Indian Journal of Animal Sciences **91** (11): 921–924, November 2021/Short communication
https://doi.org/10.56093/ijans.v91i11.118129

Genetic diversity of *Pasteurella multocida* isolates of Indian origin

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Received: 15 July 2021; Accepted: 21 July 2021

Keywords: Epidemiological typing, India, MLST, *Pasteurella multocida*

P. multocida is associated with a wide variety of diseases in animals and birds including septicaemia and pneumonic pasteurellosis in sheep and goats (Shivachandra *et al.* 2011). Multilocus sequence typing (MLST) is based on the analysis of nucleotide sequence data from 5–7 housekeeping genes (Maiden *et al.* 1998). An earlier study on *P. multocida* isolates of different host origin revealed many reported and new sequence types (STs) from India (Sarangi *et al.* 2016). In contrast, MLST analysis of Indian Haemorrhagic septicaemia (HS) causing *P. multocida* type B isolates from buffalo origin belonged to a single sequence type (ST122) (Mir *et al.* 2011, Aiswarya *et al.* 2017). The information on the genetic diversity of *P. multocida* isolates from India is limited. In the present study, we report MLST analysis of *P. multocida* isolates of different host origin with a special emphasis on small ruminants.

Twenty-one *P. multocida* isolates from different hosts (Table 1) were selected for the study. Molecular confirmation of isolates was done by species-specific and capsular types-specific PCR (Townsend *et al.* 1998, 2001). MLST analysis of the *P. multocida* strains was carried out using RIRDC *Pasteurella multocida* scheme (http://ukmirror1.pubmlst.org/pmultocida_rirdc/). The final allele-specific consensus nucleotide sequences were derived using BioEdit (Hall 1999). Allelic profiles and isolates information of the RIRDC *P. multocida* scheme (as on 28/04/2018) were retrieved from the PubMLST database (Jolley *et al.* 2004). Concatenated genes in the MLST scheme were used to create phylogeny using Interactive tree of life (iTOL) v3 (Letunic and Bork 2016).

The 21 isolates belonged to serotypes A, B and D. MLST analysis of these isolates represented 11 STs, out of which, five STs were novel. Among these, three STs (ST289, ST290 and ST291) contained two new *adk* alleles (No. 50 and 49) and one *pmi* allele (No. 71), respectively. Other two new STs (ST287 and ST288) were formed based on novel allelic combinations (Table 1). Allelic/isolate information of isolates has been submitted to RIRDC *Pasteurella multocida* database (http://ukmirror1.pubmlst.org/pmultocida_rirdc/).

All the small ruminant isolates were of capsular type A except for one sheep isolate of capsular type B. Type A small ruminant isolates (10 isolates) belonged to 6 STs, which included four novel STs (ST288, ST289, ST290 and ST291) (Table 1) and two previously reported STs from India (ST222 and ST280) (Sarangi *et al.* 2016). Among the four novel STs, three STs (ST288, ST289 and ST290) belonged to a novel clonal complex (CC229). Clonal complex CC229 includes four STs including previously reported ST229 of capsular type F from India (Sarangi *et al.* 2016). Current study also identified ST289 from a pig isolate. Hence, it may be concluded that CC229 includes STs of mostly small ruminant origin, followed by cattle and pigs, belonging to capsular types A and F (Sarangi *et al.* 2016).

In the present study, a sheep isolate exhibits sequence type ST280 belonging to CC129 and the same ST was reported from various avian species in India (Sarangi *et al.* 2016). The lone type B isolate from sheep (isolate 419) was found to be ST122 (Table 1). ST122 has been reported in large and small ruminants from India (Sarangi *et al.* 2016).

A recent study showed that isolates of ovine origin were highly diverse with respect to capsular types and MLST data as compared to abattoir isolates (Einarsdottir *et al.* 2016). All the abattoir isolates belonged to capsular type D, whereas clinical isolates showed capsular types such as A, D or F (Einarsdottir *et al.* 2016). The present study proves the existence of diverse genotypes among capsular type A isolates of small ruminant origin (Table 1).

Among the three isolates from pig, two were of capsular type A and one was capsular type D, belonging to three STs (ST129, ST289 and ST50) (Table 1). This study reported ST129 in one pig and one duck isolate, whereas the same ST was reported previously from a poultry isolate from India (Sarangi *et al.* 2016). ST280, isolated from sheep in the current study and ST129 isolated from pig and duck are genetically related (single locus variants), and belonged

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to clonal complex CC129. Similarly, a duck isolate from southern India shared same sequence type (ST129) with a pig isolate from north-eastern India. Second type A isolate from pig shared the same novel sequence type (ST289) with small ruminant isolates (Table 1).

In the present study, a capsular type D isolate from pig was represented by ST50. In Denmark (Pors *et al.* 2011), Czech Republic and Germany (https://pubmlst.org/), pig isolates which exhibited sequence type ST50 belonged to capsular type A, while pig isolates of Vietnam (Petersen *et al.* 2014), United Kingdom (Hotchkiss *et al.* 2011), Brazil and India (Sarangi *et al.* 2016) belonged to capsular type D.

In our study, ST282 is reported for two poultry isolates (capsular type A). ST282, reported only from India, seems to be strongly associated with poultry hosts (Sarangi *et al.* 2016). Current study also reports a novel sequence type (ST287) recovered from a capsular type D isolate of buffalo origin (Table 1).

Current study reported only one sequence type (ST122) among the capsular type B isolates responsible for causing HS in India. All the type B isolates belonged to four different geographical zones and hence, possibly indicate lesser genetic diversity for type B isolates with respect to host or geographical entity in India (Table 1). Previous reports also show occurrence of only ST122 among the type B strains from India (Mir *et al.* 2011, Sarangi *et al.* 2016, Aiswarya *et al.* 2017).

Concatenated MLST target gene sequences' phylogeny of the ovine isolates proves divergence of Indian isolates (belonging to CC229) as a distinct lineage. Thus, current study proves the identification of novel clonal complex – CC229 – mostly associated withsmall ruminant population as an evolutionarily distinct lineage from other reported ovine STs. Phylogeny of Indian isolates (Fig. 1) proves that

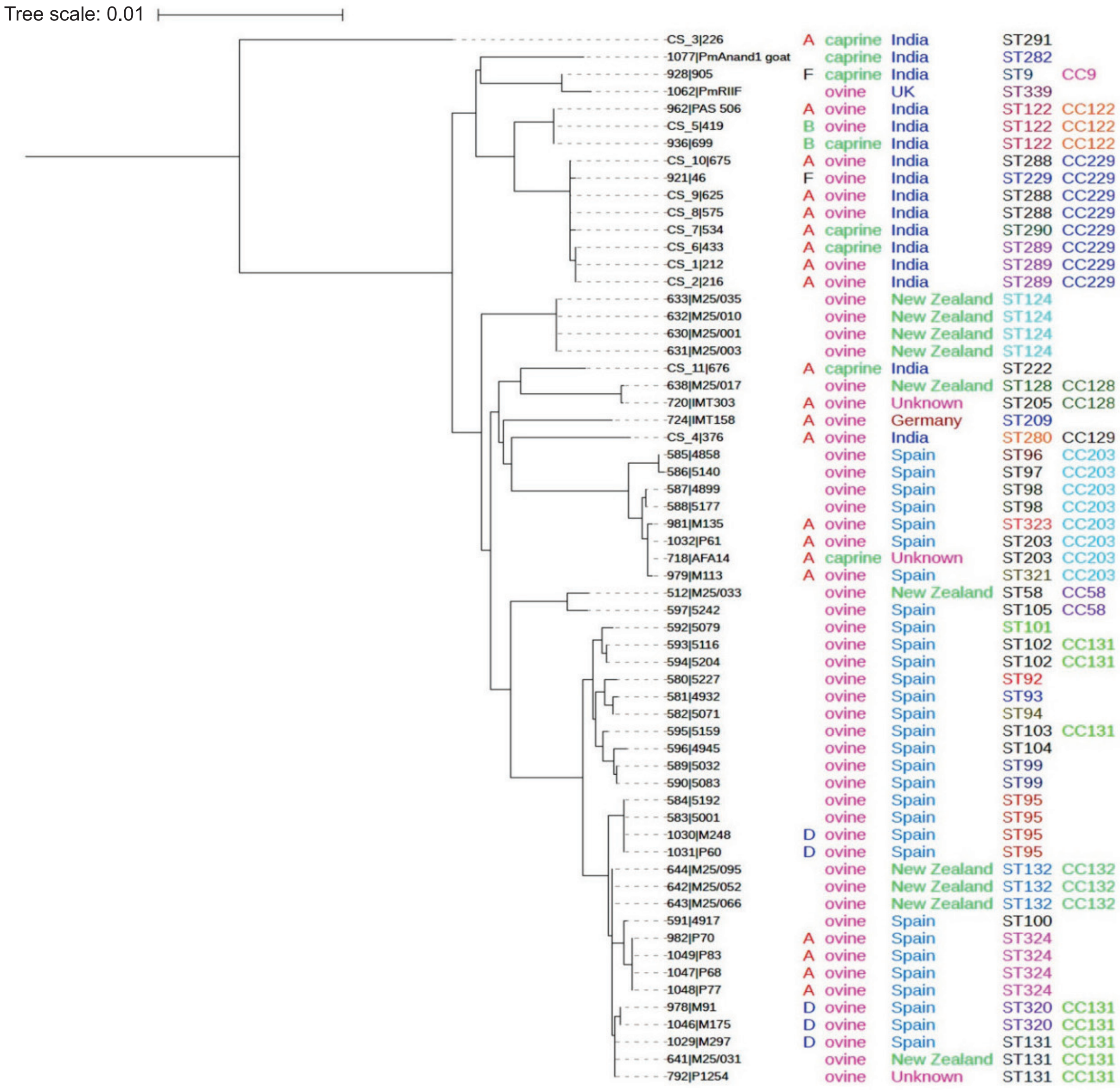

Fig. 1. Phylogenetic analysis of STs of *P. multocida* isolates of small ruminant origin. The phylogenetic tree was generated using concatenated allelic sequences of the MLST gene targets and shows the capsular type, host, country, sequence type and clonal complex details of the strains.

Table 1. *Pasteurella multocida* isolates used for MLST analysis

Isolate	Species	Year of isolation	Place	Capsular serotype	*adk*	*est*	*gdh*	*mdh*	*pgi*	*pmi*	*zwf*	Sequence type	Clonal complex
216	Sheep	2002	Srinagar	A	49*	6	25	4	8	12	17	**ST289**	CC229
212	Sheep	2002	Chennai	A	49*	6	25	4	8	12	17	**ST289**	CC229
376	Sheep	2004	Chennai	A	21	82	20	17	20	26	2	ST280	CC129
419	Sheep	2005	Chennai	B	23	37	2	4	17	21	17	ST122	CC122
625	Sheep	2008	Chennai	A	22	6	25	4	8	12	17	***ST288***	CC229
575	Sheep	2008	Chennai	A	22	6	25	4	8	12	17	***ST288***	CC229
675	Sheep	2009	Chennai	A	22	6	25	4	8	12	17	***ST288***	CC229
226	Goat	2002	Palampur	A	1	20	1	1	11	71*	17	**ST291**	
433	Goat	2006	Chennai	A	49*	6	25	4	8	12	17	**ST289**	CC229
534	Goat	2007	Chennai	A	50*	6	25	4	8	12	17	**ST290**	CC229
676	Goat	2009	Chennai	A	43	68	38	3	67	62	19	ST222	
563	Cattle	2007	Anand	B	23	37	2	4	17	21	17	ST122	CC122
593	Cattle	2008	Guwahati	B	23	37	2	4	17	21	17	ST122	CC122
222	Buffalo	2002	Mathura	B	23	37	2	4	17	21	17	ST122	CC122
797	Buffalo	2010	Kerala	D	4	61	3	4	47	43	72	***ST287***	
540	Pig	2007	Chennai	D	14	10	3	8	20	20	19	ST50	CC50
585	Pig	2008	Guwahati	A	49*	6	25	4	8	12	17	**ST289**	CC229
1027	Pig	2014	Guwahati	A	21	33	20	17	20	26	2	ST129	CC129
985	Poultry	2013	Gujarat	A	21	33	3	17	42	26	72	ST282	
942	Poultry	2013	Chennai	A	21	33	3	17	42	26	72	ST282	
960	Duck	2013	Trissur	A	21	33	20	17	20	26	2	ST129	CC129

Novel STs are mentoned in bold letters. Novel alleles of *adk* and *pmi* are indicated with asterisk. STs formed due to novel combination of alleles are indicated in bold and italic letters.

CC229 is closely related to the HS specific clonal complex – CC122.

SUMMARY

In the current study, correlation among specific capsular types, sequence types and specific hosts were observed. Study proves high diversity with respect to small ruminant isolates belonging to capsular type A. Higher sharing of sequence types among small ruminant, porcine and avian hosts indicate the close association of these isolates getting adapted for diverse host species. The study also shows that CC229, mostly from small ruminant hosts, was genetically related to HS-specific ST122 indicating recent diversification and possibly, similar genotype and pathogenesis.

ACKNOWLEDGEMENTS

Authors thank ICAR for providing financial support under AINP on Haemorrhagic Septicaemia and Director, IVRI for providing facilities.

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