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Antibiogram of bacterial isolates recovered from sheep with nasal discharge

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Abstract
Small ruminant rearing is backbone of livestock owners' economic status in rural areas of India. Among small ruminants, sheep is reared mostly for wool, milk and chevon. Most of the owners keep flocks of sheep confined in a smaller area having direct physical contact with each other. This leaves a possibility of quicker spread of diseases. Respiratory tract diseases are common among sheep in India with nasal discharge as chief complaint. People frequently use antibiotics haphazardly consulting veterinarians when they observe nasal discharge leading to resistance development. Hence, the present study was undertaken to investigate existing status of antibiogram of bacterial isolates recovered from privately-owned sheep having nasal discharge. The bacteria isolated from nasal swabs of sheep having nasal discharge included *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Klebsiella* spp. and *Salmonella* spp. Mixed presence of two different isolates was observed highest for *Staphylococcus* spp. + *Escherichia coli* followed by *Staphylococcus* spp. + *Klebsiella* spp., *Streptococcus* spp. + *Escherichia coli*, *Staphylococcus* spp. + *Salmonella* spp. and *Streptococcus* spp. + *Klebsiella* spp. All the bacterial isolates were subjected to antibiotic sensitivity test (ABST) using multiple antibiotics. Overall, the bacterial isolates recovered from sheep having nasal discharge as a clinical symptom showed variable sensitivity to antimicrobials such as Gentamicin, Ciprofloxacin, Streptomycin, Clotrimoxazole and Chloramphenicol. Comparatively lesser number of isolates showed sensitivity to Tetracycline, Cefixime, Erythromycin and Ampicillin/Sulbactam combination. All isolates were found to be resistant against Penicillin G, Ampicillin and Cefotaxime. The details have been described in the present paper.

Keywords: Antibiogram, nasal bacteria, sheep, respiratory diseases

Introduction
The current era has seen tremendous advancements in diagnostics and therapeutics of different diseases livestock animals such as cattle, buffalo, sheep and goat. India also holds a respectable position in terms of number of such animals and production (e.g., milk production and meat production). This puts responsibilities on the shoulders of the stakeholders, researchers, and academicians to evaluate existing lacunas in healthcare and management of such animals.

India holds a reputable position with regards to number of production animals such as cattle, buffalo, sheep and goat. The total livestock population in India as per the 20th Livestock Census-2019 is 535.78 million while total milk production is reported to be 187.7 million tons (MTs) in 2019[1]. Out of all livestock animals, sheep has 74.26 million of population in India[1]. Sheep hold immense importance for rural livestock owners. People generally keep such small ruminants for milk, meat and hairs/wool production. Moreover, rural scenario on small ruminant keeping shows a greater number of owners rearing having one to more than 500 animals at a single farm/flock (Picture-1). Additionally, most of the rural areas do not have standard housing, management and hygienic conditions for such animals. It is also a common scenario that small ruminants are confined in a smaller area leaving a possibility of quicker spread of diseases.

Picture 1: Privately owned farms of sheep and goats showing different types of houses, floors, different hygiene status and close/direct contact in-between animals

Sheep suffer from a wide range of systemic infectious or non-infectious diseases where diseases of respiratory tract are fairly common entities reported at veterinary hospitals. As mentioned earlier, close proximity between animals, environmental factors, housing practices, availability of veterinary healthcare services, trained manpower, lack of knowledge, inappropriate disease prevention strategies etc. affect the spread of respiratory diseases. The most common respiratory tract diseases involve rhinitis, laryngitis, tracheitis, bronchitis, tracheobronchitis, pneumonia, aspiration pneumonia, nasal bots etc. which are generally accompanied by symptoms such as nasal discharge, sneezing, coughing, open mouth breathing, dyspnea etc. Here, nasal openings or nostrils act as common portal of entry for some infectious pathogens (e.g., bacteria, fungus and virus). Unattended cases of nasal discharge may lead to further spread of the infection to lower respiratory tract which makes the treatment difficult in later stage irrespective of underlying etiology [2, 3]. Hence, it is important to collect information on common nasal bacteria present in clinical cases of sheep having nasal discharge as clinical manifestation (Picture-2).

Picture 2: Sheep with nasal discharge

Additionally, the present era is facing challenges of antimicrobial resistance (AMR). It is a common perception that rural sheep owners with nomadic lifestyle and scattered distribution often tend to use commonly available veterinary drugs with or without consulting veterinarians at home. Hence, it is also possible that owners would treat cases having nasal discharge by use of antibiotics at home which leaves a possibility of AMR and necessitates evaluation of sensitivity of commonly available antibiotics against bacterial pathogens recovered from nasal discharge at field level. Hence, investigations pertaining to existing bacterial pathogens responsible for various diseases and evaluation of their antibiogram is important.

Materials & Methods

The study was carried out at the Postgraduate Institute of Veterinary Education & Research (PGIVER) and Veterinary Hospital of Kamdhenu University, Rajpur (Nava), Himmatnagar in collaboration with (a) Central Diagnostic Laboratory at Polytechnic in Animal Husbandry, Kamdhenu University, Rajpur (Nava), Himmatnagar, (b) Private farms/flocks of sheep in villages nearby campus (*viz.*, Rajpur, Kesharpura Kampa and Khed). The study was carried out from September-2021 to March-2022. Total 60 animals were included following two categories where both categories had 30 animals of each (sheep; irrespective of age, breed and sex). [I] Category-A (Clinically healthy animals; 30 sheep) [II] Category-B (Animals with nasal discharge/respiratory symptoms; 30 sheep). Nasal swabs were collected as per methods described by Markey *et al.* (2014) [4] from sheep (30 healthy, 30 with nasal discharge) included under Categories (A) and (B) [Picture-3].

Picture 3: Collection of nasal swab from a sheep

All the nasal swabs were subjected to bacteriological cultural isolation on general/non-specific media as per methods described by Koneman *et al.* (2012) [5], Thairu *et al.* (2014) [6], Tille (2017) [7], Markey *et al.* (2014) [4] and Mondal (2019) [8]. Bacterial colonies grown on general/non-specific media were collected and subjected to bacteriological cultural isolation on specific isolation media. Bacterial colonies grown on general and specific media were taken on clean microscopic slide using sterile platinum loop to perform staining procedures by use of Gram's stain and Ziehl-Neelsen (ZN) stain for bacterial identification as per methods described by Tille (2017) [7], Markey *et al.* (2014) [4] and Mondal (2019) [8]. The bacterial isolates recovered from nasal swabs were subjected to antibiotic sensitivity test (ABST) using different antibiotic discs as per methods described by Tille (2017) [7], Markey *et al.* (2014) [4] and Mondal (2019) [8] to observe antibiogram of bacteria in cases with nasal discharge as a clinical symptom. Data generated through the study requiring statistical analysis were subjected to suitable statistical methods described by Snedecor and Cochran (1990) [9].

Results and Discussion

Bacterial isolates recovered from nasal swabs of healthy sheep (Category-A)

The most consistent bacteria isolated from nasal swabs of clinically healthy sheep (n=30) was *Staphylococcus* spp. (10.00%; 03/30) followed by *Escherichia coli* (06.67%; 02/30) and *Streptococcus* spp. (03.33%; 01/30). *Klebsiella* spp. and *Salmonella* spp. could not be isolated from healthy

sheep. Mixed presence of *Staphylococcus* spp. + *Escherichia coli* and *Streptococcus* spp. + *Escherichia coli* was observed in similar numbers of healthy sheep (03.33%; 01/30, each).

Bacterial isolates recovered from nasal swabs of sheep having nasal discharge (Category-B)

The most consistent bacteria isolated from nasal swabs of sheep having nasal discharge was *Staphylococcus* spp. (86.67%; 26/30) followed by *Streptococcus* spp. (73.33%; 22/30), *Escherichia coli* (63.33%; 19/30), *Klebsiella* spp. (36.67%; 11/30) and *Salmonella* spp. (13.33%; 04/30). Mixed presence of two different isolates was observed highest for *Staphylococcus* spp. + *Escherichia coli* (46.67%; 14/30) followed by *Staphylococcus* spp. + *Klebsiella* spp. (30.00%; 09/30), *Streptococcus* spp. + *Escherichia coli* (16.67%; 05/30), *Staphylococcus* spp. + *Salmonella* spp. (13.33%; 04/30) and *Streptococcus* spp. + *Klebsiella* spp. (06.67%; 02/30).

Antibiogram of bacterial isolates from sheep having nasal discharge

In the present study, antibiogram of bacterial isolates recovered from nasal swabs of sheep and goats having nasal discharge as a clinical symptom was observed by performing ABST using different antibiotics. The antibiogram was interpreted in terms of reducing sensitivity towards different antibiotics in percentage.

Antibiogram of bacterial isolates recovered from sheep having nasal discharge

Amongst *Staphylococcus* spp. isolates (n=26), all isolates were found sensitive to high concentration of Gentamicin (120 mcg; 100.00%; 26/26) followed by low concentration of Gentamicin (10 mcg; 96.15%; 25/26); Ciprofloxacin (92.31%; 24/26); Streptomycin (73.08%; 19/26); Cotrimoxazole (53.85%; 14/26); Chloramphenicol (50.00%; 13/26); Cefixime (26.92%; 07/26); Tetracycline (11.54%); Ampicillin/Sulbactam and Erythromycin (03.85%; 01/26, each). These isolates were resistant to Ampicillin, Cefotaxime and Penicillin G.

Amongst *Streptococcus* spp. isolates (n=22), all isolates were found sensitive to high concentration of Gentamicin (120 mcg; 100.00%; 22/22) followed by low concentration of Gentamicin (95.45%; 21/22); Ciprofloxacin and Streptomycin (72.73%; 16/22, each); Chloramphenicol (45.45%; 10/22); Cotrimoxazole (36.36%; 08/22); Cefixime (13.64%; 03/22) and Tetracycline (04.55%; 01/22). These isolates were resistant to Ampicillin, Ampicillin/Sulbactam, Cefotaxime, Erythromycin and Penicillin G.

Amongst *Escherichia coli* isolates (n=19), all isolates were found sensitive to high concentration of Gentamicin (120 mcg; 100.00%; 19/19) followed by low concentration of Gentamicin (94.74%; 18/19); Streptomycin (84.21%; 16/19); Chloramphenicol (10.53%; 02/19); Cefixime and Cotrimoxazole (05.26%; 01/19, each). These isolates were resistant to Ampicillin, Ampicillin/Sulbactam, Cefotaxime, Ciprofloxacin, Erythromycin, Penicillin G and Tetracycline.

Amongst *Klebsiella* spp. isolates (n=11), all isolates were found sensitive to high (120 mcg) and low (10 mcg) concentration of Gentamicin (100.00%; 11/11, each) followed by Ciprofloxacin (90.91%; 10/11) and Cotrimoxazole (63.64%; 07/11). These isolates were resistant to Ampicillin, Ampicillin/Sulbactam, Cefixime, Cefotaxime, Chloramphenicol, Erythromycin, Penicillin G, Streptomycin and Tetracycline.

Amongst *Salmonella* spp. isolates (n=04), all isolates were found sensitive to high concentration of Gentamicin (120 mcg; 100.00%; 04/04) followed by low concentration of Gentamicin (75.00%; 03/04); Ciprofloxacin and Cotrimoxazole (50.00%; 02/04, each). These isolates were resistant to Ampicillin, Ampicillin/Sulbactam, Cefixime, Cefotaxime, Chloramphenicol, Erythromycin, Penicillin G, Streptomycin and Tetracycline.

Table 1: Bacterial isolates recovered from healthy sheep (n=30)

Bacteria	Sheep	
	n=30	%
Staphylococcus spp.	3	0.00
Streptococcus spp.	1	3.33
Escherichia coli	2	6.67
Klebsiella spp.	0	0.00
Salmonella spp.	0	0.00
Staphylococcus spp. + *Escherichia coli*	1	3.33
Staphylococcus spp. + *Klebsiella* spp.	0	0.00
Streptococcus spp. + *Escherichia coli*	1	3.33
Streptococcus spp. + *Klebsiella* spp.	0	0.00

Table 2: Bacterial isolates recovered from sheep having nasal discharge as a clinical symptom (n=30)

Bacteria	Sheep	
	n=30	%
Staphylococcus spp.	26	86.67
Streptococcus spp.	22	73.33
Escherichia coli	19	63.33
Klebsiella spp.	11	36.67
Salmonella spp.	4	13.33
Staphylococcus spp. + *Escherichia* coli	14	46.67
Staphylococcus spp. + *Klebsiella* spp.	9	30.00
Streptococcus spp. + *Escherichia coli*	5	16.67
Streptococcus spp. + *Klebsiella* spp.	2	6.67
Staphylococcus spp. + *Salmonella* spp.	4	13.33

Table 3: Antibiogram of bacterial isolates recovered from sheep (n=30) showing sensitive results against commonly used antibiotic drugs

Antibiotic	*Staphylococcus* spp. (n=26)		*Streptococcus* spp. (n=22)		*Escherichia coli* (n=19)		*Klebsiella* spp. (n=11)		*Salmonella* spp. (n=4)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Ampicillin (AMP; 25 mcg)	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Ampicillin/Sulbactam (A/S; 10/10 mcg)	1	3.85	0	0.00	0	0.00	0	0.00	0	0.00
Cefixime (CFM; 5 mcg)	7	26.92	3	13.64	1	5.26	0	0.00	0	0.00
Cefotaxime (CTX; 30 mcg)	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Chloramphenicol (C; 30 mcg)	13	50.00	10	45.45	2	10.53	0	0.00	0	0.00
Ciprofloxacin (CIP; 5 mcg)	24	92.31	16	72.73	0	0.00	10	90.91	2	50.00
Cotrimoxazole (COT; 25 mcg)	14	53.85	8	36.36	1	5.26	7	63.64	2	50.00
Erythromycin (E;15 mcg)	1	3.85	0	0.00	0	0.00	0	0.00	0	0.00
Gentamicin (GEN; 10 mcg)	25	96.15	21	95.45	18	94.74	11	100.00	3	75.00
Gentamicin (HLG; 120 mcg)	26	100.00	22	100.00	19	100.00	11	100.00	4	100.00
Penicillin G (P; 10 units)	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Streptomycin (S; 10 mcg)	19	73.08	16	72.73	16	84.21	0	0.00	0	0.00
Tetracycline (TE; 30 mcg)	3	11.54	1	4.55	0	0.00	0	0.00	0	0.00

Conclusion

Nasal swabs from sheep having nasal discharge as a clinical symptom revealed bacterial isolates of *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Klebsiella* spp. and *Salmonella* spp. at different rates. Overall, variable sensitivity to antimicrobials such as Gentamicin, Ciprofloxacin, Streptomycin, Clotrimoxazole and Chloramphenicol was observed. Complete resistance was observed against Penicillin G, Ampicillin and Cefotaxime. These findings show alarming issue of antimicrobial resistance among free-living flocks of sheep in India. Similar antibiograms can be studied in larger areas covering more number of animals in different geographical regions to assess existing status of resistance of bacteria against commonly used antibiotics.

Conflict of Interest

Authors declare no conflict of interest with regards to funding. All the cases were included in the study after obtaining owners' consent. The project was approved and recommended by the advisory committee and Director of Research & Dean PG Studies.

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