The Haryana Veterinarian (June, 2025) **64(1),** 1-6 ISSN 0033-4359 (P) **Research Article**

MOLECULAR TYPING WITH REPETITIVE SEQUENCE-BASED PCR (REP-PCR) OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM HUMAN AND ANIMAL CLINICAL SAMPLES

SANDEEP KUMAR SHARMA*, BINCY JOSEPH, MANISH AGRAWAL[1], VIKAS GALAV[1], SHARAT CHANDER MEHTA[2] and ANIL KUMAR KATARIA[3]
Department of Veterinary Microbiology, [1]Department of Veterinary Pathology,
Post-Graduate Institute of Veterinary Education and Research (PGIVER), Jaipur
[2]National Research Centre on Equines, Indian Council of Agricultural Research (ICAR) Bikaner (Raj.), India
[3]Department of Veterinary Microbiology, College of Veterinary and Animal Science,
Rajasthan University of Veterinary and Animal sciences (RAJUVAS), Bikaner, Rajasthan, India

Received: 28.03.24; Accepted: 12.06.24

ABSTRACT

Repetitive element sequence-based PCR (*rep*-PCR) is a genotyping method that target noncoding repetitive sequences interspersed throughout the bacterial genome and abled to generate DNA fingerprinting that discriminates bacterial strains. Hence, the present study was designed to evaluate the applicability of *rep*-PCR in molecular typing of *Staphylococcus aureus* isolated from human and animal clinical samples. To achieve the target, total 157 genotypically confirmed *S. aureus* isolates obtained from various clinical samples of human, ruminants (cattle, buffalo, goat and sheep), non- ruminants (pig and camel) and companion animals (horse and dog) were included in study. The *rep*-PCR demonstrated a higher discriminatory power (D.I. 0.8892) with thirty six different rep patterns, comprising of 300 to 1400 bp band sizes. Out of the total isolates, 156 isolates were typeable with *rep*-PCR while one isolate (J9) from camel was exceptionally non-typeable. All isolates were classified into five clusters at 80% genetic similarity level and the animal and human isolates fell into different cluster with high genetic discrimination. The study provides valuable inputs in regards to suitability of *rep*-PCR as molecular tool to differentiate the *S. aureus* isolates of different origins.

Keywords: *rep*-PCR, *S. aureus*, Molecular typing, Human, Animal

How to cite: Sharma, S.K., Joseph, B., Agrawal, M., Galav, V., Mehta, S.C. and Kataria, A.K. (2025). Molecular typing with repetitive sequence-based PCR (*rep*-PCR) of *Staphylococcus aureus* isolated from human and animal clinical samples. *The Haryana Veterinarian* **64(1)**: 1-6.

Staphylococcus aureus is an important Gram positive pathogen of human and animal and is associated with various suppurative infections. In human, it is associated with food toxicity, skin infections, pneumonia, deep abscesses, osteomyelitis and endocarditis. In animals, this organism known to cause mastitis, endometritis, pyoderma, cystits, dermatitis, scirrhous cord, tick pyaemia and bumble foot in poultry along with many unique phenotypic characteristics (Naber, 2009; Sharma *et al.*, 2013; Sharma *et al.*, 2018; Verma *et al.*, 2023).

Looking to the variations in phenotypic and biochemical characteristics of *S. aureus* isolates, DNA based molecular methods have been found most effective tools for typing of the various origin. Molecular typing is an important tool to confirm the epidemiological relationships and monitoring the local and international spread (Tenover *et al.*, 1994; Zehra *et al.*, 2020). *S. aureus* strains were reported with variations in their genome so molecular typing is necessary to differentiate epidemic, nonepidemic strains and also comparison of *S. aureus* strains from human and animal origin. The ideal system for the molecular typing of *S. aureus* strains should be easy, rapid, reliable, highly discriminatory and reproducible (Diep *et al.*, 2003; Yadav *et al.*, 2015).

*Corresponding author: drsharmask01@hotmail.com

The prokaryotic chromosomes having unique repeat DNA sequences which can able to produce specific DNA fingerprint pattern for an individual strain. These repetitive sequences are existing as three types i.e. repetitive extragenic palindromic (rep) sequence, enterobacterial repetitive intergenic consensus (ERIC) sequence and BOX element sequences (Del Vecchio *et al.*, 1995). Out of these three, repetitive extragenic palindromic (*rep*) sequence based PCR method is most referred and proven methodology (Shutt *et al.*, 2005). The *rep*-PCR was proven to be a highly discriminating and rapid screening molecular typing method based on short repetitive sequence elements distributed throughout the bacterial genome to create DNA fingerprints to discriminate and to classify variety of staphylococcal infections without the need of enzyme digestion techniques (Arabestani *et al.*, 2022). Hence present study was designed to detect and establish genetic variations among different *S. aureus* isolates from human and animal samples using *rep*-PCR technique.

MATERIALS AND METHODS

Bacterial Isolates

In the present investigation, 157 genotypically confirmed (23S *rRNA* based species-specific primers) *Staphylococcus aureus* isolates were subjected to

molecular typing (Sharma *et al.*, 2017). The samples were collected in and around Bikaner region of Rajasthan and all 157 isolates belong to human clinical samples (35), meat samples (20), horse wounds (03), pig nasal swabs (02), camel wounds (08), dog skin swabs (06), sheep clinical samples (06), buffalo mastitic milk (21), goat mastitic milk (28) and cattle mastitic milk (28).

Repetitive element sequence-based PCR (*rep*–PCR)

Genomic DNA was isolated from all 157 *S. aureus* isolates as per protocol of HiPer® Bacterial Genomic DNA Extraction Kit (HTBM008). The integrity of DNA was checked by submerged agarose gel electrophoresis. Each DNA sample was mixed with one-fifth volume of the gel loading buffer. For electrophoresis, 0.8 per cent agarose gel in TBE buffer containing ethidium bromide (0.5-1.0 µg) was used. The repetitive element sequence-based PCR (*rep*-PCR) was carried out as per van Belkum *et al.* (1995). DNA amplification consisted of a cycle of pre denaturation at 94º C for 5 minute, followed by 40 cycles of 1 minute denaturation at 93º C, 1.30 minute annealing at 37º C and 1 minute extension at 72º C. A final extension step of 72º C for 10 minute was included. A negative and a positive control were also included. Each isolate was tested under the same conditions at least twice with the selected single *rep*-PCR primer (5'-TCG CTC AAA ACA ACG ACA CC- 3'). Amplified products were separated by electrophoresis in a 1.5% Metaphore- agarose gel in 1-X TBE buffer at a constant voltage of 4 V/cm and stained using ethidium bromide (0.5 µg/ml). Variable multiple *rep*-PCR product patterns were obtained (Fig. 1). The *rep*-PCR pattern and dendrogram analysis was performed as described by Reinoso *et al.* (2004) with DICE & UPGMA (Unweighted Pair Group Method with Arithmetic Mean) methods by MEGA 6 bioinformatics software. The Discriminatory Power (D)/ discriminatory index (D.I.) was calculated by formula of Simpsons index of diversity as described in the method of Hunter (1990).

RESULTS AND DISCUSSION

The study was attempted to type *S. aureus* isolates obtained from clinical samples of human and animals along with establishment of *rep*-PCR method to determine genetic variations among bacterial isolates. During *rep*-PCR screening, total 36 different *rep* patterns (Table 1) detected which comprises amplified products in the range from 300 to 1400 bp (Fig. 1) among the total 157 studied isolates. In the present investigation, all isolates differentiated with 0.8892 discriminatory index (D.I.) which indicates high level of discrimination of *S. aureus* isolates by *rep*-PCR. Out of the total isolates, one isolate (J9) from camel was exceptionally non-typeable with *rep*-PCR while remaining 156 isolates were typeable with *rep*-PCR. The pattern *rep* 30 was most common with 47 cattle and goat origin isolates followed by *rep* 17 with 13 isolates which belonged to sheep, horse and camel. Then, *rep* 7 pattern comprises 10 isolates of human origin and other remaining *rep*-patterns comprising with less than seven isolates of different origins. The production of large number of separate *rep*-patterns in isolates of different origin in the present study indicated usefulness of the method in differentiating *S. aureus* from various sources.

In the source-wise analysis of *rep*-patterns it was found that human isolates were differentiated into maximum *rep*-profiles (16 *rep*-profiles) indicating more variations while sheep isolates were grouped only into one *rep*-pattern (*rep* 17) indicating minimal variations. Other sources including variable number of *rep* profiles i.e. meat sample (11 *rep*-patterns), buffalo (8 *rep*-patterns), camel (6 *rep*-patterns) dog (5 *rep*- patterns) and remaining sources having less than four *rep*-patterns with less variabilities as described in Table 2. The phylogenetic cluster analysis of various *rep*-patterns was attempted and all 36 *rep*-patterns were classified into five clusters on the basis of 80% genetic similarity (Fig. 2). The cluster analysis indicates that most of animal origin isolates were separately clustered (I^{st} and V^{th} cluster) in comparison to human origin isolates and meat piece origin isolates (II^{nd} and IV^{th} cluster). The fifth cluster comprising 73 isolates and most of belongs to cattle, goat and buffalo while fourth cluster having 41 isolates of human and meat piece samples. Few *rep*-patterns fell into both human and animal clusters with indication of cross transmission between human and animals. It may also be concluded that human isolates could be separately differentiated from animal origin isolates even up to 80% similarity level. Similar to present study, Reinoso *et al.* (2008) have also reported 35 *rep* profiles along with seven different clusters in dendrogram analyses at 60% of relative genetic similarity level among 45 *S. aureus* strains studied by *rep*-PCR obtained from humans, bovine subclinical mastitis and food samples. In a study by Dini *et al.* (2019) the results of the *rep*-PCR analysis revealed 14 different patterns (seven common types and seven unique types) among 55 isolates of the Methicillin-resistant *S. aureus* (MRSA) and Methicillin-susceptible *S. aureus* (MSSA) strains. Reinoso *et al.* (2007) assessed genetic relationship of 52 *S. aureus* strains isolated from mammary gland infections of bovine origin and similar to present study, they also detected a total of 31 *rep*-profiles in the range size from 300 to 6000 bp during *rep*-PCR analysis. At the level of 50% similarity, it could be defined 5 clusters namely I to V and most of the strains (75%) were grouped in cluster I and suggested that *rep*-

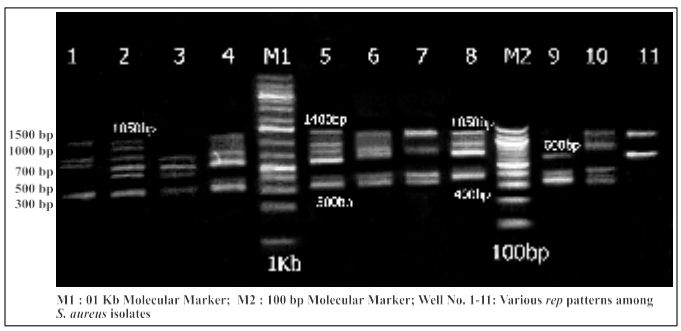
Table 1. *rep* patterns of *Staphylococcus aureus* isolates

S.No.	*rep* pattern I.D.	*rep* pattern	Number of Isolates	*S. aureus* Isolate I.D.
1.	*rep* 1	500bp, 600bp	1	H1
2.	*rep* 2	300bp, 500bp, 600bp, 700bp, 1000bp, 1050bp	1	H2
3.	*rep* 3	300bp, 700bp, 1000bp, 1050bp	6	H3, H4, H15, H21, H24, Mt15
4.	*rep* 4	300bp, 500bp, 600bp,700bp	1	H5
5.	*rep* 5	300bp, 600bp, 900 bp,1000bp, 1400bp	5	H6, H9, H10, H11, H28
6.	*rep* 6	300bp, 700bp, 1000bp	4	H14, Mt31, J2, B26
7.	*rep* 7	300bp, 700bp, 750bp, 1000bp, 1050bp	10	H29, H33, H37, H39, H40, H41, H46, H48, Mt2, Mt9
8.	*rep* 8	300bp, 700bp, 800bp, 1000bp, 1050bp	2	Mt1,Mt4
9.	*rep* 9	300bp, 600bp, 700bp, 750bp, 1000bp, 1050bp	1	H45
10.	*rep* 10	300bp, 600bp, 700bp, 1000bp, 1050bp	5	Mt3, Mt10, Mt11, Mt13, Mt14
11.	*rep* 11	300bp, 400bp, 600bp, 700bp, 1000bp	2	D13, B46
12.	*rep* 12	300bp, 400bp, 600bp, 700bp, 1050bp	3	B5, B19, B21
13.	*rep* 13	300bp, 500bp, 700bp, 750bp, 1000bp, 1050bp	1	H12
14.	*rep* 14	600bp, 900bp, 1000bp, 1400bp	1	H8
15.	*rep* 15	400bp, 700bp, 1000bp, 1050bp	1	H31
16.	*rep* 16	300bp, 600bp,700bp, 750bp	2	H30 ,H34
17.	*rep* 17	300bp, 600bp, 700bp, 1000bp	13	Mt19, Hrs3, Hrs4, Pg2, J14,J18, D10, SV2, SV3, SV4, SN3,SN4, SN14
18.	*rep* 18	300bp, 600bp, 1000bp, 1050bp	2	Mt20, Mt24
19.	*rep* 19	300bp, 400bp, 700bp, 1000bp	2	D7, B24
20.	*rep* 20	300bp, 400bp, 700bp, 750bp, 1000bp	1	D9
21.	*rep* 21	300bp, 400bp, 600bp, 1000bp	7	B27, B28, B29, B30, B31, B34, B36
22.	*rep* 22	300bp, 400bp, 600bp, 700bp	6	B10, B39, B42, B43, B55, B57
23.	*rep* 23	300bp, 350bp, 600bp, 700bp	6	AG8, G29, C3R, C8, C17, C22
24.	*rep* 24	300bp, 350bp, 600bp, 1000bp	2	G46, C15
25.	*rep* 25	300bp, 700bp, 1400bp	1	H27
26.	*rep* 26	300bp, 600bp, 700bp	3	H44, Mt27, B23
27.	*rep* 27	300bp, 800bp, 1000bp	1	Hrs1
28.	*rep* 28	600bp, 700bp, 1000bp	1	J4
29.	*rep* 29	300bp, 400bp, 700bp	1	J15
30.	*rep* 30	300bp, 350bp, 600bp	47	AG5, AG6, AG10, AG13, AG15, AG17, G1, G2, G7, G9, G10, G11, G16, G21, G24, G35, G37, G39, G40, G41, G43, G45, G47, G49, G55, C2, C5R, C7, C9, C11, C12, C13, C20, C23, C26, C29, C34, C36, C37, C39, C40, C41, C43, C46, C47, C50, C52
31.	*rep* 31	300bp, 700bp	5	H7, H16, H22, H25, J10
32.	*rep* 32	400bp, 1000bp	1	H13
33.	*rep* 33	300bp, 600bp	7	Mt22, Mt25, Mt28, J3, D4, D6, C2R
34.	*rep* 34	300bp, 1000bp	1	Mt26
35.	*rep* 35	600bp	2	Mt12, Pg4
36.	*rep* 36	300 bp	1	B1

Table 2. Group wise distribution of *rep* patterns among *Staphylococcus aureus* isolates

S.No.	Source of Isolate	S. No.	*rep* pattern I.D.	*rep* pattern	Number of Isolates	Isolate I.D.	Total No. of Isolate
1.	Human	1.	*rep*1	500bp, 600bp	1	H1	35
		2.	*rep*2	300bp, 500bp, 600bp, 700bp, 1000bp, 1050bp	1	H2	
		3.	*rep*3	300bp, 700bp, 1000bp, 1050bp	5	H3, H4, H15, H21 and H24	
		4.	*rep*4	300bp, 500bp, 600bp, 700bp	1	H5	
		5.	*rep*5	300bp, 600bp, 900bp, 1000bp, 1400bp	5	H6, H9, H10, H11 and H28	
		6.	*rep*6	300bp, 700bp, 1000bp	1	H14	
		7.	*rep*7	300bp, 700bp, 750bp, 1000bp, 1050bp	8	H29, H33, H37, H39, H40, H41, H46 and H48	
		8.	*rep*9	300bp, 600bp, 700bp,750bp, 1000bp, 1050bp	1	H45	
		9.	*rep*13	300bp, 500bp, 700bp, 750bp, 1000bp, 1050bp	1	H12	
		10.	*rep*14	600bp, 900bp, 1000bp, 1400bp	1	H8	
		11.	*rep*15	400bp, 700bp, 1000bp, 1050bp	1	H31	
		12.	*rep*16	300bp, 600bp, 700bp, 750bp	2	H30 , H34	
		13.	*rep*25	300bp, 700bp, 1400bp	1	H27	
		14.	*rep*26	300bp, 600bp, 700bp	1	H44	
		15.	*rep*31	300bp, 700bp	4	H7, H16, H22 and H25	
		16.	*rep*32	400bp, 1000bp	1	H13	
2.	Meat Piece	1.	*rep*3	300bp, 700bp, 1000bp, 1050bp	1	Mt15	20
		2.	*rep*6	300bp, 700bp, 1000bp	1	Mt31	
		3.	*rep*7	300bp, 700bp, 750bp, 1000bp, 1050bp	2	Mt2 and Mt9	
		4.	*rep*8	300bp, 700bp, 800bp, 1000bp, 1050bp	2	Mt1 and Mt4	
		5.	*rep*10	300bp, 600bp, 700bp, 1000bp, 1050bp	5	Mt3, Mt10, Mt11, Mt13 and Mt14	
		6.	*rep*17	300bp, 600bp, 700bp, 1000bp	1	Mt19	
		7.	*rep*18	300bp, 600bp, 1000bp, 1050bp	2	Mt20 and Mt24	
		8.	*rep*26	300bp, 600bp,700bp	1	Mt27	
		9.	*rep*33	300bp, 600bp	3	Mt22, Mt25 and Mt28	
		10.	*rep*34	300bp, 1000bp	1	Mt26	
		11.	*rep*35	600bp	1	Mt12	
3.	Horse	1.	*rep*17	300bp, 600bp, 700bp, 1000bp	2	Hrs3 and Hrs4	3
		2.	*rep*27	300bp, 800bp, 1000bp	1	Hrs1	
4.	Pig	1.	*rep*17	300bp, 600bp, 700bp, 1000bp	1	Pg2	2
		2.	*rep*35	600bp	1	Pg4	
5.	Camel	1.	*rep*6	300bp, 700bp, 1000bp	1	J2	8
		2.	*rep*17	300bp, 600bp, 700bp, 1000bp	2	J14 and J18	
		3.	*rep*28	600bp, 700bp, 1000bp	1	J4	
		4.	*rep*29	300bp, 400bp, 700bp	1	J15	
		5.	*rep*31	300bp, 700bp	1	J10	
		6.	*rep*33	300bp, 600bp	1	J3	
		7.	-	-	1	J9	
6.	Dog	1.	*rep*11	300bp, 400bp, 600bp, 700bp, 1000bp	1	D13	6
		2.	*rep*17	300bp, 600bp, 700bp, 1000bp	1	D10	
		3.	*rep*19	300bp, 400bp, 700bp, 1000bp	1	D7	
		4.	*rep*20	300bp, 400bp, 700bp, 750bp, 1000bp	1	D9	
		5.	*rep*33	300bp, 600bp	2	D4 and D6	
7.	Sheep	1.	*rep*17	300bp, 600bp, 700bp, 1000bp	6	SV2, SV3, SV4, SN3, SN4 and SN14	6
8.	Buffalo	1.	*rep*6	300bp, 700bp, 1000bp	1	B26	21

		2.	*rep*11	300bp, 400bp, 600bp, 700bp, 1000bp	1	B46	
		3.	*rep*12	300bp, 400bp, 600bp, 700bp, 1050bp	3	B5, B19 and B21	
		4.	*rep*19	300bp, 400bp, 700bp, 1000bp	1	B24	
		5.	*rep*21	300bp, 400bp, 600bp, 1000bp	7	B27, B28, B29, B30, B31, B34 and B36	
		6.	*rep*22	300bp, 400bp, 600bp, 700bp	6	B10, B39, B42, B43, B55 and B57	
		7.	*rep*26	300bp, 600bp,700bp	1	B23	
		8.	*rep*36	300bp	1	B1	
9.	Goat	1.	*rep*23	300bp, 350bp, 600bp,700bp	2	AG8 and G29	28
		2.	*rep*24	300bp,350bp,600bp, 1000bp	1	G46	
		3.	*rep*30	300bp, 350bp, 600bp	25	AG5, AG6, AG10, AG13, AG15, AG17, G1, G2, G7, G9, G10, G11, G16, G21, G24, G35, G37, G39, G40, G41, G43, G45, G47, G49 and G55	
10.	Cattle	1.	*rep*23	300bp, 350bp, 600bp, 700bp	4	C3R, C8, C17 and C22	28
		2.	*rep*24	300bp,350bp,600bp, 1000bp	1	C15	
		3.	*rep*30	300bp, 350bp, 600bp	22	C2, C5R, C7, C9, C11, C12, C13, C20, C23, C26, C29, C34, C36, C37, C39, C40, C41, C43, C46, C47, C50 and C52	
		4.	*rep*33	300bp, 600bp	1	C2R	



M1 : 01 Kb Molecular Marker; M2 : 100 bp Molecular Marker; Well No. 1-11: Various *rep* patterns among *S. aureus* isolates

Fig. 1. Various rep patterns (repetitive element sequence-based PCR) of *S. aureus*

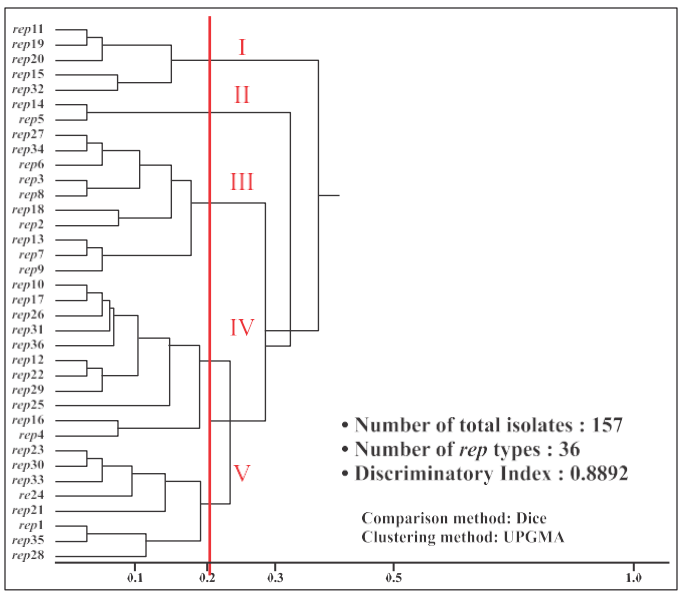


Fig. 2. Phylogenetic cluster analysis (Dendrogram analyses) of *S. aureus* rep patterns

PCR typing successfully differentiated *S. aureus* strains of bovine origin. In consent to present study, Memon *et al.* (2013) also studied *S. aureus* isolated from bovine subclinical mastitis from Eastern China by *rep*-PCR and reported 34 *rep*-profiles and seven distinct genotypes during dendrogram analysis. Pinto *et al.* (2015) had also attempted to assess clonal relationships among the *S. aureus* strains (n = 29) obtained from chilled and frozen raw poultry parts from seven different Brazilian broiler chicken processors. The isolates were grouped into three major clusters based on *rep*-PCR fingerprints typed and similar to present study they concluded that *rep*-PCR technique can able to establish a clonal relationship among the *S. aureus*.

Similar to the present study, many researchers like del Vecchio *et al.* (1995), Gardella *et al.* (2005); Nordin *et al.* (2010) and Arabestani *et al.* (2022) etc. analyzed MRSA and MSSA strains from various clinical infections to differentiate them from each other and they have also reported that *rep*-PCR was potential tool to determine genomic differences among *S. aureus* strains from different sources. A study on 31 *S. aureus* isolates from pastries food samples to demonstrate genetic diversity among isolates at Hamadan, Iran has detected eight different *rep*-PCR types with the similarity cut off ≥95%

and seven different *rep*-PCR profiles, including five common types and 2 unique types. Similar to present study, the study proved that *rep*-PCR technique is one of the important methods to demonstrate genetic diversity as well as established existence of different *rep*-types of *S. aureus* isolates (Arabestani *et al.*, 2022).

CONCLUSION

Based on different *rep*- patterns and cluster classification detected in the *rep*-PCR technique, the study may conclude that repetitive sequence-based PCR (*rep*-PCR) is found suitable and potential tool which permits differentiation of isolates to species, subspecies and strain level. Although, *rep*-PCR is more suitable molecular technique in comparison to routine bacteriological methods but looking to some limitation i.e. costs of materials, lack of advance equipped laboratories, this study warrants some other typing methods for further characterization of the isolates.

REFERENCES

Arabestani M.R., Kamarehei, F., Dini, M., Aziz Jalilian, F., Moradi, A., Shokoohizadeh, L. (2022). Characterization of *Staphylococcus aureus* isolates from pastry samples by rep-PCR and phage typing. *Iran J. Microbiol.* **14(1)**: 76-83.

Del Vecchio, V.G., Petroziello, J.M., Gress, M.J., McClesky, F.K., Melcher, G.P., Crouch, H. K. and Lupski, J.R. (1995). Molecular genotyping of methicillin-resistant *Staphylococcus aureus* via fluorophore-enhanced repetitive-sequence PCR. *J. Clin. Microbiol.* **33(8)**: 2141-2144.

Diep, B.A., Remington, F.P. and Sensabaugh, G.F. (2003). Clonal characterization of *Staphylococcus aureus* by multilocus restriction fragment typing, a rapid screening approach for molecular epidemiology. *J. Clin. Microbiol.* **41(10)**: 4559-4564.

Dini, M., Shokoohizadeh, L., Jalilian, F.A., Moradi, A. and Arabestani, M.R. (2019). Genotyping and characterization of prophage patterns in clinical isolates of *Staphylococcus aureus*. *BMC Res. Notes.* **12**: 669.

Gardella, N., Picasso, R., Predari, S.C., Lasala, M., Foccoli, M., Benchetrit, G., Famiglietti, A., Catalano, M., Mollerach, M. and Gutkind, G. (2005). Methicillin-resistant *Staphylococcus aureus* strains in Buenos Aires teaching hospitals: Replacement of the multidrug resistant South American clone by another susceptible to rifampin, minocycline and trimethoprim-sulfamethoxazole. *Rev. Argent. Microbiol.* **37(3)**: 156-160.

Hunter, P. (1990) Reproducibility and indices of discriminatory power of microbial typing methods. *J. Clin. Microbiol.* **28**: 1903-1905.

Memon, J., Yang, Y., Kashif, J., Yaqoob, M., Buriro, R., Soomro, J., Liping, W. and Hongjie, F. (2013). Genotypes, virulence factors and antimicrobial resistance genes of *Staphylococcus aureus* isolated in bovine subclinical mastitis from Eastern China. *Pak. Vet. J.* **33(4)**: 486-491.

Naber, C.K. (2009). *Staphylococcus aureus* bacteremia: Epidemiology, pathophysiology and management strategies. *Clin. Infect. Dis.* **48**: 231-237.

Nordin, S.A., Wahab, Z.A., Hussin, S. and Rahman, M.M. (2010). Molecular characterization of nosocomial methicillin resistant *Staphylococcus aureus* by Rep-PCR. *Int. J. Infect. Dis.* **9(2)**: 1-6.

Pinto, J.B., Rossatto, F.C.P., Martins, P.D. and Frazzon, A.P.G. (2015). Genetic relationships and virulence factors in *Staphylococcus aureus* isolated from raw poultry in South Brazil. *Ann. Microbiol.* **65**: 1933-1940.

Reinoso, E., Bettera, S., Frigerio, C., Di Renzo, M., Calzolari, A. and Bogni, C. (2004). RAPD-PCR analysis of *Staphylococcus aureus* strains isolated from bovine and human hosts. *Microbiol. Res.* **159**: 245-255.

Reinoso, E., Bettera, S., Odierno, L. and Bogni, C. (2007). Rep-PCR of *Staphylococcus aureus* strains isolated from bovine mastitis in Argentina. *Braz. J. Vet. Res. Anim. Sci.* **44**: 115-121.

Reinoso, E.B., El-Sayed, A., Lammler, C., Bogni, C. and Zschock, M. (2008). Genotyping of *Staphylococcus aureus* isolated from humans, bovine subclinical mastitis and food samples in Argentina. *Microbiol. Res.* **163(3)**: 314-322.

Sharma S.K., Nathawat P., Bhati T., Mohammed N., Choudhary S., Raj R., Solanki S. and Kataria A.K., (2013). Characterization of *Staphylococcus aureus* isolated from nasal discharge from pneumonic camels (*Camelus dromedarius*). *ABAH Bioflux.* **5(1)**: 38-43.

Sharma, S.K., Galav, V., Agrawal, M., Faridi, FN. and Kumar B. (2017). Multi-drug resistance pattern of bacterial flora obtained from necropsy samples of poultry. *J. Anim. Health Prod.* **5(4)**: 165-171.

Sharma, R., Singh, M., Kotwal, S.K., Malik M.A. and Chambyalia, H. (2018). Occurrence of multidrug resistant biofilm producing bacteria in veterinary clinical settings. *The Haryana Veterinarian* **57(1)**: 76-79.

Shutt, C.K., Pounder, J.I., Page, S.R., Schaecher, B.J. and Woods, G.L. (2005). Clinical evaluation of the diversi lab microbial typing system using repetitive-sequence-based PCR for characterization of *Staphylococcus aureus* strains. *J. Clin. Microbiol.* **43(3)**: 1187-1192.

Tenover, F.C., Arbeit, R., Archer, G., Biddle, J., Byrne, S., Goering, R., Hancock, G., Hebert, G.A., Hill, B. and Hollis, R. (1994). Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus*. *J. Clin. Microbiol.* **32(2)**: 407-415.

Van Belkum, A., Kluytmans, J., Leeuwen, W.V., Bax, R., Quint, W., Peters, E., Fluit,A., Vandenbroucke-Grauls, C., Van den Brule,A., Koeleman, H., Melchers, W., Meis, J., Elaichouni,A., Vaneechoutt, M., Moonens, F., Maes, N., Struelenf, M., Tenover, F. and Verbrugh, H. (1995). Multi-center evaluation of *Staphylococcus aureus* strain. *J. Clin. Microbiol.* **33(6)**: 1537-1547.

Verma, S.K., Parul, Sharma, B., Jain, U., Sharma, A., Goswami, M., Singh, P., Aashima, Rathee, A. and Agnihotri, I. (2023). Detection of biofilm forming *Staphylococcus aureus* from mastitic milk. *The Haryana Veterinarian* **62(SI-2)**: 42-45.

Yadav, R., Sharma, S.K., Yadav, J., Nathawat, P. and Kataria, A.K. (2015). Typing of *Staphylococcus aureus* obtained from mastitic milk of cattle and buffalo on the basis of coagulase (*coa*) gene RFLP patterns. *Isr. J. Vet. Med.* **70(4)**: 37-41.

Zehra, A.,Gulzar M. and Singh R. (2020). Assessment of correlation between phenotypic and genotypic resistance profile of *Staphylococcus aureus* isolated from meat samples of Punjab, India. *The Haryana Veterinarian* **59(1)**: 13-18.