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Research Article

BACTERIAL ISOLATES AND THEIR ANTIBIOGRAM FROM BUFFALOES WITH BOVINE RESPIRATORY DISEASE

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ABSTRACT

The present study was conducted for isolation, identification and antibiotic sensitivity of the bacteria isolated from buffaloes affected with respiratory disease. The nasal swabs were collected from 37 buffaloes brought for the treatment to the Veterinary Clinical Complex with a history and clinical signs of anorexia, fever, dyspnoea, coughing, nasal discharge and abnormal lung sound on auscultation of thoracic area. On the basis of morphology, staining, cultural characteristics; out of total 46 isolated organisms, *Staphylococcus* (23), *Streptococcus* (11), *Klebsiella* (6), *E. coli* (5) and *Pseudomonas* (1) were found singly as well as in mixed form (14 cases). *Staphylococci* spp. was found highly sensitivity to ofloxacin, chloramphenicol and co-trimoxazole while *Streptococci* spp. isolates showed maximum sensitivity towards moxifloxacin and amikacin. The results of this study indicated that chloramphenicol and co-trimoxazole may be preferred in clinical cases of pneumonia in buffalo.

Key words: Buffaloes, bovine respiratory disease, culture, bacterial isolates, antibiotic sensitivity, chloramphenicol

Bovine respiratory disease (BRD) is one of the most common causes of morbidity and mortality in cattle (Murray *et al*., 2017). It is a multi-factorial disease involving infectious agents, compromised host immune system and environmental factors (Grissett *et al*., 2015). The viral pathogens include bovine herpesvirus type 1 (BHV-1), parainfluenza-3 virus (PI-3), bovine viral diarrhea virus (BVDV) and bovine respiratory syncytial virus (Grissett *et al*., 2015). The bacterial pathogens most frequently associated with BRD are *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni* and *Mycoplasma bovis* (Griffin *et al*., 2010). One of the most important factors leading to relapses of BRD in cattle is delayed diagnosis and treatment (Radostits *et al*., 2007). Hence, the present research work was undertaken to identify the bacterial pathogens from respiratory tract of buffaloes affected with BRD and to study the antibiogram of bacterial isolates in order to select the most appropriate antimicrobial to control respiratory disease in buffaloes.

MATERIALS AND METHODS

Animals and Sample Collection: Thirty seven buffaloes aged between 3-8 years suffering with respiratory disease were selected at VCC, LUVAS for this investigation based on clinical signs like anorexia or inappetance, fever, dyspnoea, coughing, nasal discharge, abnormal lung sounds on auscultation of thoracic area and radiological examination. Nasal swabs were collected by inserting the sterilized cotton swabs directly into the nasal cavity aseptically.

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Bacterial Culture Examination: Bacterial isolation was performed using 5% defibrinated sheep blood agar (BA), MacConkey's lactose agar (MLA), nutrient agar (NA) and Eosin methylene blue (EMB) following method of Carter *et al*. (1995). After inoculating aseptically, the plates were incubated at 37°C overnight in incubator. Bacterial isolates were collected from these animals.

Antimicrobial Sensitivity Test: Antimicrobial susceptibility testing was performed using the disk diffusion (Quinn *et al*., 2004) method. Kanamycin (30 µg), Enrofloxacin (10 µg), Moxifloxacin (5 µg), Cefoperazone (75 µg), Cefuroxime (30 µg), Tetracycline (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Co-trimoxazole (1.25/23.75 µg), Ofloxacin (5 µg), Amikacin (30 µg), Amoxyclav (30 µg), Clindamycin (2µg), Ceftriaxone+Tazobactum (30/10 µg), Ampicillin+Cloxacillin (10 µg) (Himedia) antimicrobial disc were used to test the sensitivity and resistance pattern of bacterial isolates from nasal swab. The sensitivity was observed on the basis of zone size interpretation chart, provided by the manufacturer.

RESULTS AND DISCUSSION

The cultural examination of nasal swabs of buffaloes affected with respiratory diseases revealed 28

(75.67%) of 37 animals to be positive for either single or multiple isolates, with isolation of 46 different isolates. Different organisms isolated were *Staphylococci* (62.16%), *Streptococci* (29.72%), *Klebsiella* (16.21%), *E. coli* (13.51%) *and Pseudomonas* (2.70%) either single or mixed infection (14 cases) as shown in Table 1.

Our study showed that *Staphylococci* spp., *Streptococci* spp., *Klebsiella* spp. and *E. coli* were major pathogens isolated in BRD affected animals. Similar findings were reported by Mahmud *et al*. (2016). In contrast, Mohammadi *et al*. (2006) isolated organisms as *Staphylococcus spp.* (2.31%), *Streptococcus spp.* (3.08%), *Pseudomonas spp.* (2.31%) and *E. coli* (3.84%) from the cases of calf enzootic pneumonia. Aslan *et al*. (2002) also found on contrary *M. haemolytica* responsible for the highest isolation (25%), followed by *Klebsiella pneumoniae* (20%), β-haemolytic *Streptococci* (10%), *Staphylococcus* spp. (5%), *E. coli* (5%), on microbiological examination of the bronchoalveolar lavage samples.

Other researchers found *P. multocida, M. haemolytica and Histophilus somni* as the most common bacterial agents isolated from nasal swabs/lung sample (Onat *et al*. 2010; Portis *et al.* 2012; Garch *et al.* 2016). The difference in bacterial isolation could be due to that in present study most of the samples were collected from upper respiratory tract *i.e.* nasal cavity*;* moreover area difference may be there, as in this part of the country, vaccination against pasteurellosis is a regular practice. It may be due to work reported by above researchers has been conducted in cattle, whereas present work was in buffalo. This aspect may be explored in future studies.

A total of 46 isolates collected from nasal swabs were used for disc sensitivity testing. Overall sensitivity irrespective of isolates, maximum sensitivity was shown by chloramphenicol and co-trimoxazole (75.00%) followed by ofloxacin (72.22%), amikacin (62.22%), enrofloxacin (48.89%), cefuroxime (47.22), cefoperazone (44.44%), ciprofloxacin (41.66%), tetracycline (38.88%), moxifloxacin (35.55%), kanamycin (30.55%), amoxyclav (19.44%), clindamycin (16.67%), ceftriaxone+tazobactum (16.67%) and ampicillin+cloxacillin (5.56%) as shown in Table 2.

Chloramphenicol was determined to be highly sensitive antimicrobial agent (Diker *et al*., 1994; Kalhoro *et al*., 2010) while co-trimoxazole was determined to be highly sensitive antimicrobial agent (Welsh *et al*., 2004) in cases of BRD and respiratory diseases of animals. On contrary, Mahmud *et al*. (2016) revealed that *Staphylococcus* spp. isolated from nasal and lung swab from healthy and sick cattle were highly sensitive to erythromycin, ampicillin, amoxicillin while *E. coli were* highly sensitive to ciprofloxacin, norfloxacillin, enrofloxacin.

Present study also demonstrated that chloramphenicol and co-trimoxazole were the most sensitive against isolated bacteria. It may be because of less usage of these antimicrobials in the field.

Effective treatment and control of respiratory disease is determined by rapid and accurate identification of disease. The variation in the sensitivity of antimicrobials of the respiratory isolates may be due to choice and the indiscriminate use of antimicrobials in different stage of disease and in different locations. The result of present study indicated that culture sensitivity may be obtained

Table 1

Relative frequency of organisms isolated from nasal swabs of buffaloes (n=37) affected with respiratory disease

	Total	
Total animals		37
Positive on cultural examination		28 (75.67%)
Negative on cultural examination	9 (24.32%)	
Total bacterial isolates		46
Staphylococci spp.	(single infection+ mixed infection)	23 (10+13)
Streptococci spp.	(single infection + mixed infection)	11 (2+9)
Klebsiella spp.	(single infection + mixed infection)	6 (2+4)
E. coli	(single infection + mixed infection)	5 (0+5)
Psedomonas spp.	(single infection + mixed infection)	1 (0+1)
Animals with single infection		14 (37.83%)
Animals with mixed infection*		14 (37.83%)

**Staphylococci+Streptococci (6), Staphylococci+Klebsiella (2), Staphylococci+E. coli (1), Streptococci+E.coli (1), Staphylococci+Pseudomonas (1), Staphylococci+Klebsiella+E. coli (1), Staphylococci+Streptococci+E.coli (1)* and *Staphylococci+Streptococci+Klebsiella+E.coli (1)*

Table 2

Antimicrobial sensitivity (%) of various isolates from buffaloes (n=37) affected with respiratory disease *in-vitro*

Group of antimicrobials	Antimicrobials	*Staphylococci* spp. (23)	*Streptococci* spp. (11)	*Klebsiella spp.* (6)	*E. coli* (5)	*Pseudomonas spp.* (1)	Overall ranking
Aminoglycosides	Kanamycin	44.44	28.57	0.00	0	0	10 (30.55%)
	Amikacin	73.91	72.27	33.33	0	100	3 (62.22%)
Amphenicols	Chloramphenicol	77.77	57.14	100	75	0	1 (75.00%)
Cephalosporins	Cefoperazone	52.17	63.63	16.66	0	0	6 (44.44%)
	Cefuroxime	61.11	57.14	33.33	0	0	5 (47.22%)
	Ceftriaxone + Tazobactum	16.66	28.57	0.00	25	0	12 (16.67%)
Lincosamides	Clindamycin	16.66	28.57	16.66	0	0	12 (16.67%)
Penicillins	Amoxyclav	22.22	28.57	16.66	0	0	11 (19.44%)
	Ampicillin + Cloxacillin	5.55	0.00	16.66	0	0	13 (5.56%)
Quinolones	Ofloxacin	83.33	28.57	100	50	100	2 (72.22%)
	Enrofloxacin	60.86	54.54	16.66	0	100	4 (48.89%)
	Moxifloxacin	34.78	72.27	0.00	0	0	9 (35.55%)
	Ciprofloxacin	50.00	14.28	66.66	0	100	7 (41.66%)
Sulphonamides	Co-trimoxazole	77.77	71.42	100	50	0	1 (75.00%)
Tetracyclines	Tetracycline	55.56	14.28	50.00	0	0	8 (38.88%)

before starting the treatment to avoid antimicrobial resistance or practitioner may adopt shuttle programme with usage of antimicrobials in a particular population instead of prescribing one particular antimicrobial for a long period of time.

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