

E-ISSN: 2320-7078 P-ISSN: 2349-6800 www.entomoljournal.com JEZS 2020; 8(4): 990-994 © 2020 UEZS

© 2020 JEZS Received: 10-05-2020 Accepted: 12-06-2020

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Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

Multidrug resistance among *Klebsiella pneumoniae* passed from the gut of diarrheic goats of University farm, Maharashtra, India

Journal of Entomology and

Zoology Studies

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Abstract

A clinical investigation was conducted to determine the antibiotic sensitivity pattern of *Klebsiella pneumoniae* isolated from diarrheic goats. From 54 diarrheic goats, *Klebsiella pneumoniae* were isolated from 13 samples. Antibiotic sensitivity pattern for antibiotics revealed that highest resistance was observed in Ampicillin, Amoxycillin / Clavulanic acid, Penicillin and Tetracycline (100.0% each), followed by Co-trimoxazole (92.31% each), Cefachlor, Ceftriaxone, Cefotaxime, and Cefoperazone (61.54% each). The least antimicrobial resistance was observed in Gentamicin and Ciprofloxacin (15.38% each), followed by Chloramphenicol (7.69%). All the isolates are sensitive to Enrofloxacin and Levofloxacin. Multiple antimicrobial resistance patterns were observed involving 6 to 13 isolates. Eleven isolates were showing resistance to minimum 5 antimicrobials. Multidrug resistance genes are easily transferable to other species of Enterobacteriaceae family with potential to cause a disease and warrants a nearly possible transmission in human population. The paucity of reliable and timely information, particularly in Indian conditions, may further limit epidemiological surveillance of *Klebsiella pneumoniae* and effective stewardship efforts.

Keywords: Antibiotic sensitivity testing, diarrhea, *Klebsiella pneumoniae*, multiple drug resistance and osmanabadi goats

Introduction

Effective source of employment especially for landless agricultural labours and weaker sections of society under Indian conditions is Goat farming. Due to low resource investment, ready market throughout the year, less fluctuation in market price and fewer risks involved are the some of features that are attracting the newer entrepreneurs in goat farming. In Semiintensive system of goat management, goats are put for grazing in the field. The limitation of this system is exposure of animals to wide variety of pathogens and of all the maladies, those infecting the digestive system can cause substantial losses through low growth rate, poor feed efficiency, high morbidity and mortality resulting into substantial losses to goat farmers.

Among the pathogens of digestive tract, Klebsiella pneumoniae is a major universal pathogenic bacterium found in gastro-intestinal tract of both the human beings and the animals with certain strains being pathogenic causing various clinical entities including gastroenteritis, cystitis, meningitis, peritonitis, and septicemia. Klebsiella is one of the most virulent pathogens and is often associated with high morbidity and mortality in children (Meatherall et al., 2009) ^[10]. Klebsiella pneumoniae is transmitted by ingestion of contaminated food and water, direct contact with animals, faeces and contaminated soil and directly from one person to another. Ruminants are known to harbour not only strains pathogenic to animals but also strains that cause asymptomatic infections in animals and which can pass through the food chain to cause clinical diseases in human beings. Since, goat meat (Chevon) have been widely accepted as source of non-vegetarian diet in Maharashtra State, India, the association of Klebsiella pneumoniae in goats has significance for human infection in this part of Country. Goat meat can transmit infections and diseases either through handling or during preparation procedures or as a result of ingestion by the consumer. Domestic and wild animals are sources of Klebsiella pneumoniae but ruminants primarily goats, sheep, cattle have been identified as major reservoirs and source for human infection (Rahimi et al., 2012)^[14].

Veterinarians, there is vital need for quick For microbiological diagnosis so that adequate treatment of the Klebsiella pneumoniae infection is performed. Antibiotics are one of the most important components used in the treatment of Klebsiella pneumoniae infection. Acquired antimicrobial resistance in these bacteria is an increasing threat both in human and Veterinary medicine. Further, for the diseases caused by Klebsiella pneumoniae, indiscriminate exploitation of antimicrobials in Veterinary field as resulted into development of multidrug resistant bacteria and is particular concern as it is the most common Gram-negative pathogen associated with diarrhea in livestock. Important pathogens shared by humans and animals and their spread of antimicrobial resistance from the closed environment are increasingly perceived as a threat to public health. Monitoring antimicrobial susceptibility testing in pathogenic bacteria in animals is already recommended by OIE (Acar and Rostel, 2001)^[3]. *Klebsiella pneumoniae* strains are considered to be excellent indicators of antimicrobial resistance because as they are part of the normal microbiota of people and animals, and also occur in the environment (Aarestrup et al., 2008)^[1].

However, to our knowledge, there is no published data on the role of *Klebsiella* bacteremia and its clinical features in diarrheal goats. Considering the above facts, the present piece of research was conducted to investigate prevalence of *Klebsiella pneumoniae* from diarrheic goats at All India Coordinated Research Project (AICRP) on Osmanabadi Goats, Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri Maharashtra State, India and it's *in vitro* virulence assay with antimicrobial resistance pattern.

Materials and Methods

Animals

The whole investigation was carried out on Goats suffering from diarrhea at AICRP on Osmanabadi Goats, MPKV, Rahuri Maharashtra State (INDIA).

Husbandry and Management System

At AICRP on Osmanabadi Goats, MPKV, Rahuri Maharashtra State, India, goats were reared under semiintensive system of management. Daily, all goats were put under grazing for 5-6 hours and supplemented with green grass and concentrate diet. The goats were housed in common shed with small compartments in groups. At the time of pre and post parturient period they were kept in separate byres. Periodic vaccination against various infectious diseases viz., Enterotoxaemia, Peste des Petitis Ruminants (PPR), Hemorrhagic Septicemia, and Black Quarter is carried out for all the goats. Twenty one days prior to vaccination deworming was carried out.

Diarrheal Fecal sample collection

In the present study a total of 54 goats suffering from diarrhea during the period from June 2016 to July, 2017 were subjected to investigation. The fecal swab samples from each diarrheic goat were collected aseptically and carried to the laboratory on ice.

Isolation

Enrichment

Samples were by inoculated into MacConkey's broth tubes (M/s. Hi Media Pvt. Ltd, Mumbai, India) at 37 °C for 24 hours for enrichment followed by selective plating.

Plating on Selective Media

A loopful inoculum from MacConkey's broth was streaked onto MacConkey's agar and incubated at 37 °C for 24 hours for the characteristic pink colonies. The well separated pure colonies were picked up on nutrient agar slants as pure culture and used for standard morphological and biochemical tests.

Culture and Identification of Microorganisms

Identification of isolate was done on basis of colony morphology, Grams' staining, catalase test, Oxidase test, Indole production, Citrate utilization, Methyl Red test, Voges Proskauer test (Cheesbrough, 1994)^[7].

Antimicrobial Susceptibility Testing

The antibiotic-susceptibility profile of isolate for 14 different antibiotics was prepared using the disk diffusion method on Mueller-Hinton agar as recommended by Bauer *et al.* (1966)^[6]. In brief, isolates was grown overnight on blood agar at 37 °C, and the colonies was suspended in sterile normal saline solution (NSS) equivalent to a 0.5 McFarland standard.

The suspension (100 µl) was spread over the medium plate. Then, the antibiotic disk was transferred aseptically on to the surface of the inoculated medium, and was incubated further at antibiotics at 37 °C, for a period of 24 h. The antibiotics and their concentrations used are as follows: Ampicillin (AMP) (25 μg), Amoxyclav (AMC) (30 μg), Chloramphenicol (C) (30 µg), Cefachlor (CF), Co-Trimoxazole (Co) (30 µg), Ciprofloxacin (CP) (30 µg), Cefoperazone (CPZ) (75 µg), Ceftriaxone (CTR) (30 µg), Cefotaxime (CTX) (10 µg), Enrofloxacin (Ex) (10 µg), Gentamicin (G) (20 µg), Levofloxacin (LE) (5 µg), Penicillin G (P) (10 units) and Tetracycline (TE) (30 µg).

Results and Discussion

From 54 diarrheic goats fecal samples, 13 samples (24.07%) were found positive for *Klebsiella pneumoniae* (isolates were culturally and biochemically confirmed). Similar findings were also recorded by Gebru *et al.* (2018) ^[9] who observed that 22.2 per cent *Klebsiella pneumoniae* was isolated from diarrhea in camel calves. Already we reported association of *Klebsiella pneumoniae* in pneumonic goat in the same farm (Yadav *et al.* 2018) ^[17] indicating that goats were susceptible to said bacteria or they may be under various environmental stresses like low temperature and increased humidity in byres during winter season. Since, *Klebsiella pneumonia* is inhabitant of intestinal tracts of animals, fecal contamination of the organisms and contributes to occurrence of opportunistic infection.

Goat diarrhea continuous to be one of the most important problems faced by goat keepers, causing economic losses especially in semi-intensive system of management which is followed in this part of Country where the goats are let loose for grazing for minimum of 5 to 6 hours in the field wherein they are exposed to wide variety of animal pathogens. The most common animal pathogen found in the grazing field is pathogenic *Klebsiella pneumoniae* where the goats are at greatest risk of diarrhea.

Klebsiella pneumoniae is an intestinal commensal bacteria commonly isolated from fecal samples. Therefore, isolation of *Klebsiella pneumoniae* from feces may not be directly associated with causation of diarrhea. Since methods required for identifying pathogenic strains of *Klebsiella pneumoniae* are not established yet at the laboratory, strain identification

of *Klebsiella pneumoniae* isolates could not be carried out. However, the finding that *Klebsiella pneumoniae* were predominately recovered from diarrheic goats in the present study may be an indication for their importance in diarrheic goats as causative agent for diarrhea in the study area.

Antibiotic Resistance of *Klebsiella pneumoniae*

The results of antibiotic sensitivity testing (Table 1) showed that *Klebsiella pneumoniae* isolate was having multiple drug resistance to routinely used 14 antibiotics. Antibiotic sensitivity testing for 14 routinely used antibiotics revealed that highest resistance was observed in Ampicillin (AMP), Amoxycillin / Clavulanic acid (AMC), Penicillin (P) and Tetracycline (100.0% each), followed by Co-trimoxazole (Co) (92.31%), Cefachlor (CF), Ceftriaxone (CTR), Cefotaxime (CTX), and Cefoperazone (CPZ) (61.54% each). The least antimicrobial resistance was observed in Gentamicin (G) and (15.38%) Ciprofloxacin (CP) each), followed hv Chloramphenicol (C) (7.69%). All the isolates are sensitive to Enrofloxacin (Ex) and Levofloxacin (LE) (Table 2).

Diarrhoea associated with Klebsiella pneumoniae infections is often treated with antibiotics; however, therapy may be unsuccessful due to resistant strains in animals. In the present study highest resistance of Klebsiella pneumoniae was recored against Ampicillin, Amoxycillin / Clavulanic acid, Penicillin and Tetracycline (100.0% each). This findings are concurs with our earlier reports wherein Klebsiella pneumoniae (isolated from pneumonic goat) is resistant to Ampicillin, Amoxycillin / Clavulanic acid, Penicillin with exception of Tetracycline where it is 92. 86 per cent. The similar findings was also reported by Orhue and Aliu (2014) ^[13] who recorded 100 per cent resistance of Klebsiella isolated from different clinical specimens of human patients to penicillin and amoxyclav antibiotics. High resistance rates to tetracycline may be due to widespread application of tetracycline in Veterinary treatment. Although tetracycline initially was useful for treatment of infections with aerobic Gram negative organisms, many Enterobacteriaceae are now becoming relatively resistant to them. Resistance to tetracycline is principally plasmid-mediated and an inducible trait (Adefarakan et al., 2014) [4]. The resistance to tetracycline observed may also be as a result of decreased accumulation of tetracycline due to either acquisition of an energy-dependent efflux pathway or to decreased influx, or to decreased access of tetracycline to the ribosome (site of action) due to acquisition of ribosome protected proteins and enzymatic inactivation. The frequency of Ampicillin, Amoxycillin/ Clavulanic acid, Penicillin resistance in the current study was highest (100.0%) among the isolates. This may be due to the blanket use of inexpensive antibiotics in the goat keepers in this region of Country or may be due to production of betalactamase enzymes.

Resistance to Co-trimoxazole was 92.31 per cent; this result is not in agreement with our earlier findings of resistance to Cotrimoxazole (50%) (Yadav *et al.* 2018)^[17]. The most common mechanism of resistance to Co-trimoxazole is the acquisition of plasmid mediated, variant diaminopyrimidine folate reductase enzymes. The increase in resistance may be due to selective pressure for sustenance of these bacteria under gastro-intestinal tract wherein for treatment of diarrhea oral preferred antibiotic is Co-trimoxazole.

The study by Asha *et al.* (2017)^[5] on Klebsiella isolates observed maximum resistance (68.5% - 69.5%) to the cephalosporins which is almost similar to our present study

results that showed 61.54 per cent resistance to cephalosporins (Cefachlor, Ceftriaxone, Cefotaxime and Cefoperazone) which are now indiscriminately used in Veterinary practices. The resistance to Cepahosporins might be due to close human-animal interface through environmental cross contamination. Klebsiella was having ability to produce extended-spectrum β -lactamases (ESBL) and are resistant to many classes of antibiotics. The veterinary use of extended-spectrum β -lactams (ESBL) (second and third generation cephalosporins) over the last two decades resulted in the emergence of plasmids carrying ESBLs (in activators of the drug by hydrolysis) in *Enterobacteriaceae* family of animal origin (Aarestrup, 2006)^[2].

The least antimicrobial resistance was observed in Gentamicin (G) and Ciprofloxacin (CP) (15.38% each), followed by Chloramphenicol (C) (7.69%). Chloramphenicol is not used in animal feed as growth promoters. However, Chloramphenicol resistance in the present study is low, which might be due to Chloramphenicol less use of this antibiotic in animal diarrheal cases. All the isolates are sensitive to Enrofloxacin (Ex) and Levofloxacin (LE) (Table 2). Sensitivity to Enrofloxacin and Levofloxacin might be due to the less use of these antibiotics in clinical practice and/or veterinary medicine. So they can be effectively used against the *Klebsiella pneumoniae* infected goat diarrheal cases.

Similar or varying degree of resistance to various antibiotics by *Klebsiella pneumoniae* was also reported by Mishra *et al.* (2001) ^[11]. Their reports of resistance to various antibiotics were Ampicillin (93.3%), Cefotaxime (70.0%), Gentamicin (68.5%), and Chloramphenicol (82.0%).

In contrast to our findings, Vasantha and Leela (2015) ^[16] reported low resistance to Amoxicillin / Clavulanic acid (91.0%), Gentamicin (44.0%), Ciprofloxacin (65.0%), Co-trimoxazole (57.0%). In present findings, all the *K*. *pneumoniae* isolates was sensitive to Cefaclor antibiotic.

For all the 13 *Klebsiella pneumoniae* isolates, multiple antimicrobial resistances ranging from 5 to 11 antimicrobials were observed (Table 3). Seven major antimicrobial resistance patterns were recorded involving 6 to 13 isolates (Table 4). Eleven isolates were showing resistance to minimum 5 antimicrobials. Our earlier findings are very much concurrent to the present research investigation (Multiple drug resistance to 4 to 11 antibiotics. It was observed that all the isolates showed similar resistance pattern against Ampicillin, Amoxicillin/Clavulanic acid and Penicillin) (Yadav *et al.*, 2018)^[17].

Resistance to 5, 6, 9 and 11 antibiotics was observed in 23.08, 15.38, 53.85 and 7.69 per cent each isolates, respectively. Singh and Sharma (2001) ^[15] reported pathogenic Klebsiella isolates were having multiple drug resistance from 7 to 14 antibiotics. Mishra *et al.* (2001) ^[11] also reported multiple drug resistance of *K. pneumoniae* ranging from 2-12 antibiotics. They further reported plasmid mediated drug resistance is a common phenomena in K. pneumonia.

Antibiotic resistance pattern varies from author to author. This might be due to disease pattern, geographical variation and indiscriminate local antibiotic use. The previous reports states that Klebsiella spp. are often multidrug resistant organisms and their plasmid is a primary source of transfer of resistance genes between them (Yadav et al., 2018)^[17]. The most frequent resistances include resistance to aminoglycoside, fluoroquinolones, tetracyclines, chloramphenicol and trimethoprim / sulfamethoxazole

(Nathisuwan *et al.*, 2001) ^[12]. This was said to be due to acquisition of multidrug resistance (MDR) plasmids and *Klebsiella pneumonia* has been identified as one of the most common organisms to carry plasmid encoding extended-spectrum β -lactamases (ESBLs), and such strains are isolated with increasing frequency (Donnenberg, 2005)^[8].

The isolation of *Klebsiella pneumoniae* from diarrheic goats represents a high risk pathogen for the small ruminants and zoonotic significance for human contamination. Therefore, it

is necessary to take safety measures while handling goats and proper hygienic protocols for public health in general. In addition, use of antimicrobials in livestock production is suspected to significantly contribute to multiple drug resistance in species of bacteria that are shared by humans and animals (Acar and Rostel, 2001)^[3]. Multiple drug resistant species of enterobacteriaceae are hence creating a constant need for newer antibiotics worldwide.

C. No	Tattoo No. of Goat	Antibiotic Sensitivity Test Observations													
Sr. No.		AMC	AMP	С	CF	Со	СР	CPZ	CTR	СТХ	Ex	G	LE	Р	TE
1	TF1145	R	R	S	S	R	S	S	R	R	S	R	S	R	R
2	TM189	R	R	R	R	R	R	R	R	R	S	S	S	R	R
3	TF1008	R	R	S	S	S	R	R	R	R	S	R	S	R	R
4	TF1070	R	R	S	R	R	S	S	S	S	S	S	S	R	R
5	SM318	R	R	S	R	R	S	R	R	R	S	S	S	R	R
6	TM786	R	R	S	S	R	S	S	S	S	S	S	S	R	R
7	OF1091	R	R	S	R	R	S	R	R	R	S	S	S	R	R
8	SF262	R	R	S	R	R	S	R	R	R	S	S	S	R	R
9	SF158	R	R	S	S	R	S	S	S	S	S	S	S	R	R
10	OF1183	R	R	S	R	R	S	R	R	R	S	S	S	R	R
11	TM769	R	R	S	S	R	S	S	S	S	S	S	S	R	R
12	OF1098	R	R	S	R	R	S	R	R	R	S	S	S	R	R
13	OF882	R	R	S	R	R	S	R	R	R	S	S	S	R	R

Table 2: Antibiogram results for Klebsiell	a pneumoniae isolated from diarrheic goats
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S- No	Antibiotics	Sei	nsitive	Res	sistant	Total Isolates	
Sr. No.	Anubioucs	No.	%	No.	%	I otal Isolates	
1	Ampicillin (AMC)	0	0	13	100	13	
2	Amoxyclav (AMP)	0	0	13	100	13	
3	Chloramphenicol (C)	12	92.31	01	7.69	13	
4	Cefachlor (CF)	11	38.46	02	61.54	13	
5	Co-Trimoxazole (Co)	1	7.69	12	92.31	13	
6	Ciprofloxacin (CP)	5	84.62	8	15.38	13	
7	Cefotaxime (CTX)	5	38.46	8	61.54	13	
8	Ceftriaxone (CTR)	5	38.46	8	61.54	13	
9	Cefoperazone (CPZ)	5	38.46	8	61.54	13	
10	Enrofloxacin (Ex)	13	100	0	0	13	
11	Gentamicin(G)	11	84.62	2	15.38	13	
12	Levofloxacin (LE)	13	100	0	0	13	
13	Penicillin G (P)	0	0	13	100	13	
14	Tetracycline (TE)	0	0	13	100	13	

Table 3: Overall multiple drug resistance of Klebsiella pneumoniae isolates from diarrheic goats

Sr. No.	No. of Antimicrobials	No. of Resistant Isolates	% of Resistant Isolates (N = 41)
1	05	03	23.08
2	06	02	15.38
3	09	07	53.85
4	11	01	7.69

Table 4: Multiple antibiotic resistant pattern of Klebsiella pneumoniae isolates from diarrheic goats

Sr. No.	Resistance Pattern	No. of Antibiotics	No. of Isolates	
1	AMC, AMP, P, TE	4	13	
2	AMC, AMP, Co, P, TE	5	11	
3	AMC, AMP, G, P, TE	5	01	
4	AMC, AMP, CP, P, TE	5	09	
5	AMC, AMP, Co, CF, CPZ, CTR , CTX, P, TE	9	06	
6	AMC, AMP, CF, CPZ, CTR , CTX, G, P, TE	9	01	
7	AMC, AMP, C, CP, Co, CP, CPZ, CTR, CTX, P, TE	9	11	

Conclusion

Klebsiella pneumoniae isolated from diarrheic goats maintained under semi-intensive system of management are

found positive for multiple antibiotic-resistances. The highest rate of resistance was detected against the antibiotics used as therapeutic purposes, hence, irrational use of antibiotics in human and animals should be depressed. Knowledge of antibiogram studies will assist Veterinarians in pin point, efficacious and economic therapeutic / empirical treatment of goats suffering from diarrhea. To identify the shifting trends in antibiotic resistance regular antibiotic susceptibility studies should be conducted. So, continuous surveillance through antibiotic sensitivity testing of multidrug resistant pathogen and understanding patterns of antimicrobial resistance is the need of current time in order to prevent therapeutic failure and reduce antibiotic selective pressure. To prevent and control emergence of multiple drug resistant pathogens, it is vital to propose and execute fast detection techniques with maximum accuracy in addition to safeguarding the best use of existing antimicrobials.

Acknowledgement

The author is thankful to Director of Research, Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri for providing necessary facilities for carrying out this study.

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