

## Prevalence and Antimicrobial Resistance of *Klebsiella* species Associated with Bovine Diarrhea

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**ABSTRACT:** Diarrhea in the production animals is a major threat to the industry because of the losses due to poor growth, mortality, low production and cost involved in veterinary intervention. *Klebsiella* sp. is a gram-negative coliform bacterium that lives in the intestines of humans and animals as commensal. *Klebsiella* sp. is considered as an opportunistic pathogen that potentially could cause diarrhea. Understanding distribution of bovine diarrhea causing pathogen *Klebsiella* spp. can contribute to the selection of suitable antimicrobials. Fecal samples collected from 47 dairy cattle from Livestock Research Centre, SVPUAT, Meerut and villages adjoining to Meerut city, Uttar Pradesh were examined to study the frequency of *Klebsiella* spp and to determine the associated risk factors, resistance of isolated strains to various antimicrobial agents. Nearly 29.79% of the faecal samples were positive for *Klebsiella* spp. Herd size and age group were found to be significantly associated ( $p < 0.05$ ) with the occurrence of these pathogens. A high level of resistance was observed against oxytetracycline, gentamicin, streptomycin and ceftriaxone. In contrast, enrofloxacin and amikacin were effective against *Klebsiella* spp. The high prevalence of AMR in India is a very important concern for strategy programs to control bovine diarrhea caused by *Klebsiella* spp.

**Keywords:** Antibiotyping, Cattle, India, *Klebsiella*, Prevalence.

### INTRODUCTION

Animal life and productivity are seriously threatened by pathogens like bacteria, viruses, protozoa, and parasites (Dhama *et al.*, 2013; Anita *et al.*, 2014). In particular, diarrhoea in newborn calves poses a serious threat to the livestock sector due to direct losses *viz.*, deaths, stunted growth, and high treatment costs (Izzo *et al.*, 2011; Malik *et al.*, 2012; Lee *et al.*, 2021). Due to multiple etiology, the treatment of bovine diarrhoea is difficult (Bartels *et al.*, 2010; Izzo *et al.*, 2011; Cho and Yoon 2014). Faecal shedding of *Klebsiella* (Munoz *et al.*, 2006; Zadoks *et al.*, 2011; Cheng *et al.*, 2021) could occur in as many as 80% of healthy dairy cows and may contribute to the exposure of animals to these pathogens and to occurrence of mastitis (Roberson *et al.*, 2004; Munoz and Zadoks 2007; Olde Riekerink *et al.*, 2008). *Klebsiella* spp. is considered as an opportunistic bacterial pathogen having high frequency

and diversity of antimicrobial resistance (AMR) genes. In addition to its own clinical pathology, *Klebsiella* is the species within which several new AMR genes (carbapenem-resistance genes KPC, OXA-48 and NDM-1) were first discovered before spreading to other pathogens (Wyres and Holt 2018). *Klebsiella pneumoniae* belongs to a group of bacteria frequently associated with antimicrobial resistance (AMR) known as ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp (Wyres and Holt 2018; Rice, 2010; Masse *et al.*, 2020). Food-producing animals are known to be reservoirs for zoonotic pathogens including *Klebsiella pneumoniae* and other commensals (Carattoli, 2008; Iweriebor *et al.*, 2015; Valentin *et al.*, 2015; Gao *et al.*, 2015) and such animals have been reported to serve as potential

sources of infections to mankind especially in rural areas (Smet *et al.*, 2010) and this is of major concern. Presently, various kinds of antibiotics are used for the treatment, prevention of disease or infection, growth enhancement and increasing the feed conversion efficiency in livestock (Woolhouse *et al.*, 2015; Argudin *et al.*, 2017). Increased antimicrobial resistance in the microorganisms may result from the indiscriminate and imprudent use of antibiotics (Berge *et al.*, 2005; Sobhy *et al.*, 2020). To the best of the author's knowledge, there were no previous studies conducted on diarrhoea in bovines due to *Klebsiella* spp as well as on their antibiotypes in the study area. However, there are many instances of bovine diarrhoea in the study area, which frequently necessitates veterinary assistance due to the severe morbidity and mortality it causes. Therefore, the present study was designed with the aim to isolate, identify the *Klebsiella* spp and their antibiotyping from clinical cases of bovine diarrhoea in Meerut, Uttar Pradesh.

## MATERIALS AND METHODS

**Study Area and Sampling.** This cross-sectional was conducted on the clinical cases of bovine diarrhea of adult and calves at an organised dairy farm of SVPUAT and in & around Meerut city of Uttar Pradesh, India. Total of 47 faecal samples were collected during the study period (2020-21) and transported to the laboratory in sterile faecal containers under ice packs.

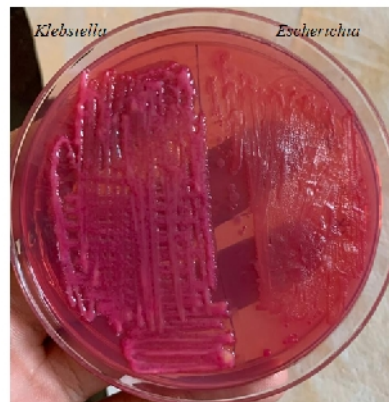
**Isolation and Identification of *Klebsiella* spp.** The 47 faecal samples were incubated in NA (HiMedia, Mumbai) at 37°C for 24hrs. After incubation, the inoculums were streaked onto MacConkey Lactose agar (HiMedia, Mumbai) and incubated at 37°C overnight. Characteristic colonies of *Klebsiella* spp were picked up and subjected to presumptive identification using Gram's staining and biochemical characteristics (Edwards and Ewings 1986).

**Antibiogram.** The confirmed isolates of *Klebsiella* spp were further checked for drug sensitivity pattern against 07 commonly used antimicrobials enrofloxacin (10 mcg), gentamicin (10 mcg), streptomycin (10 mcg), amikacin (30 mcg), oxytetracycline (30 mcg), ceftriaxone (10 mcg) and metronidazole (5 mcg) by disc diffusion method as per the guidelines of NCCLS-2018.

## RESULTS

Out of 47 faecal samples, 14 samples produced large, opaque, flat colonies with large shiny, mucoid and dark pink color colonies on MLA (Fig. 1). The presumptive *Klebsiella* colonies were subjected to Gram's staining and biochemical tests. Staining revealed pink colored, small rods, round ended parallel edged Gram negative cocobacilli bacteria. Further confirmation of these isolates were done by biochemical tests and the IMViC pattern was found as - - + +. Out of 47 faecal samples of diarrheic animals, the presences of *Klebsiella* in 14 samples were confirmed. The overall positivity of *Klebsiella* spp was 29.79%. The positivity of *Klebsiella* spp was significantly higher in adult animals (35.48%) in comparison to that of calves (18.75%). Eight isolates

were checked for antimicrobial agent resistance patterns. All the *Klebsiella* isolates were resistant to metronidazole. Overall, very high percent of isolates were resistant to Oxytetracycline (75.00%), followed by Gentamicin, streptomycin and ceftriaxone (50.00% each), while lesser percent of isolates were resistant to and enrofloxacin (37.50%) whereas least percent of isolates were resistant to amikacin (25.00%). Drug sensitivity analysis of all the 08 *Klebsiella* isolates revealed eight different biotypes (Table 1). The study revealed isolates with resistance against one antibiotic to the isolates with resistance against all the 07 antibacterials used.



**Fig. 1.** *Klebsiella* on MacConkey Lactose agar.

**Table 1: Antibiotyping of *Klebsiella* isolates in diarrheic dairy animals.**

Sr. No.	Animal No.	<i>Klebsiella</i> antibiotype Pattern (Sensitivity)
1.	K-7	S,A
2.	K-8	E,A
3.	K-21	E,G,S,A,O,C
4.	K-25	E,S,A,C
5.	K-27	C
6.	K-32	Resistant to All
7.	K-33	E,G,S,A,O
8.	K-34	E,G,A,C

(A; amikacin, C; ceftriaxone, E; enrofloxacin, G; gentamicin, M; metronidazole, O; oxytetracycline and S; streptomycin)

## DISCUSSION

Diarrhoea is a major cause of concern not only for the animal health professionals, practicing in the field but also for the dairy industry and animal owners due to enormous economic losses owing to calf death, growth retardation, and treatment costs (Izzo *et al.*, 2011; Lee *et al.*, 2021). A dairy is considered as successful, when the animals are healthy and their progeny is safe and sound, but diarrhoea is the main curse to this. The treatment of diarrhoea in animals is a challenge to veterinarians due to poor immunological, physiological status and deprived body resistance of animals. In the present study, the prevalence of *Klebsiella* spp was detected as 29.79%. In the present cross-sectional study, the prevalence of faecal shedding of *Klebsiella* spp. was 29.79%. Faecal shedding and gastrointestinal carriage of *Klebsiella* spp has also been reported from

animals including cattle (Munoz *et al.*, 2006; Zadoks *et al.*, 2011; Agga *et al.*, 2021; Bandyopadhyay *et al.*, 2021), dogs (Stolle *et al.*, 2013; Donati *et al.*, 2014; Liu *et al.*, 2017) as well as human beings (Thom, 1970; Degener *et al.*, 1983; Podschun and Ullmann 1998). The faecal shedding of *Klebsiella* could occur in as many as 80% of healthy dairy cows and this may lead to the exposure of cows to this pathogen and development of mastitis in the animals (Munoz *et al.*, 2006; Zadoks *et al.*, 2011). The faecal shedding of *Klebsiella* occurs randomly and often intermittently rather than persistently by dairy cows (Munoz and Zadoks 2007).

Antibiogram study revealed that amikacin (75%) to be the most effective antibiotics, followed by enrofloxacin (62.5%), gentamicin (50%), streptomycin (50%) and ceftriaxone (50%) against *Klebsiella* spp. The similar antibiogram pattern was also previously reported in earlier studies (Bedada and Hiko, 2011; Kumar and Sharma 2002). The highest efficacy of amikacin and enrofloxacin against *Klebsiella* spp isolates could be attributed to less common use in bovines in the study area. Amikacin and enrofloxacin were found to be effective drugs for the treatment of *Klebsiella* infection in bovines in the area of study. This is in agreement with previous studies (Dhakal *et al.*, 2007; Kumar and Sharma 2002; Kaur *et al.*, 2015), who also reported gentamicin, ciprofloxacin and enrofloxacin as effective choice of antibiotics in clinical cases of bovine mastitis. In the present study, all the *Klebsiella* isolates were resistant to metronidazole, while a very high percent of isolates were resistant to Oxytetracycline (75.00%). Banerjee *et al.* (2020) reported that *Klebsiella* isolates from rectal swabs of healthy dogs, cats, sheep and goats reared as companion or household animals in India were phenotypical resistant against chloramphenicol, tetracycline, doxycycline, co-trimoxazole, ampicillin, cefotaxime/ clavulanic acid. Indiscriminate and extensive use of broad spectrum antibiotics in clinical cases of gastrointestinal infections, mastitis, metritis and other infection of livestock and humans has led to both increased carriage of *Klebsiella* and subsequently the development of multidrug resistant strains (Mathew *et al.*, 1975). These strains are highly virulent and have the ability to spread rapidly (Jack and Richmond 1970). The high level of antibiotic resistant in the *Klebsiella* isolates might be due to the presence of capsule that gives some level of protection to the cells, presence of multidrug resistance efflux pump, easy spread of organism, efficient at acquiring and disseminating resistance plasmid (Donati *et al.*, 2014; Lee *et al.*, 2021; Bandyopadhyay *et al.*, 2021). Moreover, the patterns of antimicrobial usages at the farms/ villages were not known.

Thus, the data from this study gives an idea about the antimicrobial resistance pattern using a small number of samples and may not completely reflect the overall picture of antimicrobial resistance in the livestock. Animals colonized with the clinically important group of bacteria reduces the therapeutic options for disease incidence and may serve as potential sources of infections in human beings (Smet *et al.*, 2010).

## CONCLUSION

The results of the present study indicated that amikacin may be the most effective antibiotic in case of diarrhoea in cattle caused by *Klebsiella* in cow. This further suggested the crucial need for improvements in the antibiotic stewardship programs to limit the rise of resistant microorganisms. Antibiotics are crucial for treatment of animals but unnecessary or overuse of antibiotics constitutes a serious problem for one health. The data in the present study contributes to diagnosis, treatment and control of bovine diarrhoea in Uttar Pradesh, India, in particular, and emphasis the need of good managerial practices to avoid the entry of commensal and nosocomial pathogens in host to induce any diseases conditions. The occurrence of *Klebsiella* can be managed by improving the environmental contamination.

## FUTURE SCOPE

This study has some limitations like sample size, antibiotyping on the basis of fixed number of commonly used antimicrobial agents, differential studies on the basis of age of animals and molecular characterization of the organisms. Therefore, the study may also be conducted further with more samples, different species, and breed of dairy animals and in large geographical areas.

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**Conflict of Interest.** None.

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