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The prevalence of enterobacterales with MDR status and their associated risk factors in goat population

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Abstract
Studies on the prevalence of pathogenic Enterobacteriaceae and their antibiogram status were carried out in goat population affected with pneumonia and enteritis in the villages around Jaipur, Rajasthan. A total numbers of 126 samples i.e. 72 nasal and 54 enteric samples from goat population were subjected for identification of Enterobacteriaceae pathogens and their antibiotic susceptibility test (ABST). The major pathogens identified in this study were *Escherichia coli*, *Proteus*, *Salmonella*, *Klebsiella*, *Providencia* species. The ABST revealed drug sensitivity of 94.4% to amikacin, 88.8% to gentamicin, 83.3% to ofloxacin, 44.4% to tetracycline and doxycycline, 38.8% to streptomycin and ampicillin/sulbactam, 27.7% to ceftriaxone, 22.2% to amoxicillin/clavulinic acid, 16.6% to co-trimoxazole, 11.1% to ampicillin and imipenam, 5.5% to penicillin – G and cefixime, and 0% to cefotaxime. It was found that the isolated colonies of enterobacterales showed 100% sensitivity to levofloxacin only. The study conveyed the prevalence of major enterobacterales and their associated risk factors for the development MDR in goat population.

Keywords: Enterobacteriaceae, goats, antibiogram, risk factors

Introduction
Livestock plays an important role in the socio-economic life of India and a significant role in the national economy. It is a major source of income to the marginal and landless farmers in India. Sheep and goats are important species of livestock in India, on account of their short generation intervals, higher rates of prolificacy, and their easy marketing. Since sale of these animals are easy, rural people are engaged in this industry in India. According to 19th live-stock census of India (2012), sheep population contributes 12.71% and goat population contributes around 26.40% of the total livestock population. In Rajasthan state the goat populations is 20.84 million as per 2019 census which represents 3.81%, decrease from the previous census in 2012, Rajasthan produced a total of 3.10 million tons of goat milk and 95.23 thousand tons of goat meat in 2022-23. (Poonam Ahari & Hina Asharf Waiz 2024) [19]. Statistics shows an overall decline of 33.33% in the total livestock population as compared to previous censes of 2007.The total sheep population in the country was 65.06 million in 2012, declined by about 9.07% and goat population has declined by 3.82% over the previous census with a population of 135.17 million in 2012. (19th Livestock Census-2012.All India Report). Maru *et al.* (1990) [13] reported that the population decline and economic losses are associated with various diseases in sheep and goats. Paczosa, M.K *et al.* (2016) [18] reported the pneumonic and enteric problems in goat population. Respiratory diseases cause substantial losses in goat industry (Bordeanu A.D *et al.* 2012) [4] Antimicrobial resistance is one of the most reported health challenges and associated deaths could rise to 10 million by 2050 (Mukana *et al.* 2023) [14]. Indian subcontinent is considered to be hub of food and water borne tropical diseases specially caused by members of Enterobacteriaceae family owing to fluctuation in temperature and humidity (Garofalo *et al.* 2019) [6]. Hence an attempt was made to know the enterobactericeae involved in the causation of pneumonia and enteritis in goats and their antibiogram status in goats around villages in Jaipur, Rajasthan State, India.

Materials and Methods

Clinical examination of goats and Collection of clinical samples

A total numbers of 126 samples i.e. 72 nasal and 54 enteric samples from goat population were collected after clinical examination. All the above animals were vaccinated for H.S, B.Q and FMD and dewormed regularly. A total numbers of 126 samples i.e. 72 nasal and 54 enteric samples from goat population from Chawandya (2 flocks) Rampura (2 flocks) and Nyankidhani (2 flocks) villages were subjected for identification of Enterobacteriaceae pathogens and their antibiotic susceptibility test (ABST). The nasal samples were taken from the diseased animals with nasal discharge, coughing and sneezing. Faecal samples were collected from animals having enteritis.

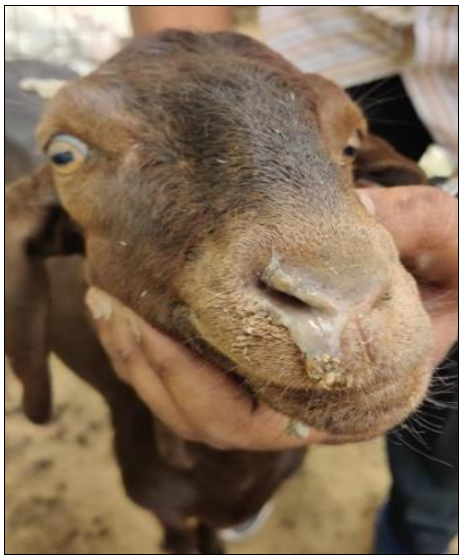


Fig 1: Goat with pneumonia

Bacteriological examination: Culture and biochemical studies.

Culture studies were carried out in MacConkey's and EMB agar media using nasal and enteric materials swabs. The nature of growth and cultural characters of colonies were studied. Preliminary morphological identification was done on Gram's staining for their characteristics. Enterobacteriaceae were identified for their gram negative, non-spore forming rods, and facultative anaerobes and capable of fermenting sugars to various end products.

Antibiogram assay

Isolates were swabbed on the media; discs placed and were incubated at 37 °C aerobically for 24 hours. Afterwards, zone of inhibition was observed and recorded. The following discs were used- Amikacin (AK), Amoxicillin-Clavulanate (AMC), Ampicillin (AMP), Ampicillin/Sulbactam (A/S), Cefixime (CFM), Cefotaxime (CTX), Ceftriaxone (CTR), Co-trimoxazole (COT), Doxycycline (DO), Gentamicin (GEN), Imipenem (IPM), Levofloxacin (LE), Ofloxacin (OF), Penicillin –G (P), Streptomycin (S) and Tetracycline (TE).

The susceptibility of all isolates to different antibiotics was achieved by using disk diffusion method on Muller-Hilton agar media, (CLSI 2020) [5].

Results

Bacterial species identified

The bacteria identified in this study as per the morphological identification by culture and grams method of staining. i.e. *Escherichia coli, Proteus, Salmonella, Klebsiella,* and *Providencia* species.

Fig 2: *Klebsiella* colonies

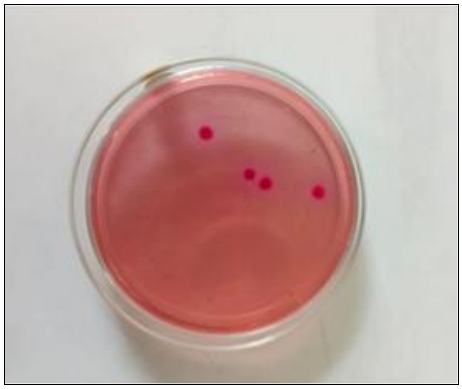
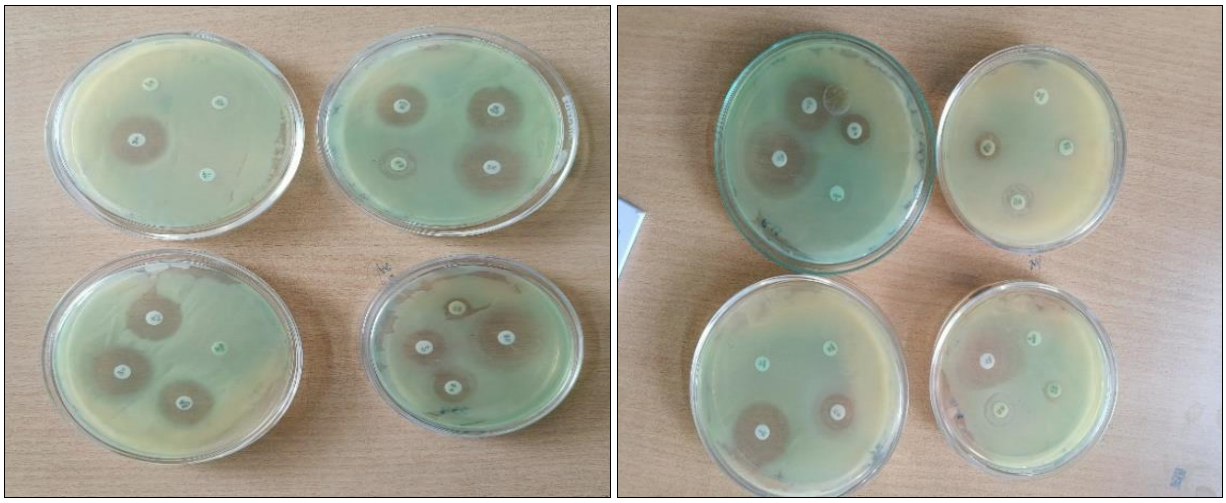


Fig 3: *E. coli* colonies

Fig 4: *Salmonella* colonies

Fig 5: Mixed colonies



(6) (7)

Fig 6, 7: Antibiogram -MDR status

Antimicrobial sensitivity assay

It was found that the isolated colonies of Enterobacteriaceae showed 100% sensitivity only to levofloxacin. They showed 94.4% drug resistance to amikacin, 88.8% to gentamicin, 83.3% to ofloxacin, 44.4% to tetracycline and doxycycline, 38.8% to streptomycin, ampicillin/sulbactam, 27.7% to ceftriaxone, 22.2% to amoxicillin/clavulanate, 16.6% to co-trimoxazole, 11.1% to ampicillin and imipenem 5.5% to penicillin-G, and cefixime, and 0% to cefotaxime (Fig 6 Fig 7 & Table).

Table 1: Antibiotic assay

S. No.	Antibiotic(s)	Sensitivity	S. No	Antibiotic(s)	Sensitivity
1.	Levofloxacin (LE 5 mcg)	100%	9.	Ceftriaxone (CTR 30 mcg)	27.7%
2.	Amikacin (AK 30 mcg)	94.4%	10.	Amoxiclav (AMC 30 mcg)	22.2%
3.	Gentamicin (GEN 10 mcg)	88.8%	11.	Co-trimoxazole (COT 25 mcg)	16.6%
4.	Ofloxacin (OF 5 mcg)	83.3%	12.	Ampicillin (AMP 10 mcg)	11.1%
5.	Tetracycline (TE 30 mcg)	44.4%	13.	Imipenem (IPM 10 mcg)	11.1%
6.	Doxycycline (DO 30 mcg)	44.4%	14.	Penicillin- G (P 10 units)	5.5%
7.	Streptomycin (S 10 mcg)	38.8%	15.	Cefixime (CFM 5 mcg)	5.5%
8.	Ampicillin/Sulbactam (A/S 10 mcg)	38.8%	16.	Cefotaxime (CTX 30 mcg)	0.0%

Discussion

The study was carried out in goat population in clinically positive cases of pneumonia and enteritis in Chawandya (2 flocks), Rampur (2flocks) and Nyankidhani (2 flocks) villages nearer to Jaipur, Rajasthan state.

Identification of bacterial isolates in goats

Bordeanu A-D *et al.* (2012) [4] identified bacterial strains in the respiratory tract of goats in different seasons in Romania. *E. coli* was the major strains isolated followed by *K. pneumoniae*, *Staphylococcus xylosus and Staphylococcus leutus, Enterobacter aerogenes, Serratia* species and *Rahnella aquatilis* from the goat's respiratory tract. Megra T *et al.* (2006) [12] isolated the aerobic flora of the respiratory tract of the goat population in Eastern Ethiopiaand isolated *S. aureus* (17.2%), *P. multocida* (11.9%), *C. tuberculosis* (8.8%), *Bacillus* sp, (7.4%), *A. pyogenes*(6.7%), *E. coli* (6.9%) and *Micrococcus* (1.0%).

Identification of bacterial isolates in goats with enteric problem was reported by many authors. AL-Darraji, A.J.Z and Yousif, A.A (2012) [1] isolated *Citrobacter braacki, Proteus mirabilis, Proteus vulgaris, Enterobacter cloacae, Klebsiella pneumoniae, Salmonella enteritidis, Salmonella Newport,* and *Providencia rettgeri* from enteric goats in Baghdad.

Mukuna W *et al.* (2023) [14] isolated *Chryseomonas luteola, Citrobacter freundii, E. coli, Enterobacter aerogenes, Enterobacter cloacae* and *Yersinia enterocolitica* from Nashville, USA from the goats manure and soil. Hossein Esmaeili *et al.* (2024) [8] isolated *E. coli* frequently followed by *S.aureus, Pseudomonas, Bacillus* and *Klebsiella* in Saanen and Alpine goats with enteric problem. Nagalaa *et al.* 2019 [16]; Azizi *et al.* (2013) [2] reported variation in incidence and percentage of causative agents in small ruminants affected with pneumonia due to various factors such as geographical area, seasonal variations, stress, changes in management, transportation, immune status of infected animals and the different isolation procedures. Good-Ray. K.A (2006) [7] reported the intensified pneumonia in sheep and goat due to various pathogens, parasites along with host's physiology and immunology. The major pathogens identified in these studies were *Escherichia coli, Proteus, Salmonella, Klebsiella, Providencia* species.

Antibiogram assay

Antibiogram assay was carried out for respiratory problems in goats by many authors.

Hazim O. Khalifa *et al.* (2021) [9] studied the prevalence of Enterobacteriaceae Narita, Japan in the respiratory diseases of sheep and goats. The identified isolates were *E. coli* 47.4%, *Klebsiella* spp isolates 26.3%, *Enterobacter* spp isolates 23.7%, as single *Citrobacter koseri* and *Serratia marcescens* isolates 1.3%.The authors reported that most of the isolates i.e. 9.2% were multidrug –resistant, with high resistance rates to Beta-lactams and quinolones, and 11.8% and 6.6% isolates were phenotypically positive for AmpC and ESPL respectively.

Antibiogram assay was carried out for enteric problems in

goats by many authors.

Mukana.W *et al.* (2023) [14] reported that all Enterobacteriaceae from soil, manure and water in cattle and goat farms was highly resistant to novobiocin (100%), erythromycin (100%), vancomicin, & tetracycline (100%). Kanamicin resistance in all Enterobacteriaceae isolates ranged from 0-33.3%. Generally cefpodoxime and nalidixic acid showed relatively low resistance from 0-16.7% and all Enterobacteriaceae isolates from environment were susceptible to imipenem.

In this study it was found that the isolated colonies of Enterobacteriaceae *i.e. Escherichia coli, Proteus, Salmonella, Klebsiella,* and *Providencia* species showed 100% sensitivity only to levofloxacin. The antibiogram showed 94.4% drug resistance to amikacin, 88.8% to gentamicin, 83.3% to ofloxacin, 44.4% to tetracycline and doxycycline, 38.8% to streptomycin and ampicillin/sulbactam, 27.7% to ceftriaxone, 22.2% to amoxicillin, 16.6% to co-trimoxazole, 11.1% to ampicillin and Imipenem, 5.5% to penicillin and cefixime, and 0% to cefotaxime (Table). The ABST revealed sensitivity to levofloxacin alone.

Attributed factors for the development of MDR

The factors associated for the development of MDR in food animals discussed by many authors. Vanderhaeghen W *et al.* (2017) [22] reported antimicrobial use in livestock for prevention and control of diseases. Boeckal Van *et al.* (2017) [3] reported 131,109 tons of antimicrobials were used globally in food animals. Murphy C *et al.* (2018) [15] reported that the antimicrobial use contributes and creates antimicrobial resistance genes in commensal and pathogenic bacteria. Khalifa H O *et al.* (2021) [9] opined that extensive use of antimicrobials in the veterinary field for the prevention of infectious diseases and to increase the animal production led to the dissemination of antimicrobial resistance. Khalifa H.O *et al.* 2020 [10]; Soliman. A.M. *et al.* (2016) [20] reported the close contact between animals and humans facilities cross-transmission especially enterobacterales to acquire and disseminate several unique resistance mechanisms.

Megra T *et al.* (2006) [12] reported several bacterial species inhabit the respiratory passageways of apparently normal goats which include commensals also and subject the animals to pathogenic role due to poor management conditions. Mukana W *et al.* (2023) [14] studied the antimicrobial susceptibility profile of pathogenic and commensal bacteria recovered from cattle and goats, and suggested cattle and goats as reservoirs of MDR bacteria. Manyi-Loh, C *et al.* (2018) [11] reported the antibiotics use in food animal production for evolving AMR in humans. Olufemi F.O *et al.* (2017) [17] reported the prevalence of highly resistant commensals in the animal body, and the reservoir status of healthy animals for bacterial resistance.

As per the studies available the isolation of bacteria varies from place to place and the antibiogram also varies accordingly. The attributed risk factors for the development of antimicrobial resistance were invariable use of antibiotics in veterinary field, development of resistant bacterial genes inside the body, reservoir status of cattle and goats for MDR, presence of highly resistant commensals and bacteria in the animals' body and changing environmental factors. This study confirmed the prevalence of MDR and it' associated risk factors among goat population.

Conclusion

The study revealed the drugs resistance pattern of the routine antibiotics which are in use in goat in and around Jaipur. It indicates the invariable use of antibiotics in goat population in these studied areas. A detailed study needed to ascertain the multiple drug resistance pattern (MDR) which are prevailing in goat population and thereby to reduce the antibiotic residue in meat and milk to avoid zoonotic implications. This study highlighted the potential role of goats in disseminating AMR determinants and resistant bacteria to humans and emphasizes the need for prudent use of antibiotics in veterinary practices and one health approach to combat this problem.

Conflict of Interest

Not available.

Financial Support

Not available.

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