

Original Research Article

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Isolation, Identification and Antimicrobial Sensitivity of *Klebsiella pneumoniae* from Cattle and Buffaloes

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ABSTRACT

Klebsiella is an opportunistic bacterial pathogen of man and animals known for presence of diversity of Anti Microbial Resistance (AMR). The present study was undertaken to isolate and study the anti-microbial resistance in *Klebsiella* isolates from cattle and buffaloes. Sixty (60) samples, comprising of 33 mastitic milk and 27 diarrhoeic faecal samples were collected aseptically from cattle and buffaloes. From these 60 samples, 11 (18.33%) *Klebsiella* species were isolated and identified on the basis of cultural, morphological and biochemical characteristics. All these isolates were later confirmed as *Klebsiella pneumoniae* by PCR assay using species specific primers for *Klebsiella pneumoniae*. Out of these 11 isolates, 8 belonged to diarrhoeic faecal and 3 belonged to mastitic milk samples. The anti-microbial sensitivity test revealed that all *Klebsiella pneumoniae* isolates were resistant to penicillin, amoxicillin, cefuroxime, and ampicillin/sulbactam (100.0%), followed by streptomycin (83.3%), amikacin (75.0%), cefalexin (58.3%), ceftriaxone (41.6%), gentamicin (25%), imipenem, co-trimoxazole (Sulph-trimethoprim), and ciprofloxacin (16.6% each), and norfloxacin and ofloxacin (8.3% each). However, no resistance was observed against tetracycline. Multiple Drug Resistance (MDR) was recorded in all the isolates.

Keywords

Antimicrobial
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Introduction

Klebsiella pneumoniae, a member of the family *Enterobacteriaceae*, is pervasive in the natural environment and benignly colonizes the gastrointestinal tracts of humans and animals. It is an opportunistic pathogen capable of causing a wide range of diseases in humans and different animal species (Davis and Price 2016), especially hospitalized or otherwise immunocompromised individuals

(Gorrie *et al.*, 2017). The bacterium causes coliform mastitis in cattle, pneumonia and suppurative conditions in foals, cervicitis and metritis in mares, and urinary tract infections in dogs. In animals, *Klebsiella* have not only been isolated from clinical conditions, but also from apparently healthy animals. *Klebsiella* is a member of ESKAPE group of bacteria, which is a global major threat to the clinicians due to the rapid emergence of multiple drug resistant isolates of bacteria.

Among different niches from which *Klebsiella* species can acquire anti-microbial resistance (AMR) genes, animals have a considerable share. Thus *Klebsiella* species of animal origin can be a key trafficker of drug resistance genes. The present study was undertaken to isolate *Klebsiella* from dairy cattle and buffaloes and to assess the antimicrobial resistance pattern of these isolates by *in vitro* method.

Materials and Methods

Sample collection

Faecal samples

A total of 27 faecal samples were collected from the diarrhoeic as well apparently healthy cattle and buffaloes presented to the Veterinary Clinical Complex, GADVASU and from dairy farms located in and around Ludhiana city of Punjab, India. The rectal swabs were taken aseptically using sterile swabs and immediately transferred in the sterile tubes. All the samples were immediately brought to lab in a collection box containing ice packs and immediately processed for the isolation of bacteria.

Mastitis milk samples

A total of 33 Milk samples were collected from cattle and buffaloes affected with mastitis. These animals were presented at Veterinary Clinical Complex, GADVASU, Ludhiana. The milk samples were collected aseptically in sterile containers after discarding the first few streaks of milk from the teats. Milk from all the teats was pooled together for the further processing of samples.

Isolation of bacteria

All the faecal samples brought were streaked on McConkey Lactose agar (MLA) and

incubated at 37 °C for 18-24 hrs for the isolation of bacteria. Mastitis milk samples were inoculated initially on Brain Heart Infusion agar and incubated overnight at 37 °C. Subsequently single isolated bacterial colonies were streaked on MLA and incubated at 37 °C for 18-24 hrs for the isolation of bacteria. Lactose fermenting colonies on MLA were further subjected to Gram's staining. Following this a battery of various biochemical tests like Catalase, Oxidase, Indole, Methyl Red, Vogues Proskauer, Citrate utilization, Urease production, Motility on SIM media, and Carbohydrate fermentation test was applied for identification of the *Klebsiella* species.

Genotypic confirmation of *Klebsiella pneumoniae*

The phenotypically identified *Klebsiella* species were confirmed genotypically by PCR using *Klebsiella pneumoniae* specific primers. For this purpose whole bacteria DNA of each bacterial isolates was extracted by using DNA isolation kit (Chrom-Aid Genomic DNA isolation kit, BTL Research Labs) as per the manufacturer's instructions and the purity of DNA was checked using nanodrop spectrophotometer.

The *Klebsiella pneumoniae* species specific primers having reference number CP024838.1 with a sequence of (5'-3') F: ATGGCCGGGCATGGTACTTC, R: ACCGGAGGTGATGTTTTTCGGT (Sekhri 2019). PCR reaction mixture was prepared that consisted of 12.5 µl mastermix (2X GoTaq® Master Mix) (Promega, USA) 1µl of 20 pmol/ul of each forward and reverse primers (Metabion International, Germany), 1µl of template DNA and finally the reaction volume was made up to 25 µl using Nuclease free water (Promega, USA) PCR was performed on a thermocycler (Veriti, ABI, USA) with the following conditions: an initial

denaturation at 94°C for 5 minutes and 35 cycles each of denaturation at 94°C for 45 seconds, annealing at 60°C for 1 minute and extension at 72°C for 1 minute. This was followed by a final extension at 72°C for 10 minutes.

The PCR product was run on a 1.5% agarose gel prepared in the molecular biology lab at 80 V for 1 h. After the gel electrophoresis, DNA bands were visualized and the images were captured by using Gel Documentation System (AlphaImager, Innotech).

Antibiotic sensitivity testing

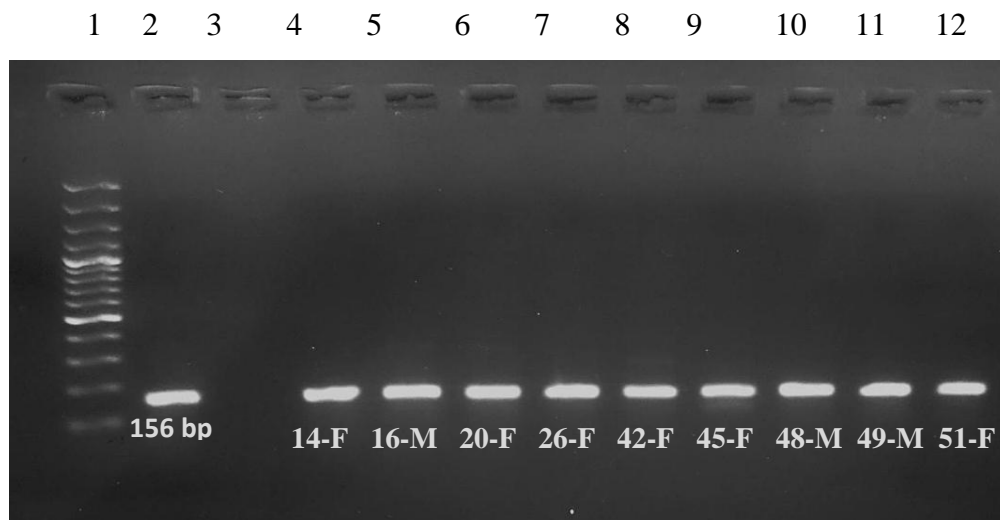
The *in vitro* Antibiotic sensitivity test (AST) of all *Klebsiella pneumoniae* isolates was performed according to disc diffusion method of Bauer *et al.*, (1966). Fifteen antibiotic discs viz., Penicillin (10 units), Streptomycin (10 mcg), Ampicillin/Sulbactam (10/10 mcg), Amoxicillin (10 mcg), Imipenem (10 mcg), Cefalexin (30 mcg), Cefuroxime (30 mcg), Ceftriaxone (30 mcg), Tetracycline (30 mcg), Gentamicin (10 mcg), Amikacin (30 mcg), Co-trimoxazole (25 mcg), Norfloxacin (10 mcg), Ofloxacin (5 mcg), Ciprofloxacin (5 mcg) were used in the study.

Results and Discussion

From 60 samples, 11(18.33%) *Klebsiella* isolates were isolated and identified on the basis of cultural, morphological, and biochemical characteristics. These *Klebsiella* isolates on subjecting to PCR using species specific primers revealed the presence of an expected amplicon of 156 bp size (Fig. 1) and, hence, confirmed genotypically as *K. pneumoniae*. The incidence of *K. pneumoniae* was 29.6 (8/27) and 9.1 (3/33) percent in faecal and mastitic milk samples, respectively.

The *in vitro* AST revealed that all (100%) *K. pneumoniae* isolates were resistant to penicillin, amoxicillin, cefuroxime, and ampicillin/sulbactam, followed by streptomycin (83.3%), amikacin (75.0%), cefalexin (58.3%), ceftriaxone (41.6%), gentamicin (25%), imipenem, co-trimoxazole (Sulph-trimethoprim), and ciprofloxacin (16.6% each), and norfloxacin and ofloxacin (8.3% each). All the isolates were found to be multidrug resistant as each of the isolate was resistant to minimum one agent of at least three antimicrobial groups.

Fig.1 Gel electrophoresis of amplicon (156bp) of *Klebsiellapneumoniae* isolates



In present research *K. pneumoniae* was isolated from 29.6% (8/27) of faecal samples. Bobbadi *et al.*, (2019) found 29.4 % samples of animal intestines to harbor *Klebsiella* spp., which is similar to our findings. In close corroboration of present results, Montso *et al.*, (2019) found *K. pneumoniae* species in 32% samples of cattle faeces. However, Sousa *et al.*, (2019) could isolate *K. pneumoniae* from only 15% faecal samples of domestic and wild animals. Although *Klebsiella* is a member bacteria of normal gut microflora of animals, its prevalence in intestines and, therefore, faeces may vary with age, season, bacteriological quality of feed and water at farms, type of bedding material (inorganic versus organic), use of antibiotics as growth promoter and prevailing clinical diseases, if any, at the time of collection of samples (Xiong *et al.*, 2018). The fecal shedding of *Klebsiella* by dairy cows is intermittent and associated with transient rather than persistent presence of the organism in the gastrointestinal tract (Munoz and Zadoks, 2007).

K. pneumoniae was recovered from 9.0% (3/33) of mastitic milk samples. *K. pneumoniae* is one of the major pathogens of coliform bovine mastitis (Podder *et al.*, 2014). In this research pursuit, *K. pneumoniae* were isolated from 9% samples of mastitis milk, which is in agreement to 8.6 % reported by Osman *et al.*, (2014), but lower than the reports of Kastande *et al.*, (2013), and Masse *et al.*, (2020). *K. pneumoniae* is an opportunistic pathogen, and therefore udder and teat cleanliness scores, faecal shedding of bacteria, season, and type of bedding material are the important predisposing factors for mastitis (Munoz *et al.*, 2006), which could be the reason for variations recorded.

On comparison of results of AST with previous reports we found that our findings are comparable to that of Briss and Duijkeren

(2005) who found resistance in *Klebsiella* isolates against ampicillin to be 99%. Similarly, Jones *et al.*, (2005) also recorded 100% resistance to ampicillin in *Klebsiella* isolates, which is in agreement with our findings.

Montso *et al.*, (2019) isolated 196 isolates of *E. coli* and *K. pneumoniae* and found that upto 100% of the isolates were MDR. Resistance to various antimicrobial agents in 67 *K. pneumoniae* isolates was observed and all were MDR isolates (Sousa *et al.*, 2019). All these findings are in corroboration to our observations.

The present study is evident of Multi Drug Resistant *Klebsiella pneumoniae* in cattle and buffalos in the area of study.

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References

- Davis, G. S., and Price, L. B. 2016. Recent research examining links among *Klebsiella pneumoniae* from food, food animals, and human extra intestinal infections. *Current Environmental Health Reports*, 3(2): 128–35.
- Gorrie, C. L., Mirceta, M., Wick, R. R., Edwards, D. J., Thomson, N. R., Strugnell, R. A. 2017. Gastrointestinal carriage is a major reservoir of *Klebsiella pneumoniae* infection in intensive care patients. *Clinical Infectious Diseases* 65: 208–15.
- Bauer, A. W., Kirby, W. M., Sherris, J. C., Turck, M. 1966. Antibiotic

- susceptibility testing by standardized single disk method. *American Journal of Clinical Pathology* 45: 433-96.
- Sekhri I. 2019. Prevalence and pattern of antimicrobial resistance in *Pseudomonas aeruginosa* isolated from mastitic milk. *Thesis submitted to Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana*.
- Bobbadi, S., Kiranmayi, C. B., Nelapati, S., Tumati, S. R., Kandhan, S., Gottapu, C.,Boddu, S.V. 2019. Occurrence and genetic diversity of ESBL producing *Klebsiella species* isolated from livestock and livestock products. *Journal of Food Safety*, 12738.
- Montso, K. P., Dlamini, S. B., Kumar, A.,Ateba, C. N. 2019. Antimicrobial resistance factors of extended-spectrum beta-lactamases producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from cattle farms and raw beef in north-west province, South Africa. *BioMed research international* 2019: 1-13.
- Sousa, A. T. H. I., Makino, H., Bruno, V. C. M., Candido, S. L., Nogueira, B. S., Menezes, I. G., Nakazato, L. Dutra, V. 2019. Antimicrobial resistance profile of *Klebsiella pneumoniae* isolated from domestic and wild animals. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* 71(2): 584-593.
- Xiong, W., Wang, Y., Sun, Y., Ma, L., Zeng, Q., Jiang, X., Li, A., Zeng, Z., Zhang, T. 2018. Antibiotic-mediated changes in the fecal microbiome of broiler chickens define the incidence of antibiotic resistance genes. *Microbiome* 6: 34.
- Munoz, M. A., and Zadoks, R. N. 2007. Patterns of Fecal Shedding of *Klebsiella* by Dairy Cows. *Journal of Dairy Science* 90: 1220–24.
- Podder, M. P., Rogers, L., Daley, P. K., Keefe, G. P., Whitney, H. G., Tahlan K. 2014. *Klebsiella* Species Associated with Bovine Mastitis in Newfoundland. *PLOS ONE* 9(9): e106518.
- Massé, J., Dufour, S. Archambault, M. 2020.Characterization of *Klebsiella* isolates obtained from clinical mastitis cases in dairy cattle. *Journal of Dairy Science* 103(4): 3392-400.
- Munoz, M. A., Ahlström, C., Rauch, B. J.,Zadoks, R. N. 2006. Fecal Shedding of *Klebsiella pneumoniae* by Dairy Cows. *Journal of Dairy Science* 89(9): 3425-3430.
- Brisse, S. and Duijkeren, E. 2005.Identification and antimicrobial susceptibility of 100 *Klebsiella* animal clinical isolates. *Veterinary Microbiology* 105(3-4): 307–12.
- Jones, L. A., McIver, C. J., Kim, M. J., Rawlinson, W. D., White, P. A. 2005. The *aadB* Gene Cassette Is Associated with *bla_{SHV}* Genes in *Klebsiella* Species Producing Extended-Spectrum β -Lactamases. *Antimicrobial Agents and Chemotherapy* 49(2): 794-97.

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