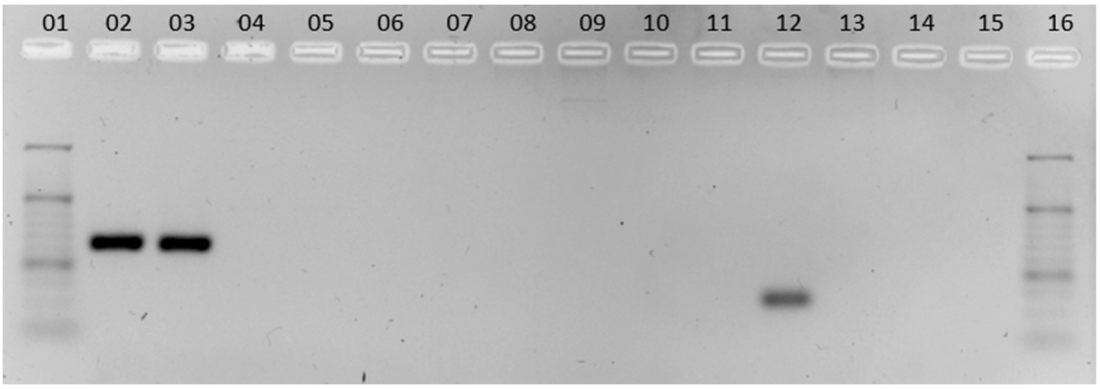
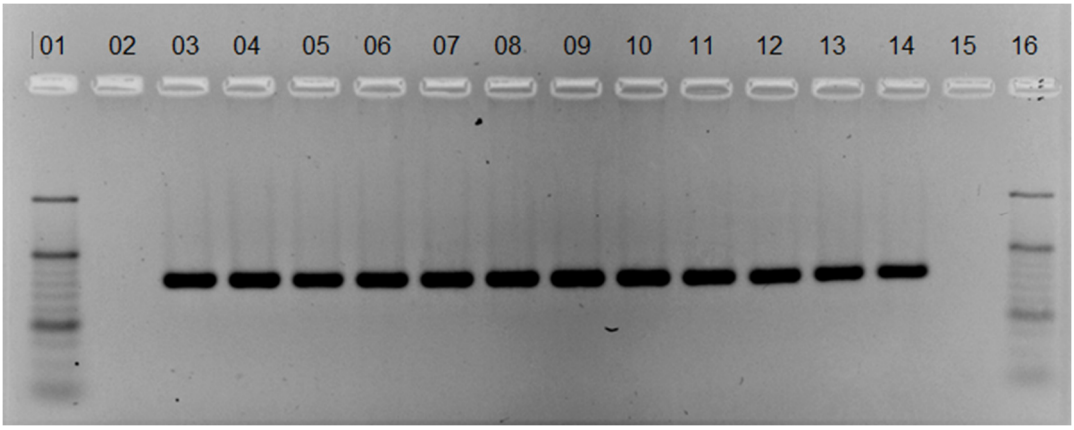
Supplementary Materials



Sraga1	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Srdys1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Srube3	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Staur3	-	-	-	-	-	-	-	-	-	-	-	-	-	-

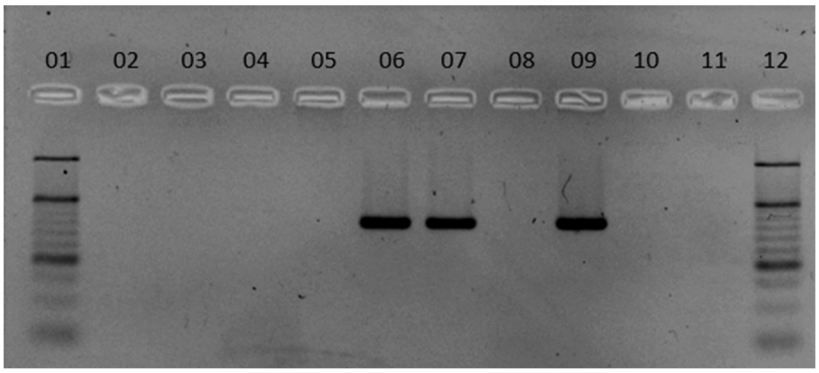
Fig. S1. Multiplex PCR results. Lane 01, 50 bp DNA ladder (with the following distribution – 50, 100,150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000); Lane 02, *Streptococcus uberis* 105PP2021; Lane 03, *Streptococcus uberis* 112PP2021; Lane 04, *Aerococcus viridans* 3 006PP2021; Lane 05, *Aerococcus viridans* 2 007PP2021; Lane 06, *Aerococcus viridans* 3 008PP2021; Lane 07, *Enterococcus faecium* 003PP2021; Lane 08, *Enterococcus faecium* 004PP2021; Lane 09, *Enterococcus faecalis* 022PP2021; Lane 10, *Staphylococcus epidermidis* 001PP2022; Lane 11, *Staphylococcus sciuri* 001PP2020; Lane 12, *Staphylococcus sciuri* 002PP2021; Lane 13, *Staphylococcus sciuri* 003PP2021; Lane 14, *Staphylococcus xylosus* 001PP2021; Lane 15, *Staphylococcus xylosus* 002PP2022; Lane 16, 50 bp DNA ladder.



Sraga1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Srdys1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Srube3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Staur3	-	+	+	+	+	+	+	+	+	+	+	+	+	-

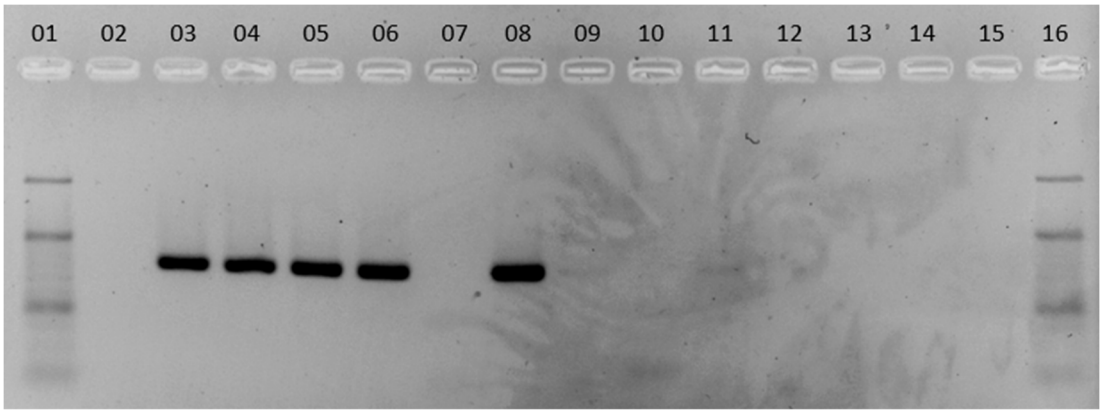
Figure S2. Multiplex PCR results. Lane 01, 50 bp DNA ladder (with the following distribution – 50, 100,150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000); Lane 02, *Staphylococcus xylosus* 003PP2022; Lane 03, *Staphylococcus aureus* ATCC 6538P Rosenbach; Lane 04, *Staphylococcus aureus* PCM 2267; Lane 05, *Staphylococcus aureus* PCM 458/2195; Lane 06, *Staphylococcus aureus* PCM 2101; Lane 07, *Staphylococcus aureus* PCM 2054; Lane 08, *Staphylococcus aureus* PCM 1650; Lane 09, *Staphylococcus aureus* PCM 1116; Lane 10, *Staphylococcus aureus* PCM 1115; Lane 11, *Staphylococcus aureus* PCM 1102;

Lane 12, *Staphylococcus aureus* PCM 565; Lane 13, *Staphylococcus aureus* PCM 502; Lane 14, *Staphylococcus aureus* PCM 1937; Lane 15, NTC; Lane 16, 50 bp DNA ladder.



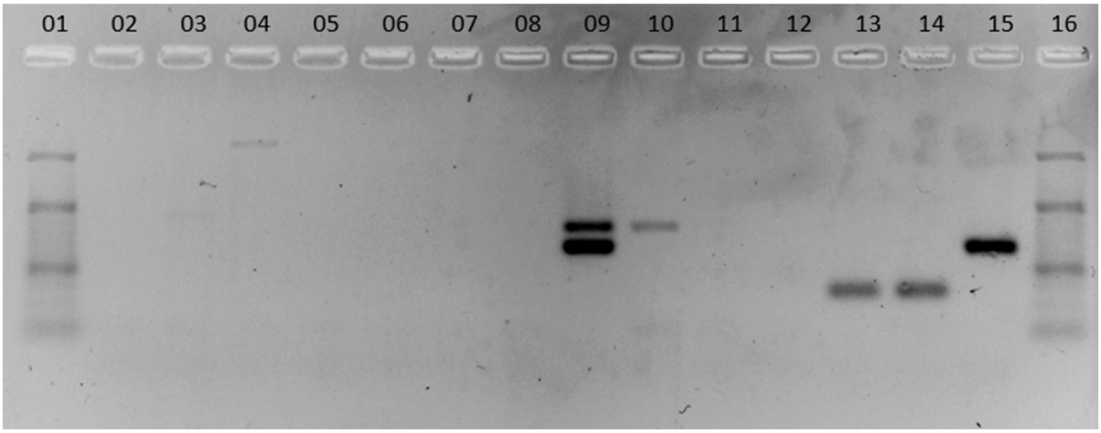
Sraga1	-	-	-	-	-	-	-	-	-	-
Srdys1	-	-	-	-	-	-	-	-	-	-
Srube3	-	-	-	-	-	-	-	-	-	-
Staur3	-	-	-	-	+	+	-	+	-	-

Figure S3. Multiplex PCR results. Lane 01, 50 bp DNA ladder (with the following distribution – 50, 100,150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000); Lane 02, *Enterococcus* 009PP2022; Lane 03, *Enterococcus* 010PP2022; Lane 04, *Enterococcus* 012PP2022; Lane 05, *Enterococcus* 013PP2022; Lane 06, *Staphylococcus* 008PP2022; Lane 07, *Staphylococcus* 009PP2022; Lane 08, *Staphylococcus* 010PP2022; Lane 09, *Staphylococcus* 011PP2022; Lane 10, *Staphylococcus* 012PP2022; Lane 11, NTC; Lane 12, 50 bp DNA ladder.



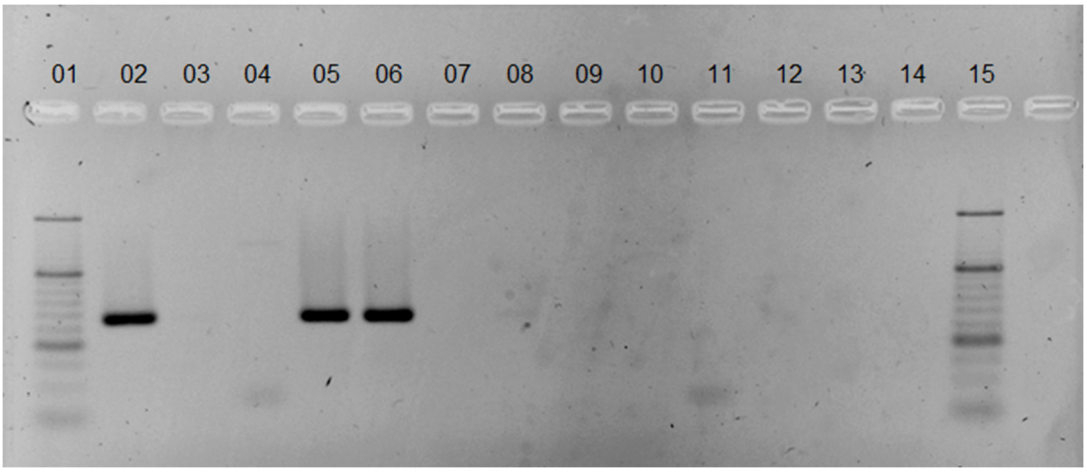
Sraga1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Srdys1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Srube3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Staur3	-	+	+	+	+	-	+	+	-	+	-	-	-	-

Figure S4. Multiplex PCR results. Lane 01, 50 bp DNA ladder (with the following distribution – 50, 100,150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000); Lane 02, *Enterococcus* 011PP2022; Lane 03, *Staphylococcus* 013PP2022; Lane 04, *Staphylococcus* 014PP2022; Lane 05, *Staphylococcus* 015PP2022; Lane 06, *Staphylococcus* 016PP2022; Lane 07, *Staphylococcus* 017PP2022; Lane 08, *Staphylococcus* 018PP2022; Lane 09, *Staphylococcus* 019PP2022; Lane 10, *Staphylococcus* 023PP2022; Lane 11, *Staphylococcus* 025PP2022; Lane 12, *Staphylococcus* 026PP2022; Lane 13, *Staphylococcus* 027PP2022; Lane 14, *Staphylococcus* 030PP2022; Lane 15, *Staphylococcus* 031PP2022; Lane 16, 50 bp DNA ladder.



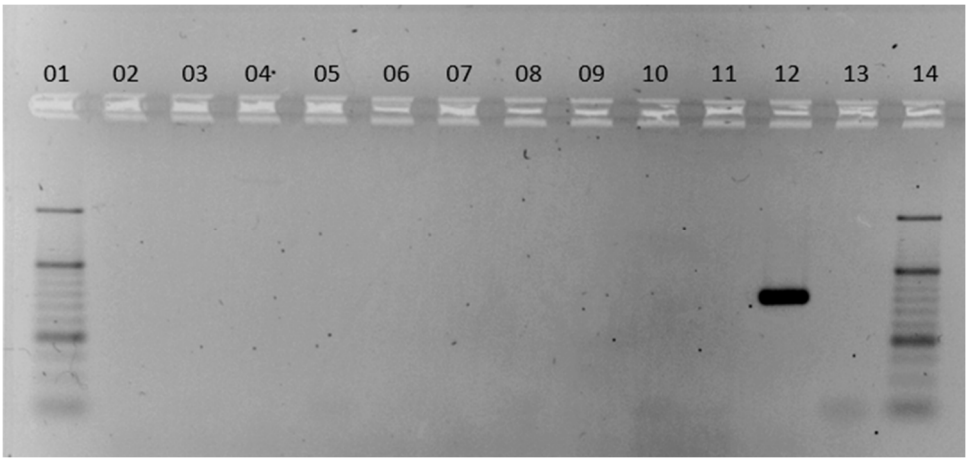
Sraga1	-	-	-	-	-	-	-	-	-	-	-	+	+	-
Srdys1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Srube3	-	-	-	-	-	-	-	+	-	-	-	-	-	+
Staur3	-	-	-	-	-	-	-	+	+	-	-	-	-	-

Figure S5. Multiplex PCR results. Lane 01, 50 bp DNA ladder (with the following distribution – 50, 100,150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000); Lane 02, *Staphylococcus* 032PP2022; Lane 03, *Staphylococcus* 034PP2022; Lane 04, *Staphylococcus* 038PP2022; Lane 05, *Staphylococcus* 039PP2022; Lane 06, *Staphylococcus* 040PP2022; Lane 07, *Staphylococcus* 041PP2022; Lane 08, *Staphylococcus* 042PP2022; Lane 09, *Staphylococcus* 043PP2022; Lane 10, *Staphylococcus* 044PP2022; Lane 11, *Staphylococcus* 045PP2022; Lane 12, *Staphylococcus* 046PP2022; Lane 13, *Streptococcus agalactiae* 021PP2018; Lane 14, *Streptococcus agalactiae* 022PP2021; Lane 15, *Streptococcus* 003PP2017; Lane 16, 50 bp DNA ladder.



Sraga1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Srdys1	-	-	+	-	-	-	-	-	-	+	-	-	-	
Srube3	+	-	-	+	+	-	-	-	-	-	-	-	-	
Staur3	-	-	-	-	-	-	-	-	-	-	-	-	-	

Figure S6. Multiplex PCR results. Lane 01, 50 bp DNA ladder (with the following distribution – 50, 100,150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000); Lane 02, *Streptococcus* 004PP2017; Lane 03, *Streptococcus* 006PP2017; Lane 04, *Streptococcus* 008PP2018; Lane 05, *Streptococcus* 009PP2019; Lane 06, *Streptococcus* 014PP2022; Lane 07, *Streptococcus* 017PP2022; Lane 08, *Streptococcus* 018PP2022; Lane 09, *Staphylococcus warneri* ATCC 27836; Lane 10, *Staphylococcus epidermidis* ATCC 14990; Lane 11, *Streptococcus dysgalactiae* ATCC 12394; Lane 12, *Aerococcus viridans* ATCC 11563; Lane 13, *Enterococcus faecalis* ATCC 29212; Lane 14, NTC; Lane 15, 50 bp DNA ladder.



Sraga1	-	-	-	-	-	-	-	-	-	-	-	-
Srdys1	-	-	-	-	-	-	-	-	-	-	-	-
Srube3	-	-	-	-	-	-	-	-	-	-	-	-
Staur3	-	-	-	-	-	-	-	-	-	-	+	-

Figure S7. Multiplex PCR results. Lane 01, 50 bp DNA ladder (with the following distribution – 50, 100,150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000); Lane 02, *Staphylococcus* 047PP2022; Lane 03, *Staphylococcus* 048PP2022; Lane 04, *Staphylococcus* 049PP2022; Lane 05, *Staphylococcus* 050PP2022; Lane 06, *Staphylococcus* 051PP2022; Lane 07, *Staphylococcus* 052PP2022; Lane 08, *Staphylococcus* 053PP2022; Lane 09, *Staphylococcus* 054PP2022; Lane 10, *Staphylococcus* 055PP2022; Lane 11, *Staphylococcus* 056PP2022; Lane 12, *Staphylococcus* 057PP2022; Lane 13, NTC; Lane 14, 50 bp DNA ladder.

Table S1. Bacterial genomes assemblies used for test designing as true positives to detect conservative core sequence for analyzed species. .

True Positive Streptococcus uberis	*True Positive Streptococcus dysgalactiae*	*True Positive Staphylococcus aureus*	*True Positive Streptococcus agalactiae*
GCF_000975325.1	*GCF_000307185.1*	*GCF_900004855.1*	*GCF_000007265.1*
GCF_000975335.1	*GCF_000317855.1*	*GCF_900017485.1*	*GCF_000012705.1*
GCF_000975345.1	*GCF_000970985.1*	*GCF_900017495.1*	*GCF_000167755.1*
GCF_000975355.1	*GCF_000971005.1*	*GCF_900017505.1*	*GCF_000186445.1*
GCF_000975405.1	*GCF_001038445.1*	*GCF_900017515.1*	*GCF_000196055.1*
GCF_000975415.1	*GCF_001072165.1*	*GCF_900017525.1*	*GCF_000264975.1*
GCF_000975435.1	*GCF_001682735.1*	*GCF_900017535.1*	*GCF_000287955.1*
GCF_000975445.1	*GCF_001682745.1*	*GCF_900017545.1*	*GCF_000287975.1*
GCF_000975485.1	*GCF_001682755.1*	*GCF_900017555.1*	*GCF_000287995.1*
GCF_000975495.1	*GCF_001682765.1*	*GCF_900017565.1*	*GCF_000288015.1*
GCF_000975505.1	*GCF_001682815.1*	*GCF_900017575.1*	*GCF_000288035.1*
GCF_000975545.1	*GCF_001682825.1*	*GCF_900017585.1*	*GCF_000288055.1*
GCF_002814135.1	*GCF_001704435.1*	*GCF_900017595.1*	*GCF_000288075.1*
GCF_009706695.1	*GCF_001704445.1*	*GCF_900017605.1*	*GCF_000288095.1*
GCF_009706705.1	*GCF_001704455.1*	*GCF_900017615.1*	*GCF_000288115.1*

GCF_009706755.1	*GCF_001704515.1*	*GCF_900017625.1*	*GCF_000288135.1*
GCF_009706785.1	*GCF_001704525.1*	*GCF_900017635.1*	*GCF_000288155.1*
GCF_009706805.1	*GCF_002094215.1*	*GCF_900017645.1*	*GCF_000288175.1*
GCF_009706815.1	*GCF_003202195.1*	*GCF_900017655.1*	*GCF_000288195.1*
GCF_009706825.1	*GCF_003967135.1*	*GCF_900017665.1*	*GCF_000288215.1*
GCF_009706845.1	*GCF_008121775.1*	*GCF_900017675.1*	*GCF_000288235.1*
GCF_009706885.1	*GCF_008121785.1*	*GCF_900017685.1*	*GCF_000288255.1*
GCF_009706895.1	*GCF_008121795.1*	*GCF_900017695.1*	*GCF_000288275.1*
GCF_009706905.1	*GCF_008121855.1*	*GCF_900017705.1*	*GCF_000288295.1*
GCF_009706935.1	*GCF_008693725.1*	*GCF_900017715.1*	*GCF_000288315.1*
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GCF_009706985.1	*GCF_009389575.1*	*GCF_900017965.1*	*GCF_000288355.1*
GCF_009706995.1	*GCF_009389595.1*	*GCF_900017975.1*	*GCF_000288375.1*
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GCF_009707045.1	*GCF_009650255.1*	*GCF_900018005.1*	*GCF_000288435.1*
GCF_009707085.1	*GCF_009650275.1*	*GCF_900018015.1*	*GCF_000288455.1*
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Table S2. The analysis of affinity primers in single PCR and multiplex PCR were calculated by Primer-Dimer software.

Single PCR primer-primer interaction			
Forward Primer Name	**Reverse Primer Name**	**Structure**	**dG [kcal/mole]**
Sraga_1_F	Sraga_1_R	heterodimer	-3,58
Srdys_1_F	Srdys_1_R	heterodimer	-0,08
Srube_3_F	Srube_3_R	heterodimer	0
Staur_3_F	Staur_3_R	heterodimer	-1,8
Multiplex PCR primer-primer interaction			
Sraga_1_F	Sraga_1_R	heterodimer	-3,58
Sraga_1_F	Srdys_1_F	heterodimer	-2
Sraga_1_F	Srdys_1_R	heterodimer	0
Sraga_1_F	Srube_3_F	heterodimer	-0,06
Sraga_1_F	Srube_3_R	heterodimer	-1,31
Sraga_1_F	Staur_3_F	heterodimer	-2,11
Sraga_1_F	Staur_3_R	heterodimer	-0,05
Sraga_1_R	Srdys_1_F	heterodimer	-3,58
Sraga_1_R	Srdys_1_R	heterodimer	-3,58
Sraga_1_R	Srube_3_F	heterodimer	-3,58
Sraga_1_R	Srube_3_R	heterodimer	-3,58
Sraga_1_R	Staur_3_F	heterodimer	-3,58
Sraga_1_R	Staur_3_R	heterodimer	-3,58
Srdys_1_F	Srdys_1_R	heterodimer	-0,08
Srdys_1_F	Srube_3_F	heterodimer	-0,08
Srdys_1_F	Srube_3_R	heterodimer	-0,08
Srdys_1_F	Staur_3_F	heterodimer	-1,8
Srdys_1_F	Staur_3_R	heterodimer	-0,08
Srdys_1_R	Srube_3_F	heterodimer	-2,18
Srdys_1_R	Srube_3_R	heterodimer	-2,33
Srdys_1_R	Staur_3_F	heterodimer	-2,37
Srdys_1_R	Staur_3_R	heterodimer	0
Srube_3_F	Srube_3_R	heterodimer	0

Srube_3_F	Staur_3_F	heterodimer	-1,8
Srube_3_F	Staur_3_R	heterodimer	-0,4
Srube_3_R	Staur_3_F	heterodimer	-1,8
Srube_3_R	Staur_3_R	heterodimer	-2,39
Staur_3_F	Staur_3_R	heterodimer	-1,8

Table S3. The statistics of designed PCR tests.

Species	***S. agalactiae***	***S. dysgalactiae***	***S. uberis***	***S. aureus***
Primers	Sraga_1	Srdys_1	Srube_3	Staur_3
Condition positive (P)	1466	132	108	14204
Condition negative (N)	15153	16487	16511	4616
True positive (TP)	1466	132	108	14193
True negative (TN)	15123	16484	16511	4562
False positive (FP), Type I error	30	3	0	53
False negative (FN), Type II error	0	0	0	12
Sensitivity $(\frac{TP}{TP+FN})$	1	1	1	0.9992
Specificity $\frac{TN}{TN+FP})$	0.9980	0.9998	1	0.9883
Precision $(\frac{TP}{TP+FP})$	0.9799	0.9778	1	0.9963
Prevalence $(\frac{P}{P+N})$	0.0882	0.0079	0.0065	0.7547
Accuracy $(\frac{TP+TN}{P+N})$	0.9982	0.9998	1	0.9965

Table S4. Characteristics of strains used in the study.

Strain	**Taxonomy reclassification after bioinformatics analysis**	**Animal species**	**Origin**
S. warneri ATCC 27836	*n/a*	Human	no info
A. viridans ATCC 11563	*n/a*	no info	Environmental
E. faecalis ATCC 29212	*n/a*	no info	no info
S. aureus ATCC 6538P	-	no info	no info
S. aureus PCM 1102	-	Human	Clinical material
S. aureus PCM 1115	-	Human	Clinical material
S. aureus PCM 1116	-	Human	Clinical material
S. aureus PCM 1650	-	Human	Clinical material
S. aureus PCM 1937	-	Human	Clinical material
S. aureus PCM 2054	-	no info	no info
S. aureus PCM 2101	-	no info	no info
S. aureus PCM 2267	-	no info	no info
S. aureus PCM 458/2195	-	no info	no info
S. aureus PCM 502	-	Human	Pleural fluid
S. aureus PCM 565	-	Human	Empyema
S. epidermidis ATCC 14990	*n/a*	no info	no info
A. viridans 007PP2021	*A. urinaeequi* 002PP2021	Dairy cow	Udder milk
A. viridans 008PP2021	*M. sciuri* 004PP2021	Dairy cow	Udder milk
E. faecalis 022PP2021	-	Dairy cow	Udder milk
E. faecium 003PP2021	-	Dairy cow	Udder milk
E. faecium 004PP2021	-	Dairy cow	Udder milk
Enterococcus sp. 009PP2022	*n/a*	Dairy cow	Udder milk
Enterococcus sp. 010PP2022	*n/a*	Dairy cow	Udder milk

Strain	Taxonomy reclassification after bioinformatics analysis	Animal species	Origin
Enterococcus sp. 011PP2022	*n/a*	Dairy cow	Udder milk
Enterococcus sp. 012PP2022	*n/a*	Dairy cow	Udder milk
Enterococcus sp. 013PP2022	*n/a*	Dairy cow	Udder milk
S. agalactiae 021PP2018	*n/a*	Dairy cow	Udder milk
S. agalactiae 022PP2021	*n/a*	Dairy cow	Udder milk
S. agalactiae 023PP2021	-	Dairy cow	Udder milk
S. agalactiae 024PP2021	-	Dairy cow	Udder milk
S. agalactiae 025PP2021	*S. dysgalactiae* 004PP2021	Dairy cow	Udder milk
S. agalactiae 026PP2021	*S. dysgalactiae* 005PP2021	Dairy cow	Udder milk
S. agalactiae 027PP2021	-	Dairy cow	Udder milk
S. dysgalactiae 001PP2016	*n/a*	Dairy cow	Udder milk
S. dysgalactiae 002PP2016	*E. casseliflavus* 002PP2016	Dairy cow	Udder milk
S. dysgalactiae 003PP2016	*S. uberis* 120PP2022	Dairy cow	Udder milk
S. epidermidis 001PP2022	*S. haemolyticus* 004PP2022	Swine	Skin wound
S. sciuri 001PP2020	-	Dairy cow	Udder milk
S. sciuri 002PP2021	*S. agalactiae* 028PP2022	Dairy cow	Udder milk
S. sciuri 003PP2021	*S. equorum* 003PP2022	Dairy cow	Udder milk
S. uberis 041PP2021	-	Dairy cow	Udder milk
S. uberis 045PP2021	-	Dairy cow	Udder milk
S. uberis 059PP2021	-	Dairy cow	Udder milk
S. uberis 067PP2021	-	Dairy cow	Udder milk
S. uberis 071PP2021	-	Dairy cow	Udder milk
S. uberis 103PP2021	-	Dairy cow	Udder milk
S. uberis 105PP2021	-	Dairy cow	Udder milk
S. uberis 112PP2021	-	Dairy cow	Udder milk
S. xylosus 001PP2021	*S. equorum* 001PP2022	Dairy cow	Udder milk
S. xylosus 002PP2022	*S. equorum* 002PP2022	Dairy cow	Udder milk
S. xylosus 003PP2022	*M. vitulinus* 001PP2022	Dairy cow	Udder milk
Staphylococcus sp. 008PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 009PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 010PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 011PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 012PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 013PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 014PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 015PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 016PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 017PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 018PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 019PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 023PP2022	*n/a*	Swine	Skin wound
Staphylococcus sp. 025PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 026PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 027PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 030PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 031PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 032PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 034PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 038PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 039PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 040PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 041PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 042PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 043PP2022	*n/a*	Dairy cow	Udder milk

Strain	Taxonomy reclassification after bioinformatics analysis	Animal species	Origin
Staphylococcus sp. 044PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 045PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 046PP2022	*n/a*	Dairy cow	Udder milk
Staphylococcus sp. 047PP2022	*S. pseudintermedius* 001PP2023	Dog	Urine
Staphylococcus sp. 048PP2022	*S. pseudintermedius* 002PP2023	Dog	Eye
Staphylococcus sp. 049PP2022	*S. simulans* 002PP2022	Cat	Throat
Staphylococcus sp. 050PP2022	*S. pseudintermedius* 003PP2023	Dog	Ear
Staphylococcus sp. 051PP2022	*S. pseudintermedius* 004PP2023	Dog	Vagina
Staphylococcus sp. 052PP2022	*S. pseudintermedius* 005PP2023	Dog	Vagina
Staphylococcus sp. 053PP2022	*S. pseudintermedius* 006PP2023	Dog	Vagina
Staphylococcus sp. 054PP2022	*S. pseudintermedius* 007PP2023	Dog	Urine
Staphylococcus sp. 055PP2022	*S. pseudintermedius* 011PP2023	Cat	Urine
Staphylococcus sp. 056PP2022	*S. pseudintermedius* 012PP2023	Dog	Skin
Staphylococcus sp. 057PP2022	*n/a*	Dog	Wound
Staphylococcus sp. 059PP2022	*M. sciuri* 005PP2023	Dog	Larynx
Streptococcus sp. 003PP2017	*n/a*	Dairy cow	Udder milk
Streptococcus sp. 004PP2017	*n/a*	Dairy cow	Udder milk
Streptococcus sp. 006PP2017	*n/a*	Dairy cow	Udder milk
Streptococcus sp. 008PP2018	*n/a*	Dairy cow	Udder milk
Streptococcus sp. 009PP2019	*n/a*	Dairy cow	Udder milk
Streptococcus sp. 014PP2022	*n/a*	Dairy cow	Udder milk
Streptococcus sp. 017PP2022	*n/a*	Dairy cow	Udder milk
Streptococcus sp. 018PP2022	*n/a*	Dairy cow	Udder milk
NTC – No Template Control			

n/a – not applicable, strain was not sequenced; dash - no change in taxonomy after sequencing