

Table 1 Primer sequences used for PCR amplification for bacterial species causing pneumonia in small ruminants

Bacterial species	Target Gene	Primer	Sequence (5'→3')	Amplicon size (bp)	References
<i>Mannheimia haemolytica</i>	<i>sodC</i>	Forward	AAGGTGGCAAGCTCACAGCAG	230	Ewers (2008)
		Reverse	TGAGTGGTTATCGCCGCCT		
<i>Pasteurella multocida</i>	<i>KMT1</i>	Forward	ATCCGCTATTTACCCAGTGG	457	Townsend et al. (1998)
		Reverse	GCTGTAAACGAACTCGCCAC		
<i>Escherichia coli</i>	<i>uidA</i>	UAL-754	AAAACGGCAAGAAAAAGCAG	147	Bej et al., (1990)
		UAR-900	ACGCGTGGTTACAGTCTTGCG		
<i>Klebsiella pneumonia</i>	<i>gyrA</i>	Forward	CGCGTACTATACGCCATGAACG TA	441	Brise and Verhoef (2001)
		Reverse	CGCGTACTATACGCCATGAACG TAACCGTTGATCACTTCGGTCAG G		
<i>Staphylococcus aureus</i>	<i>nuc</i>	Forward	GCGATTGATGGTGATACGGTT	270	Louie et al. (2002)
		Reverse	AGCCAAGCCTTGACGAACTAAA GC		
<i>Pseudomonas aeruginosa</i>	<i>O-antigen acetylase gene.</i>	PA431CF	CTGGGTGCGAAAGGTGGTTGTTA TC	232	Choi et al. (2013)
		PA431CR	GCGGCTGGTGCGGCTGAGTC		
<i>Proteus mirabilis</i>	<i>zapA</i>	Forward	ACCGCAGGAAAACATATAGCCC	540	Stankowska et al. (2008)
		Reverse	GCGACTATCTTCCGCATAATCA		

Table 2 PCR conditions used for detection of each bacterial species in this study

PCR conditions	<i>M. haemolytica</i>	<i>P. multocida</i>	<i>E. coli</i>	<i>K. pneumonia</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>P. mirabilis</i>
Initial denaturation	95°C-15min	95°C - 4min	94°C for 5min	94°C - 4 min	94°C-5min	95°C-3min	95°C-2min
Denaturation	94°C - 30Sec	95°C -1min	94°C for 10Sec	94°C- 30Sec	94°C -30Sec	95°C-1min	95°C-30 Sec
Annealing	55°C - 45Sec	55°C- 1min	55 for 10Sec	55°C - 40Sec	55°C -30Sec	63°C-30Sec	59°C-30Sec
Extension	72°C - 1min	72°C- 1min	72°Cfor 1min	72°C - 1min	72°C - 1min	72°C-1min	72°C-1min
Amplification	35 cycles	30 cycles	35 cycles	30 cycles	35 cycles	35cycles	30 cycles
Final extension	72°C-10min	72°C -9min	72°C for 10min	72°C- 10min	72°C- 10min	72°C-10min	72°C-5min

Table 3 Total number and percentages of the examined animals and affected animals

Species	Number of examined animal		Affected animals	
	Total number	Total percentage%	Number	Percentage%
Sheep	3667	91.30	218	86.17%
Goat	394	9.70	35	13.83%
Total	4061	100%	253	100%

Table 4 Prevalence of isolated bacterial species from affected pneumonic lungs in sheep and goats (n=253).

No	Bacterial	No. of affected lungs		No. isolated Bacteria with percentage (%)	
		Sheep (218)	Goat (35)	Sheep	Goat
1	<i>Pasteurella multocida</i>	15	3	15 (6%)	3 (4%)
2	<i>Mannhaemia haemolytica</i>	6	2	6 (2.4 %)	2(2.6%)
3	<i>Staphylococcus aureus.</i>	66	9	85(34 %)	30 (40%)
4	<i>Escherichia coli</i>	47	7	55 (22%)	15 (20%)
5	<i>Klebsiella pneumonia</i>	10	3	12 (4.8%)	3 (4%)
6	<i>Pseudomonous spp.</i>	1	1	2 (0.8%)	2 (2.6%)
7	<i>Proteus spp.</i>	5	2	7 (2.8 %)	3 (4%)
8	<i>Staphylococcus aureus</i> and <i>E. coli</i>	40	5	40 (16%)	10 (13.33%)
9	Negative for Bacterial isolation	28	5	28 (11.2)	5 (6.94%)
10	Total	218	35		

Table 5 The biochemical investigation results for all isolated bacteria from pneumonic lungs

Isolated bacteria	Biochemical reactions of isolated bacteria													
	Gram' s reaction	Motility	Catalase	Oxidase	Simmon Citrate	Urease	Indole	Methyl red	Vogas- ,	Lactose	Triple sugar		Iron Agar	
											Slope	Butt	H ₂ S	Gas
<i>P. multocida</i>	G-	-	+	+	-	-	+	-	-	-	N/A	-	-	
<i>M. haemolytica</i>	G-	-	+	+	-	-	-	-	-	+	N	-	-	
<i>S. aureus.</i>	G+	-	+	+	-	-	-	+	+	-	N	-	+	
<i>E. coli</i>	G-	+	+	-	-	-	+	+	-	+	Y/Y	-	-	
<i>K. pneumonia</i>	G-	-	+	-	+	-	-	-	+	+	Y/Y	-	+	
<i>Pseudomonas spp.</i>	G-	+	+	+	+	-	-	-	-	-	R/Y	-	-	
<i>Proteus spp.</i>	G-	+	+	-	-	+	-	+	+	-	R/Y	+	+	

Table 6 Characteristics of isolated bacteria from pneumonic lungs in different culture media.

No.	Isolated bacteria	Blood Agar	MacConkey Agar	Mannitol Salt Agar
1	<i>Pasteurella multocida</i>	Gamma-hemolytic, round smooth or mucoid. Gram negative coccobacilli	No growth	No growth
2	<i>Mannhaemia haemolytica</i>	B-hemolytic, Gram-negative, non-motile, small rod- coccobacilli. Bipolar stained with Gram's stain, smooth and greyish colony (1-2 mm width)	Growth	No growth
3	<i>Staphylococcus aureus</i>	β-heamolytic ,Gram positive cocci	No growth	Golden yellow colonies with yellow zones
4	<i>Escherichia coli</i>	Beta (β) heamolytic, Gram-negative, big, circular, gray and moist colony.	Colonies are circular, moist, smooth and of entire margin, flat and pink colored, lactose fermenting.	No growth
5	<i>Klebsiella pneumonia</i>	Non- hemolytic (gamma-hemolytic), mucoid colonies.	Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped and mucoid colonies	No growth
6	<i>Pseudomonous spp.</i>	Weak alpha hemolysis	Gram-negative, rod shaped, colorless colony due to the lack of lactose fermenting.	No growth
7	<i>Proteus spp.</i>	Gram-negative rod-bacilli, Swarming growth of <i>Proteus</i> on chocolate agar and (gamma-hemolytic) on blood agar, motile, aerobic and facultative anaerobic.	Pale or colorless (NLF) colonies	No growth