

Rapid diagnosis for *S. aureus* strains that will provide information on the likelihood of persistent or non-persistent intramammary infection.

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Introduction

MASTITIS: Inflammation of the mammary gland.

Most widespread diseases affecting dairy cattle.

Frequent use of antibiotic.

Staphylococcus aureus
MOST COMMON PATHOGEN CAUSING MASTITIS.

VIRULENT STRAIN

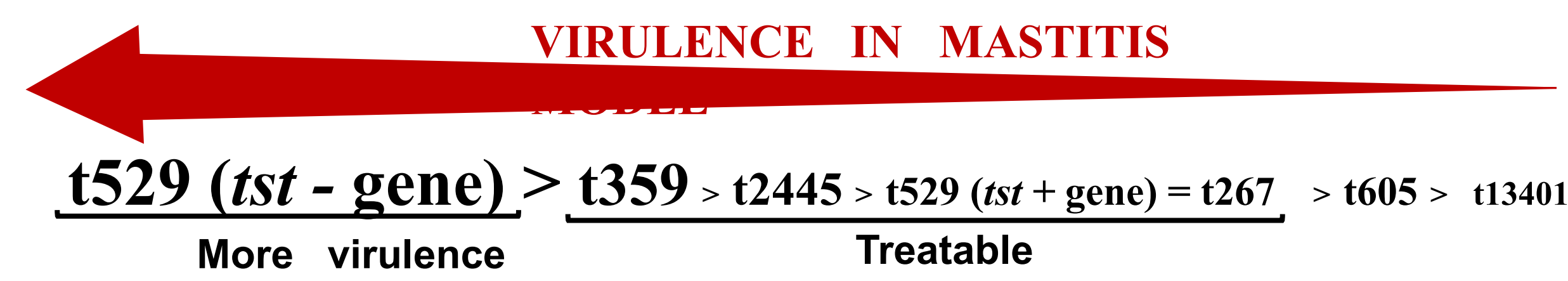
PERSISTENT STRAIN

- Can damage gland due to severe inflammation.
- Most commonly found among bovine mastitis isolates in Canada.
- Enterotoxin G (*seg*) and the toxic shock syndrome toxin (TSST-1) (*tst*) are superantigens.
- Disturb the host immune response by causing a non-specific polyclonal activation of immune cells

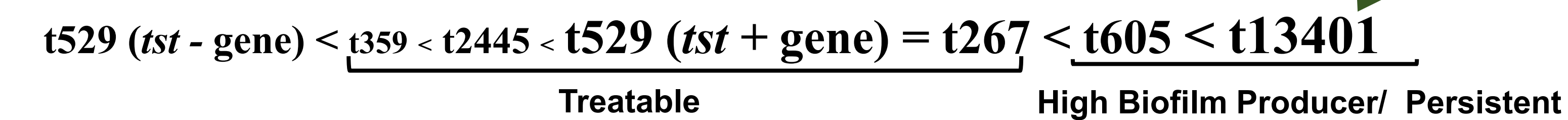
- High biofilm producers.
- Protection against antibiotics.
- Affects the efficacy of the host's immune system.
- Can persist in the mammary gland for up to 1 year.
- Cause difficult-to-treat chronic infections.

The 6 major Canadian *spa* types associated with mastitis: t529 (*tst* +/*tst* -), t359, t2445, t267, t605, t13401

Pichette-Jolette, S. et al. 2019. Vet. Microbiol. 228:188-195.
Demontier, E. et al. 2021. J. Dairy Sci. 104:11904-11921 <https://doi.org/10.3168/jds.2020-19904>



PERSISTENT INTRAMAMMARY INFECTION IN COWS



t529
Great importance
- Persistent infection
- High virulence
Depending (*tst* +/*tst* -) gene

Predicting disease outcome by determining the type of *S. aureus* strain could help make better on-farm management decisions and thereby significantly reduce economic losses caused by IMI in milk production.

Methods

Compare virulence among *S. aureus spa* t529 strains (*tst* + or *tst* -) in a mouse model of intramammary infection (IMI).

- Gland colonization.
- Inflammation and redness of mammary glands.

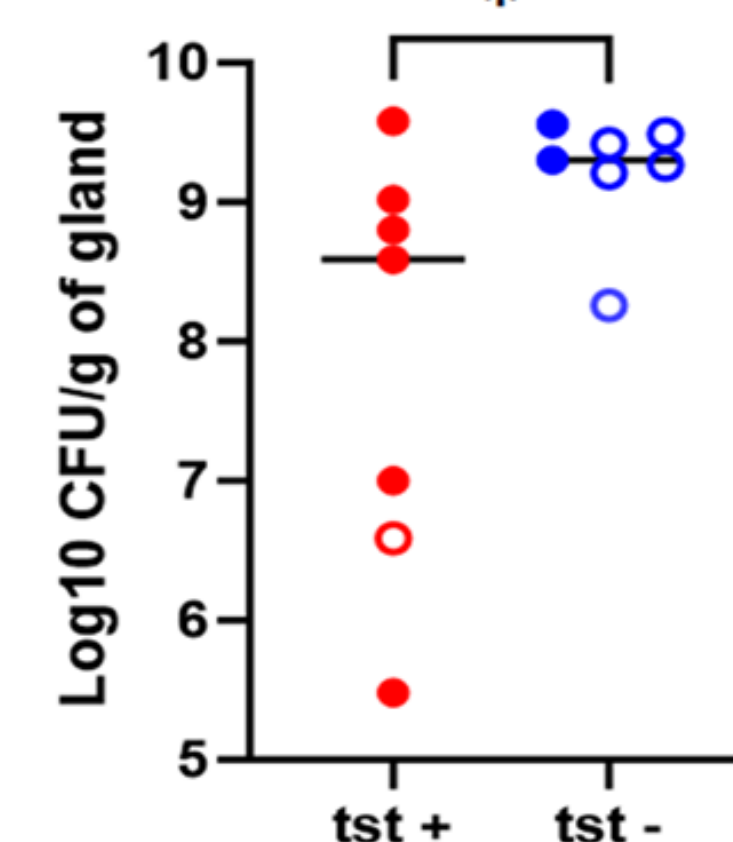
Rapid molecular methods can detect problematic strain.

- Simple PCR
- Multiplex PCR

Results

Compare virulence among *S. aureus spa* t529 strains (*tst* + or *tst* -) in a mouse model of intramammary infection.

Gland colonization



Samples used by Demontier et al., 2021 in similar study.

P < 0,05

S. aureus (*spa* type t529)

Figure 1: Bacterial load in mouse mammary glands 20 h after IMI.

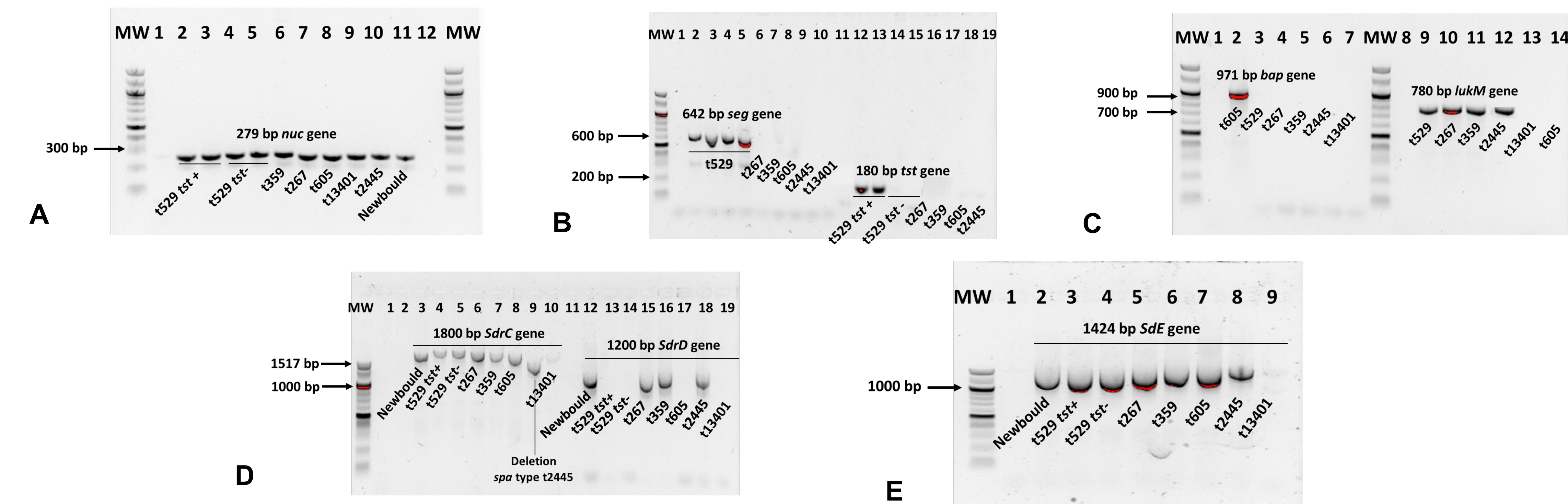


Figure 2: Sensitivity and specificity of the primers according to Table 1. A: *nuc* gene, B: *seg* and *tst* genes, C: *bap* and *lukM* genes, D: *SdrC* and *D* genes and E: *SdrE* gene. Molecular weight pattern (MW) N0467S (NEB).

• **Multiplex PCR**

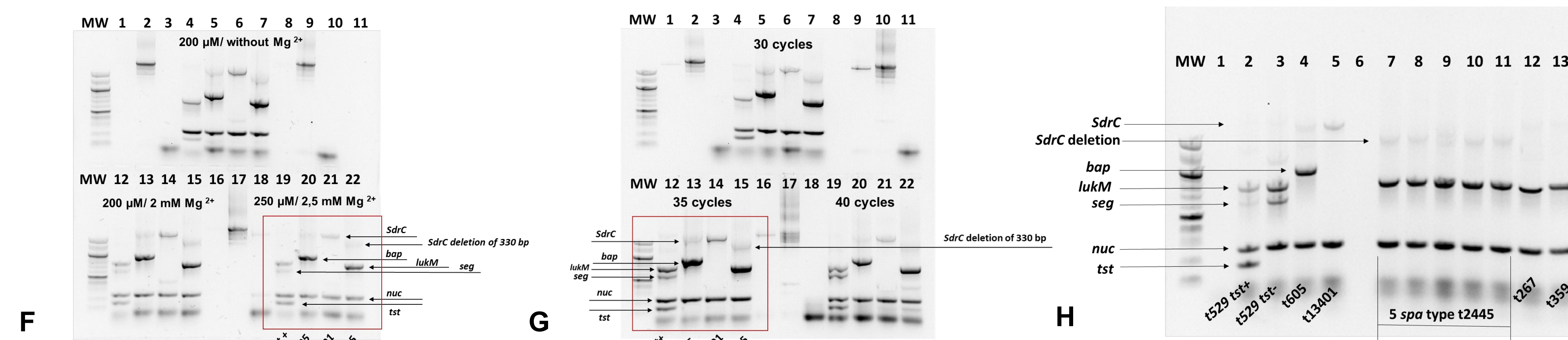


Figure 3: Evaluation of F: different concentrations of dNTPs and $MgCl_2$ and the 2 minutes extension time, G: the different reaction cycles and H: the results obtained with the optimized Multiplex PCR for each *spa* types. Molecular weight pattern (MW) N0467S (NEB).

Rapid molecular methods can detect problematic strain.

• **Simple PCR**

Table 1. Specific genes for 6 *spa* types of *Staphylococcus aureus*.

<i>Spa</i> type	<i>lukM</i>	<i>bap</i>	<i>seg</i> / <i>tst</i> *	<i>SdrC</i>	<i>SdrD</i>	<i>SdrE</i>
t13401	-	-	-	+	-	-
t605	-	+	-	+	-	+
t529	+	-	+ / + or -	+	-	+
t2445	+	-	-	+	+	+
t359	+	-	-	+	+	+
t267	+	-	-	+	+	+

* The *tst* + or - results will depend on the strains to be selected

Conclusion

Gland colonization

- Significant differences ($P < 0.05$) between the two groups of *spa* type t529 (*tst* + / *tst* -).
- Intense reddening of the mammary glands was only observed in three strains all belonging to the *tst*- group, which also coincides with the strains that presented the highest bacterial loads recovered from the mammary glands.
- The t529 *tst* +, *spa* type, which possess the superantigen gene *tst*, showed a lower bacterial load than that observed in the t529 *tst* - strains.
- This result was similar to that obtained by Demontier et al., 2021, when they performed the first comparative study on the virulence of t529 *tst* + and *tst* - strains, although they used only one *tst* + strain.
- The results confirm that presence of *tst* + reduces the apparent virulence and possibly favors persistence.

Simple PCR

- All the primers tested were sensitive and specific against the strains studied.
- The reactivity of the t2445 strains to the *SdrC* gene shows a band of lower molecular weight than the rest of the *spa* type, indicating the existence of a deletion of about 300 bp, which is present only in this type of strains.

Multiplex PCR

- The bands that appear in the Figure 3 F-H referring to the *SdrC* gene are not of great intensity, and it is precisely the element that differentiates the *spa* type t2445 from the rest of the *spa* types due to the presence of the deletion.
- The remaining t359 and t267 strains, although showing a different banding pattern than the t529, t605, t13401 and t2445 are not differentiated from each other, for the moment, using the primers shown in Table 1.

References

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