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Isolation, Identification and Antibiogram Studies of *Pasteurella multocida* Isolated from Sheep and Goats in Siwa Oasis

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Abstract: A total of seven hundred and fifty nasal swabs were collected, 523 from apparently healthy and 227 from diseased sheep and goats for bacteriological examination. Another 303 grossly pneumonic lungs were collected from dead and slaughtered animals. The isolation rate of *P. multocida* was 10.16% (107 out of 1053 samples). All the isolates were confirmed through biochemical characterization. The antibiogram profile of *P. multocida* isolates revealed that the potential antibiotics against *P. multocida* were Erythromycin 93.3%, Rifampicin 90% and Ciprofloxacin 86.6%, where the less effective antibiotics were Oxytetracycline 26.6% and Amoxicillin13.3%.

Key words: Sheep · Isolation · *P. multocida* and Antibiogram

INTRODUCTION

Pasteurella multocida is one of the most common pathogens of sheep and goats throughout the world where outbreaks usually lead to high mortality and great economic loss to the ruminant industry [1, 2]. It is a commensal germ in the upper respiratory tract of many animals [3, 4].

The Gram-negative bacterium *Pasteurella multocida* constitutes a heterogeneous species associated with a wide range of diseases in many animals. Isolates are classified into five groups based on capsular antigen (Capa, B, D, E and F) [5].

Pasteurellosis caused by *Pasteurella multocida* is an acute septicaemic disease characterized by high morbidity and mortality in cattle, sheep, goat and poultry resulting in severe economic losses [6].

Other pathological conditions were induced by *P. multocida* as atrophic rhinitis, mastitis, peritonitis, meningitis, ear and eye infections as well as cancer that could be induced due to effect of *P. multocida* toxins [7].

Individual animals may be affected or outbreaks can occur, with losses due to mortality, reduced production-poor or delayed growth in fattening lambs with a greater feed consumption for finishing and ill thrift and poor milk production in adult ewes in addition to treatment costs [8].

This study was conducted for isolation and identification of the *Pasteurella multocida* from sheep and goats in Siwa Oasis with regard to their antibiogram activity.

MATERIALS AND METHODS

Samples: Seven hundred and fifty nasal swabs were collected including 336 and 146 from apparently healthy sheep and goats and 187 and 81 from diseased sheep and goats, respectively. Another 303 gross lung samples were collected from slaughtered animals as 51 and 15 pneumonic lung samples and and 192 and 45 from grossly apparently healthy lungs from sheep and goats, respectively (Table 1). All samples were transferred in Brain heart infusion broth medium to laboratory, plated onto 10% sheep blood agar (SBA) and incubated at 37°C overnight.

Clinical Symptoms of Studied Animals: Nasal discharge, Coughing, hyperpnea, Pyrexia (Fever might reach 40°C in case of septicaemia).

Postmortem Lesions of Lung Samples: Purulent bronchopneumonia accompanied with pleurisy, Serous or fibrinous fluid in the pleural and pericardium cavity.

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Table 1: Total number of examined animals

	Living		Slaughtered or dead		
Species	Diseased	App. healthy	Diseased	App. healthy	Total
Sheep	187	336	51	192	766
Goats	81	146	15	45	287
Total	268	482	66	237	1053

Table 2: Antimicrobial disks used in the sensitivity test

Antimicrobial disks	Symbol	Concentration (µg)
Oxytetracycline	OT	30
Chlormphenicol	C	30
Erythromycin	E	15
Cephalexin	CP	30
Spectinomycin	Sp	100
Ciprofloxacin	CF	5
Gentamycin	G	10
Amoxycillin	AM	20
Neomycin	N	30
Nalidixic acid	NA	30

Primary Isolation: Culture and morphological identification of suspected *P. multocida* isolates were carried out according to standard biochemical tests [9]. The cells were stored in Brain Heart Infusion (BHI) with 30% glycerol at-70°C.

Antibiotic Sensitivity Testing of the Isolates: The sensitivity of isolates was tested against the following antimicrobial disks (Oxoid) agents as shown in Table 2 using Disk diffusion technique [10].

RESULTS

***P. multocida* Isolation:** Following incubation at 37°C for 24 h, small glistening mucoid dewdrop-like colonies were appeared on blood agar medium. Microscopic observations revealed that all isolates were Gram-negative coccobacilli and biochemical analysis confirmed that the isolates were indole, catalase and oxidase positive but, citrate, MR, VP and gelatin liquefaction negative. Growth test on MacCankey agar was negative and the isolates showed no motility and non-hemolytic effects on blood agar. Bipolar organisms were shown when stained with Leishman's stain as shown in Fig. 1.

Isolation Rate of *P. multocida* from Apparently Healthy and Diseased Sheep Cases: The examination of 766 samples collected from apparently healthy (528) and diseased (238) (Living, slaughtered or dead) sheep for *P. multocida* revealed the isolation of 67 positive isolates with recovery rate of 8.7%. It appeared that the highest rate of isolation was found among the samples from slaughtered and dead animals with the isolation of 28 *P. multocida* out of 243 samples (11.5%). The isolation rate was comparatively low in samples from living animals (39 out of 523; 7.4%), (Table 3). The rate of *P. multocida* isolation in the diseased sheep reached (36 out of 238; 15.1%) which was comparatively higher than those from apparently healthy ones (31 out of 528; 5.8%) (Table 4).

Isolation Rate of *P. multocida* from Apparently Healthy and Diseased Goat Cases: The examination of 287 samples collected from apparently healthy (191) and diseased (96) (Living, slaughtered or dead) goats for *P. multocida* revealed the isolation of 40 positive isolates with recovery rate of 13.9%. It appeared that the highest rate of isolation was found among the samples from slaughtered and dead animals with the isolation of 13 *P. multocida* out of 60 samples (21.6%). The isolation rate was comparatively low in samples from living animals (27 out of 227; 11.8%), (Table 5). The rate of *P. multocida* isolation in the diseased sheep reached (22 out of 96; 22.9%) which was comparatively higher than those from apparently healthy ones (18 out of 191; 9.4%) (Table 6).

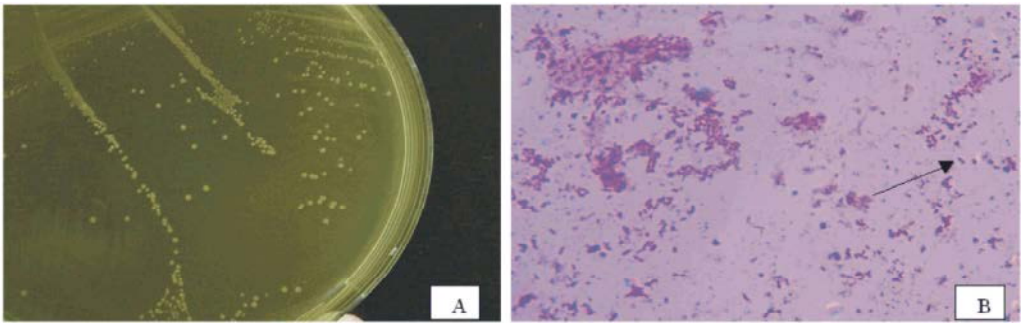

Fig. 1: A: Colonial morphology of *Pasteurella multocida*. B: bipolar organisms on brain heart infusion agar stained with Leishman's stain.

Table 3: The isolation rate of *P. multocida* in apparently healthy and diseased (Slaughtered, dead and living) sheep

	Apparently Healthy			Diseased			Total		
		positive			positive			Positive	
Conditions of the examined animals	No. of samples	No	%	No. of samples	No	%	No. of samples	No	%
Slau. or dead	192	15	7.8	51	13	25.4	243	28	11.5
Living	336	16	4.7	187	23	12.2	523	39	7.4
Total	528	31	5.8	238	36	15.1	766	67	8.7

Table 4: Occurrence of *P. multocida* in different samples of apparently healthy and diseased sheep

	Apparently healthy			Diseased			Total		
		Positive			Positive			Positive	
Type of samples	No of samples	No	%	No of samples	No	%	No of samples	No	%
Nasal swab	336	16	4.7	187	23	12.2	523	39	7.4
Lung tissue	192	15	7.8	51	13	25.4	243	28	11.5
Total	528	31	5.8	238	36	15.1	766	67	8.7

Table 5: The isolation rate of *P. multocida* in apparently healthy and diseased (Slaughtered, dead and living) goats

	Apparently Healthy			Diseased			Total		
		positive			positive			Positive	
Conditions of the examined animals	No. of samples	No	%	No. of samples	No	%	No. of samples	No	%
Slau. or dead	45	8	17.7	15	5	33.3	60	13	21.6
Living	146	10	6.8	81	17	20.9	227	27	11.8
Total	191	18	9.4	96	22	22.9	287	40	13.9

Table 6: Occurrence of *P. multocida* in different samples of apparently healthy and diseased goats

	Apparently healthy			Diseased			Total		
		Positive			Positive			Positive	
Type of sample	No of samples	No	%	No of samples	No	%	No of samples	No	%
Nasal swab	146	10	6.8	45	17	37.7	191	27	14.1
Lung tissue	81	8	9.8	15	5	33.3	96	13	13.5
Total	227	18	7.9	60	22	36.6	287	40	13.9

Table 7: Comparison between sheep and goats in frequency of *P. multocida* in different samples examined

	Sheep			Goats		
		positive			positive	
Type of sample	No of samples	No	%	No of samples	No	%
Nasal swab	523	39	7.4	191	27	14.1
Lung tissue	243	28	11.5	96	13	13.5
Total	766	67	8.7	287	40	13.9

Table 8: Antibiogram of *P. multocida* isolated from different specimens of clinically infected and contact apparently healthy animals

		P. multocida			
		Sensitive		Resist	
Antimicrobial disks	Concentration (µg)	No	%	No	%
Oxytetracycline	30	8/30	26.6	22/30	73.4
Chloramphenicol	30	24/30	80	6/30	20
Erythromycin	15	28/30	93.3	2/30	6.7
Cephalexin	30	25/30	83.3	5/30	16.7
Ampicillin	10	23/30	76.6	7/30	23.4
Ciprofloxacin	5	26/30	86.6	4/30	13.4
Enrofloxacin	10	19/30	63.3	11/30	36.7
Amoxicillin	30	4/30	13.3	26/30	86.7
Rifampicin	5	27/30	90	3/30	10
Amikacin	30	17/30	56.6	13/30	43.4

%: was Calculated according to the number of examined isolates (30)

Frequency of *P. multocida* in different types of examined samples in sheep and goats: In comparison between sheep and goats, the isolation percentage of *P. multocida* isolates from different types of samples was a relatively higher rate in goat samples (13.9%) (Nasal swabs 14.1%, lung tissues 13.5%), than that of sheep (8.7%) (Nasal swabs 7.4%, lung tissues 11.5% (Table 7).

Antibiogram and *P. multocida* Sensitivity: The antibiogram profile of thirty *P. multocida* isolates revealed that the potential antibiotics against *P. multocida* were Erythromycin (28/30) 93.3%, Rifampicin (27/30) 90% and Ciprofloxacin (26/30) 86.6%, where the less effective antibiotics were Oxytetracycline (8/30) 26.6% and Amoxicillin (4/30) 13.3%, as shown in Table 8.

DISCUSSION

Respiratory infection in sheep due to Pasteurella multocida is a crucial contributor to economic losses in the sheep industry in most parts of the world. Pasteurella multocida was considered among the primary etiological agent that incriminated in pneumonia and septicaemia in sheep and goats, Davies *et al.* [10] *Pasteurella multocida* is the causative agent of numerous relevant diseases worldwide like hemorrhagic septicemia in cattle and buffaloes, enzootic bronchopneumonia in cattle and sheep, fowl cholera, snuffles in rabbit, atrophic rhinitis in swine [12].

The present study revealed the occurrence of *P. multocida* in the examined animals 10.16%. The colonial morphology of the isolated strains was proven to be smooth mucoid or rough colony round 1-2ml in diameter, yellowish, glistening, translucent and non haemolytic. There is no growth on MacConkey agar [13]. The microscopical examination proved that it was Gram negative coccobacilli occur singly, in pairs or less frequently in short chain. *P. multocida* showed bipolarity with Leishman's stain [14]. Our study has showed that *p. multocida* inhibited and colonized the nasal passages of apparently healthy and clinically sick sheep and goats (Tables 3, 4, 5 and 6). These results are in agreement with Ikede [15] where he isolated similar bacteria from pneumonic caprine lungs; apparently healthy respiratory tract and nasal cavity of goats [16] and with fewer reports from apparently healthy sheep [17].

Concerning to the rate of *Pasteurella multocida* isolation in sheep and goats, it was 10.16% (107 out of 1053), 67 positive isolates with recovery rate of 8.7% in sheep, as shown in table 3, while it was 40 positive isolates with recovery rate of 13.9% in goats, as shown in table 5. In comparison between sheep and goats, the isolation percentage of *P. multocida* isolates from different types of samples was a relatively higher rate in goat samples (13.9%) (Nasal swabs 14.1%, lung tissues 13.5%), than that of sheep (8.7%) (Nasal swabs 7.4%, lung tissues 11.5%, (Table 7). Shayegh *et al*. [18] isolated *Pasteurella multocida* from goats with recovery rate of 3.5%. These results are nearly similar to that obtained by Shayegh *et al.* [19] who recovered *Pasteurella multocida* from sheep with recovery rate of 9.07% respectively.

Antibiotics are important remedies in modern farm animal production. The use of these chemical agents should be based on an accurate diagnosis since there is an increasing incidence of bacterial resistance to antibiotics in humans. This phenomenon was attributed to the use of anti-microbial drugs in food-producing animals. Also, there is a concern about possible residues in animal products [20].

As shown in Table 8 results of antibacterial sensitivity against *P. multocida* against different broad spectrum antibiotics revealed that the potential antibiotics against p. multocida were Erythromycin 93.3%, Rifampicin 90% and Ciprofloxacin 86.6%, this assumption is supported by the reports of Laxmi *et al.* [21] who added that enrofloxacin and chloramphenicol were the best drugs of choice infection in sheep and goats.

Where the less effective antibiotics were Oxytetracycline 26.6% and Amoxicillin13.3%, while sulphadiazine and cloxacillin were the less effective as reported by Kalorey *et al.* [22].

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