and antibiotic discs were aseptically placed on the agar surface. Samples were incubated aerobically for 18–24 hours at 37°C and the zones of inhibition were measured to the nearest millimetre. After inhibition zone measurement, organisms were classified as Sensitive (S), Intermediate sensitive (I) or Resistant (R) as per defined by CLSI (2017).

Results and Discussion

Out of 68 samples, 33 isolates were obtained after growth on Simmon's citrate agar (SCAI) with 1% inositol, where they showed yellow mucoid dome shaped colonies, all isolates were further confirmed by growth on Eosin Methylene Blue (EMB) agar where 100% isolates showed dark mucoid colonies without metallic sheen, expressed pink mucoid lactose fermenting colonies on Mac-Conkey agar and exhibited yellow mucoid colonies on Bromo Cresol Purple (BCP) agar. The phenotypically identified isolates were confirmed on the basis of *peh*X species specific gene primers with amplicon size of 344bp, which confirmed all isolates as *Klebsiella oxytoca* (Fig.1). In the present study, 100% isolates were observed with typical IMViC pattern of *K. oxytoca* viz. +-++. All the isolates were able to reduce the nitrates and utilise the malonate including hydrolysation of the aesculin and decarboxylation of the lysine. To study the phenotypic expression of antibiotic resistance, antibiogram of the *K. oxytoca* isolates were carried out, against the selected 26 commonly used antibiotics in veterinary therapeutics. Out of the total antibiotics, cefepime and imipenem were most effective with 100% efficacy while amoxycillin, ampicillin, bacitracin, clindamycin, oxacillin, rifampicin, sulfadiazine and vancomycin were found to be least effective. In the studied isolates, more than 90.0% sensitivity was observed for norfloxacin and gentamicin while for other sensitivity ranged from 36.3% to 87.8%.

Being ubiquitous, *Klebsiella oxytoca* has shown their presence in water, soil and on plants (Podschun & Ullmann, 1998). This bacteria has also been detected on the mucosal membranes of both healthy and diseased mammals including human (Darby *et al,* 2014), animals, poultry (chicken) and has also been reported in various reptiles such as green anoles, garter snakes reptile, wild tuatara and green turtles by different scientists (Goldstein *et al,* 1981; Santoro *et al,* 2006; Gartrell *et al,* 2007; Nthenge *et al,* 2008; Jackson, 2016).

Similar to present study, Santoro *et al* (2006) observed that 31.0% of green turtles were positive for *K. oxytoca*. Similarly, 20% & 17.0% occurrence was reported in garter snakes and green anoles by Goldstein *et al* (1981), Jackson (2016), respectively. However, lower prevalence (3.0%) was reported by Gartrell *et al* (2007) in wild tuatara. Present study as well as past studies such as haemorrhagic colitis (diarrhoea) in hospitalised patients (Cheng *et al,* 2012; Rath and Padhy, 2014), pneumonia, urinary tract and skin infections (Herzog *et al*, 2014) suggests certain adaptations on part of opportunistic *K. oxytoca* strains that are not only infecting animals but also humans as well.

Biochemical profile of Klebsiella isolates in present study is similar to profiles observed by Davis *et al* (1987), Rath and Padhy (2014) and Trivedi *et al* (2015) in various studies. However, few variations were also reported by Monnet and Freney (1994) who pointed out that 97.0% isolates were positive for indole production. Further, looking into the variability pattern of other biochemical tests such as positive gelatin liquefaction (Power and Calder,

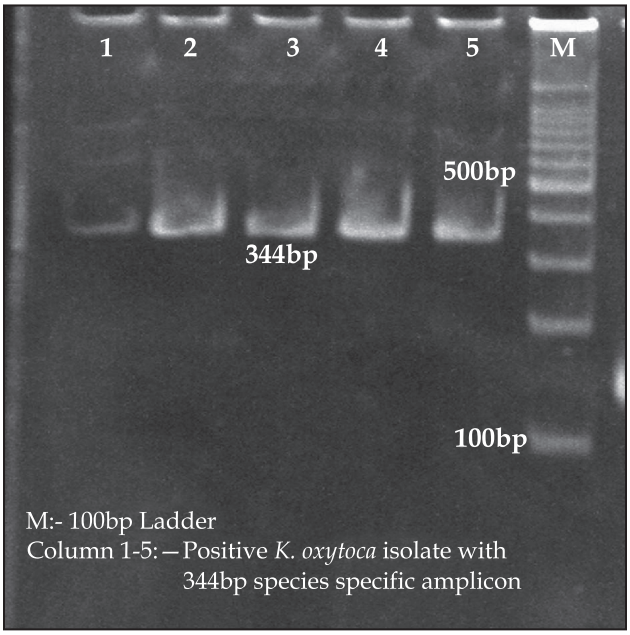

Fig 1. *peh*X species specific gene based genotyping of *Klebsiella oxytoca* (344bp) isolates (8% native PAGE)

1983), growth on 10°C (Kovtunovych *et al,* 2003) and negative citrate utilisation (Trivedi *et al,* 2015), the genotypic characterisation becomes significant. In the present study all isolates were characterised as *K. oxytoca* with species specific *peh*X gene. Similar to present study, Chander *et al* (2011) and Kovtunovych *et al* (2003) have also found that this genotypic method has good repeatability, sensitivity and specificity.

Similar to present study, other researchers have also reported good efficacy of third generation cephalosporins, β-lactamase inhibitor combinations, fluoroquinolones, trimethoprim-sulfomamides, gentamicin and carbapenem antibiotics (Brisse and Duijkeren, 2005; Rath & Padhy, 2014). Similarly, complete resistance to ampicillin and other beta lactams was reported by Trivedi *et al* (2016) in human clinical samples (Darby *et al,* 2014) in lab animals and non-human primates isolates and (Nthenge *et al,* 2008) also detected similar resistance pattern among *K. oxytoca* isolates obtained from chicken samples. In agreement to present study, ciprofloxacin and colistin efficacy was reported by Labrador & Araque (2014) and Singh *et al* (2016) for *K. oxytoca* isolates of human origin. However, indifferent to present study Singh *et al* (2016) observed some resistance for imipenem, meropenem, gentamicin and ciprofloxacin among human isolates. Labrador and Araque (2014) have also detected similar resistance pattern and further explained that the strains of *K. oxytoca* are resistant to amino-penicillins and carboxy-penicillins due to the production of β-lactamases and other similar enzymes. In present study and also reported in earlier studies (Darby *et al,* 2014; Sato *et al,* 2015) *K. oxytoca* has been observed with resistance to many antibiotics such as beta lactams, fluoroquinolone, cephalosporins, aminoglycosides etc.

Conclusion

Klebsiella oxytoca exists with variable phenotypic characteristics and genotypic confirmation with species specific primers based on *peh*X gene may be an important tool for molecular epidemiology as well as field diagnostics. Looking into the scarcity of literature of *K. oxytoca* with special reference to camel, this study suggests further genotypic characterisation of *K. oxytoca* isolates including investigation of virulence genes as well as study of antibiotics resistance dynamics of animal origin isolates.

Acknowledgements

We acknowledge the support and facilities provide by Head, Department of Veterinary Microbiology and Biotechnology and Dean, Post Graduate Institute of Veterinary Education and Research (PGIVER), Jaipur for this study.

References

Bauer AW, Kirly WMM, Sherris JC and Turck M (1966). Antibiotic susceptibility testing by a standardised single disk method. Americal Journal of Clinical Pathology 45:493-496.

Brisse S and Duijkeren E (2005). Identification and antimicrobial susceptibility of 100 Klebsiella animal clinical isolates. Veterinary Microbiology 307-312. doi: 10.1016/j.vetmic.2004.11.010.

Chander Y, Ramakrishnan MA, Jindal N, Hanson K and Goyal SM (2011). Differentiation of Klebsiella pneumonia and *K. oxytoca* by multiplex polymerase chain reaction. International Journal of Applied Research in Veterinary Medicine 9(2):138-142.

Cheng VC, Yam WC, Tsang LL, Yau MC, Siu GK, Wong SC, Chan JF, To KK, Tse H, Hung IF, Tai JW, Ho PL and Yuen KY (2012). Epidemiology of *Klebsiella oxytoca*-associated diarrhoea detected by simmons citrate agar supplemented with inositol, tryptophan, and bile salts. Journal of Clinical Microbiology 50:1571-1579.

Cheesebrough M (2000). Distric Laboratory Practice in Tropical Countries. Chambridge University Press. pp 434.

CLSI (2017). Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute 27:32-39.

Darby A, Lertpiriyapong K, Sarkar U, Seneviratne U, Park DS, Gamazon ER, Batchelder C, Cheung C, Buckley EM, Taylor NS, Shen Z, Tannenbaum SR, Wishnok JS and Fox JG (2014). Cytotoxic and pathogenic properties of *Klebsiella oxytoca* isolated from laboratory animals. PLoS One. doi:10.1371/journal.pone.0100542.

Davis JK, Gaertner DJ, Cox NR, Lindsey JR, Cassell GH, Davidson MK, Kervin KC and Rao GN (1987). The role of *Klebsiella oxytoca* in utero-ovarian infection of B6C3F1 mice. Laboratory Animal Science 37(2):159-166.

Fenosa A, Fuste E, Ruiz L, Veiga-Crespo P, Vinuesa T, Guallar V, Villa TG and Vinas M (2009). Role of TolC in *Klebsiella oxytoca* resistance to antibiotics. Journal of Antimicrobial Chemotherapy 63(4):668-674.

Gartrell BD, Youl JM, King CM, Bolotovski I, McDonald WL and Nelson NJ (2007). Failure to detect *Salmonella* species in a population of wild tuatara (*Sphenodon punctatus*). New Zealand Veterinary Journal 55:134-136.

Goldstein EJ, Agyare EO, Vagvolgyi AE and Halpen E (1981). Aerobic bacterial flora of garter snakes: Development of normal flora and pathogenic potential for snakes and humans. Journal of Clinical Microbiology 13:954-956.

Herzog AT, Schneditz G, Leitner E, Feierl G, Hoffmann KM, Schwetz IZ, Krause R, Gorkiewicz G, Zechner EL and Högenauer C (2014). Genotypes of *Klebsiella oxytoca* isolates from patients with nosocomial pneumonia are distinct from those of isolates from patients with antibiotic-associated haemorrhagic colitis. Journal of

Clinical Microbiology 52(5):1607-1616. doi: 10.1128/jcm.03373-13.

Jackson KA (2016). Prevalence of *Klebsiella oxytoca* in Anolis carolensis of Louisiana. Department of Biology, McNeese State University, Lake Charles, Louisiana. Vector-Borne And Zoonotic Diseases 16(12):800-801. doi: 10.1089/vbz.2016.2023

Kovtunovych G, Lytvynenko T, Negrutska V, Lar O, Brisse S and Kozyrovska N (2003). Identification of *Klebsiella oxytoca* using a specific pcr assay targeting the polygalacturonase *peh*X gene. Research in Microbiology 154:587-592. doi:10.1016/S0923-2508(03)00148-7

Labrador and Araque (2014). First description of KPC-2-producing *Klebsiella oxytoca* isolated from a pediatric patient with nosocomial pneumonia in Venezuela. Hindawi Publishing Corporation. Case Reports in Infectious Diseases. Volume 2014; Article ID 434987, 4 pages. doi: 10.1155/2014/434987

Monnet D and Freney J (1994). Method for differentiating *Klebsiella planticola* and *Klebsiella terrigena* from other *Klebsiella* species. Journal of Clinical Microbiology 32: 1121-1122.

Nthenge AK, Nahashon SN, Chen F and Adefope N (2008). Prevalence and antimicrobial resistance of pathogenic bacteria in chicken and guinea fowl. Poultry Science 87: 1841-1848. doi:10.3382/ps.2007-00156

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

Power JT and Calder MA (1983). Pathogenic significance of *K. oxytoca* in acute respiratory tract infection. *Thorax* 38(3):205-208.

Rath and Padhy (2014). Prevalence of two multidrug-resistant Klebsiella species in an Indian teaching hospital and adjoining community. Journal of Infection and Public Health 7:496-507. doi: 10.1016/j.jiph.2014.05.002

Sambrook J and Russell DW (2001). Molecular Cloning, a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Santoro M, Hernandez G, Caballero M and Garcia F (2006). Aerobic bacterial flora of nesting green turtules (*Chelonia Mydas*) from tortuguero national park, Costa Rica. Journal of Zoo and Wildlife Medicine 37:549–552.

Sato T, Hara T, Horyama T, Kanazawa S, Yamaguchi T and Maki H (2015). Mechanism of resistance and bacterial susceptibility in extended spectrum beta lactamases phenotype *Klebsiella pneumoniae* and *Klebsiella oxytoca* isolated between 2000 and 2010 in Japan. Journal of Medical Microbiology 64:538-543. doi: 10.1099/jmm.0.000057

Singh L, Cariappa MP and Kaur M (2016). *Klebsiella oxytoca*: An emerging pathogen? Medical Journal Armed Forces India 1-3. doi: 10.1016/j.mjafi.2016.05.002

Stojowska K and Beata Krawczyk (2016). A new assay for the simultaneous identification and differentiation of *Klebsiella oxytoca* strains. Applied Microbiology and Biotechnology 100:10115-10123. doi: 10.1007/s00253-016-7881-1

Trivedi MK, Patil S, Shettigar H, Bairwa K and Jana S (2015). Phenotypic and biotypic characterisation of *Klebsiella oxytoca*: An impact of biofield treatment. Journal of Microbial and Biochemical Technology 7:(4)202-205. doi:10.4172/1948-5948.1000205

Trivedi MK, Branton A, Trivedi D, Nayak G, Shettigar H, Gangwar M amd Jana S (2016). Characterisation of antimicrobial susceptibility profile of biofield treated multidrug-resistant *Klebsiella oxytoca*. Applied Microbiology 1(1):1-6. doi:10.4172/2471-9315.1000101

Van Kregten E, Westerdaal NAC and Willers JMN (1984). New, simple medium for selective recovery of *Klebsiella pneumoniae* and *Klebsiella oxytoca* from human faeces. Journal of Clinical Microbiology 20:936-941.