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Antibiotic sensitivity testing of *Pasteurella multocida* strains isolated from small ruminants

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Abstract
Respiratory infection of sheep and goat due to *Pasteurella multocida* is a crucial contributor to production losses in the small ruminant industry in most parts of the world. The current study was designed to isolate *P. multocida* from pneumonic cases of sheep and goats and to determine the antibiotic susceptibility pattern of the isolates. A total of 12 isolates were recovered from 287 samples collected from pneumonic sheep and goat in Telangana. The isolates were confirmed by PM-PCR using *kmt1* gene primers. The isolates were screened for antibiotic sensitivity or resistance pattern using 21 antibiotics. The antibiotic sensitivity test of the isolates had shown that gentamicin was the resistant drug followed by erythromycin, tetracycline, Methicillin, pencillin G, nalidixic acid, co-trimoxazole, ampicillin, Cefotaxime, Lincomycin, kanamycin and amoxiclav. All the isolates were 100% sensitive to ceftriaxone, cefoperazone/sulbactum, ceftiofur, cloxacillin, ciprofloxacin, enrofloxacin, levofloxacin and tetracycline (30 µg/disc).

Keywords: *Pasteurella multocida*, *P. multocida*, PM-PCR, ABST, antobiogram

1. Introduction
India is a home for various livestock species and the country stands third in sheep and first in goat population in the world. The total sheep population is estimated to be 74.26 million and goat population is148.88 million. Despite the huge population, the current productivity and commercialization of sheep and goat remain very low due to diseases, inadequate feed and poor infrastructure. The persistence of animal diseases such as bronchopneumonia has continued to be a major constraint to the small ruminant productivity. It causes huge economic losses and reduced performance during and after the illness. Stresses, viral infections, nutritional and environmental conditions are the predisposing factors that enhance the vulnerability of sheep and goat to respiratory illness. Common bacterial agents which cause pneumonia include *P. multocida*, *M. haemolytica* and *Mycoplasma* species (Prabhakar *et al*., 2010) [7]. Though use of antibiotics is a successful method to control *Pasteurella* infection, the treatment is expensive, lengthy and ineffective due to the development of antibiotic resistance limiting antibiotic potency. Increase in antibiotic resistance is a threat to health sector globally. Surveillance on the spread and emergence of antibiotic resistance is therefore inevitable. In the present study, prevalence of *P. multocida* with more emphasis on antibiotic sensitivity profile of *P. multocida* strains isolated from pneumonic sheep and goat was investigated.

2. Materials and Methods
2.1 Collection of samples
A total of 287 samples including nasal swabs (n=267) and lung tissues (n=20) were collected from pneumonic sheep and goat in Telangana during 2020-21. All the nasal swabs were added with 2 ml of Amies transport medium and lung samples were collected in sterile screw capped sample collection bottles and all the samples were immediately transported to the laboratory on ice.

2.2 Sample processing
All the samples were immediately plated on to Brain Heart Infusion (BHI) agar, 5% sheep blood agar (BA) and MacConkey agar (MCA) and then the plates were incubated at 37 ^{o}C for 18-24 h.

2.3 Isolation and identification of *Pasteurella multocida*

The colonies showing typical morphology of *P. multocida* were further subjected to Gram's staining and biochemical characterization as per the methods described by Cruickshank (1975) [3]. After preliminary identification by cultural, morphological and biochemical tests, isolates were confirmed by *Pasteurella multocida* species specific PCR (PM-PCR) using KMT1 gene primers as described by Townsend *et al.* (1998) [10].

2.4 Antibiotic Sensitivity Test (ABST)

After confirmation by PM-PCR, all the isolates were subjected to antibiotic sensitivity test. The ABST was performed using 21 antibiotic discs by disc diffusion method described by Bauer *et al*. (1966) [1] with slight modifications. The pure cultures were inoculated in BHI broth and incubated for 12-18 hrs. The turbidity of test cultures was adjusted to McFarlands tube no. 0.5 and then the same cultures were used for inoculation onto Mueller Hinton agar (MHA). The cultures were streaked on MHA in such a way that a smooth, homogenous lawn culture was obtained. The antibiotic discs (n=21) were placed with the help of sterile forceps and pressed gently to ensure full contact with the media and then the plates were incubated at 37 ºC for 18-24 h. The zone of inhibition for different antibiotics was recorded and results were interpreted in terms of sensitivity and resistant using the criteria chart provided by CLSI (2016) [2]. The details of the antibiotics used and result of ABST for each antibiotic is presented in Table 1.

3. Results and Discussion

The isolates produced small, smooth, round, glistening translucent colonies on BHI agar and non hemolytic colonies on blood agar. No growth was observed on MacConkey agar. On Gram's staining, the colonies showed small, Gram-negative coccobacilli organisms. Upon cultural and morphological examination, 12 isolates were tentatively identified as *P. multocida* (Quinn *et al*., 1994) [8]. All the 12 isolates were positive for Indole, catalase, oxidase and nitrate reduction tests and showed acidic reaction in TSI test without gas and H_2S production. All the isolates produced an expected amplicon of 460 bp size after PM-PCR, thus the isolates were confirmed as *P. multocida*.

The antibiotic sensitivity or resistance pattern of the isolates had shown that, most of the antibiotics tested were not effective against the *P. multocida* strains. The resistance to gentamicin, erythromycin, tetracycline, methicillin, pencillin G, nalidixic acid, co-trimoxazole, ampicillin, cefotaxime, lincomycin, kanamycin and amoxiclav was found to be 66.67%, 41.67%, 25%, 25%, 16.67%, 16.67%, 16.67%, 16.67%, 8.34%, 8.34%, 8.34% and 8.34%, respectively. All the isolates of the current study were 100% sensitive to amoxycillin/sulbactum, ceftriaxone, cefoperazone/sulbactum, ceftiofur, cloxacillin, ciprofloxacin, enrofloxacin, levofloxacin and tetracycline (30 µg/disc). This assumption is supported by the reports of Marru *et al.* (2013) [6]. They reported that, chloramphenicol followed by sulfamethoxazole and tetracycline were the most effective antibiotics whereas gentamicin and vancomycin were ineffective. Hung *et al.* (2020) [5] reported that cephalexin, cefotaxime, ceftriaxone, of loxacin, pefloxacin, ciprofloxacin, and enrofloxacin were active against *P. multocida* while amoxicillin and tetracycline were inactive. In contrary to our study, Tigga *et al.* (2014) [9] reported high sensitivity of the isolates to amoxicillin, gentamicin, enrofloxacin, sulphadiazine, ofloxacin, trimethoprim, chloramphenicol and oxytetracycline and resistance to ceftizoxim, cephalexin and cloxacillin. Furian *et al.* (2016) [4] reported high sensitivity to gentamicin and amoxicillin and stated that these are the most effective antibiotics against *P. multocida*.

Table 1: Antibiogram profile of *P. multocida* isolates

S. No	Name of the Antibiotic & Concentration (µg/disc)	Sensitive (%)	Resistant (%)
1	Amoxycillin / Sulbactum (AMS 30/15)	100	0
2	Ceftriaxone (CTR 30)	100	0
3	Cefoperazone / Sulbactum (CFS 75/30)	100	0
4	Ceftiofur (EFT 30)	100	0
5	Cloxacillin (COX30)	100	0
6	Ciprofloxacin (CIP 5)	100	0
7	Enrofloxacin (EX 5)	100	0
8	Levofloxacin (LE 5)	100	0
9	Tetracycline (TE 30)	100	0
10	Amoxyclav (Amoxicillin/Clavulanic acid) (AMC 20/10)	91.66	8.34
11	Kanamycin (K 30)	91.66	8.34
12	Lincomycin (L 15)	91.66	8.34
13	Cefotaxime (CTX 5)	91.66	8.34
14	Ampicillin (AMP 10)	83.33	16.67
15	Co-Trimoxazole (Trimethoprim/Sulphamethoxazole) (COT 25)	83.33	16.67
16	Nalidixic Acid (NA 30)	83.33	16.67
17	Penicillin G (P 1 Unit)	83.33	16.67
18	Methicillin (MET 5)	75.00	25.00
19	Tetracycline (TE 10)	75.00	25.00
20	Erythromycin (E 15)	58.33	41.67
21	Gentamicin (GEN 10)	33.33	66.67

Fig 1: Isolate showing resistance to Cotrimoxazole

4. Conclusions

In conclusion, the present investigation described the prevalence of *P. multocida* and antibiotic sensitivity pattern of *P. multocida* isolates from pneumonic cases of sheep and goat. The phenotypic and molecular assays have confirmed that *P. multocida* is one of the etiological agents identified from pneumonic cases in the study area. The results of antibiotic sensitivity revealed that the potential antibiotics against *P. multocida* were amoxycillin/sulbactum, ceftriaxone, cefoperazone/sulbactum, ceftiofur, cloxacillin, ciprofloxacin, enrofloxacin, levofloxacin and tetracycline. As the isolates were reported to be resistant for many of the antibiotics, a prior ABST is necessary before going for treatment against *P. multocida*. Besides, continuous outbreak monitoring and surveillance of antimicrobial susceptibility is indispensable to decide on the drug of choice attributable to the development of multidrug-resistant strains. Therefore, the current findings suggest further comprehensive studies to investigate strain distribution, the antigenic relationship among strains to understand the molecular epidemiology, and other bacterial pathogens associated with bronchopneumonia in small ruminants at the national level to design a cost-effective control strategy.

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