LWT - Food Science and Technology 173 (2023) 114287

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Development and application of multiple polymerase spiral reaction (PSR) assays for rapid detection of methicillin resistant *Staphylococcus aureus* and toxins from rice and flour products

Junyan Liu [a,b,1], Tengyi Huang [c,1], Yuzhu Mao [d], Thanapop Soteyome [e], Gongliang Liu [a,b], Gamini Seneviratne [f], Birthe V. Kjellerup [d], Zhenbo Xu [c,*]

[a] College of Light Industry and Food Science, Guangdong Provincial Key Laboratory of Lingnan Specialty Food Science and Technology, Academy of Contemporary Agricultural Engineering Innovations, Zhongkai University of Agriculture and Engineering, Guangzhou, 510225, China
[b] Key Laboratory of Green Processing and Intelligent Manufacturing of Lingnan Specialty Food, Ministry of Agriculture, Guangzhou, 510225, China
[c] Department of Laboratory Medicine, The Second Affiliated Hospital of Shantou University Medical College, Shantou, Guangdong, China
[d] Department of Civil and Environmental Engineering, University of Maryland, College Park, MD 20742, United States
[e] Home Economics Technology, Rajamangala University of Technology Phra Nakhon, Bangkok, Thailand
[f] National Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka

ARTICLE INFO

Keywords:
Methicillin-resistant *Staphylococcus aureus* (MRSA)
Polymerase spiral reaction (PSR)
Food poisoning
Rapid detection

ABSTRACT

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is an important foodborne pathogen and produces a variety of toxins causing serious illnesses. This study aimed at developing a rapid detection assay for MRSA and toxins targeting on two housekeeping genes (*mecA* and *femA*) and 5 virulence factors (*sea*, *seb*, *sec*, *see* and *pvl*) using the polymerase spiral reaction (PSR), compared with PCR. For naked-eye result inspection, calcein was added and the PSR reaction can be completed at 65°C within 60 min. The PSR assay was subjected to optimization, evaluation, and application in 3 different types of food sample made from rice and flour, designated rice and flour product. The limit of detection of PSR assay for the *mecA*, *femA*, *sea*, *seb*, *sec*, *see* and *pvl* genes was 4.2 pg/μL, 0.42 pg/μL, 630 pg/μL, 63 pg/μL, 12.3 pg/μL, 1.015 pg/μL and 53 pg/μL, respectively, which was higher than the sensitivity of PCR. In rice and flour products, the detection limit of PSR assay was 10^4 CFU/mL for *mecA*, and 10^3 CFU/mL for *femA*, *sea*, *seb*, *sec*, *see* and *pvl* genes. In conclusion, optimized PSR assay serves as an efficient tool for rapid, cost-effective and accurate testing of MRSA and is suitable for point-of-care detection.

1. Introduction

Food safety is a significant issue that affects everyone throughout the world. Unsafe food causes more than 200 diseases, ranging from diarrhea to cancers (Radovanovic, 2011; Gao et al., 2022). Amongst, foodborne pathogen is one of the leading causes of food mediated infection and diseases (Gizaw, 2019). *Staphylococcus aureus* is an important food pathogen in many countries, and it is responsible for Staphylococcal Food Poisoning (SFP). Methicillin-resistant *S. aureus* (MRSA) is one of most widely distributed human and animal pathogens which can produce many toxins and cause a range of serious illnesses (Su et al., 2014; Tacconelli et al., 2009). MRSA was previously limited as a clinical pathogen, but recently it can be associated with food safety shown by its occurrence in retailed meat products thus causing a risk for occupational staff in the food industry (Kadariya et al., 2014; Köck et al., 2012; Voss et al., 2005). Since food products contaminated with MRSA may not exhibit any visual spoilage appearance or bad smell, it is challenging for consumers to detect the contaminated foods.

The standard method for *S. aureus* in food industry is CFU counting. However, it is time consuming and unable to identify virulence gene carriage. Current methods available for detection of MRSA include standard clinical testing, molecular methods such as polymerase chain reaction (PCR) and real-time fluorescence PCR (Corrente et al., 2007; Palavecino, 2020). PCR inhibitors will compromise the sensitivity of PCR present in food samples (Monteiro et al., 1997), these disadvantages have significantly restricted further application. Recently, isothermal amplification methodologies have received much attention due to omission of thermocyclers, presence of simple protocols, fast analysis

* Corresponding author. Department of Laboratory Medicine, The Second Affiliated Hospital of Shantou University Medical College, Shantou, Guangdong, China.
E-mail address: zhenbo.xu@hotmail.com (Z. Xu).
[1] These authors contribute equally to the study.

https://doi.org/10.1016/j.lwt.2022.114287
Received 9 August 2022; Received in revised form 6 December 2022; Accepted 8 December 2022
Available online 9 December 2022

and they have an analytical performance compared to PCR(Dean et al., 2001; Euler et al., 2012; Tomita et al., 2008). A novel isothermal amplification strategy, Polymerase Spiral Reaction (PSR), exhibits by Jiang et al. (2016) present high performance in gene amplification by completing DNA amplification under constant temperature, which is simple, rapid, high sensitive and cost effectiveness (Liu et al., 2015). Using only one pair of primers that span three distinct sequences of a target gene, the entire procedure can be completed within 60 min in a water bath or a heating block. The measurement of PSR products is based on turbidity, electrophoresis of amplicons, a double-strand DNA-specific fluorescent dye for color change or visual observation by applying calcein or SYBR Green II into the reaction tube. Until now, PSR assays were used for detection of pathogens (Jiang et al., 2016; Liu et al., 2018). However, there has been no mention of report of PSR assays developed or applied for detection of MRSA.

In this study, we aimed to develop a rapid, accurate and cost-effective testing method for detecting on MRSA strains and virulence genes based on PSR, including the species specific, methicillin-resistant determinant MRSA, as well as 5 common toxins. Also, the developed PSR assays have been further subjected to optimization, evaluation and application in 3 different rice and flour products.

2. Materials and methods

2.1. Study design

A comprehensive of MRSA detection had been designed, (i) *femA* has been aligned among all *Staphylococcus* strains and the *S. aureus* species-specific region was selected to differentiate between *S. aureus* and coagulase-negative *Staphylococci* or strains other than *S. aureus*; (ii) *mecA* was selected as methicillin resistant determinants; (iii) four types of staphylococcal enterotoxins (SEs) responsible for staphylococcal food poisoning (SFP) were chosen, including *sea*, *seb*, *sec* and *see*. (iv) *pvl* was selected as a major pathogenic gene in MRSA. *Pvl* gene has been shown to strongly linked to community-acquired MRSA (CA-MRSA) (Lindsay, 2009). Since foodborne MRSA (such as livestock associated MRSA) falls more often into the scope of CA-MRSA than hospital-associated MRSA (HA-MRSA), it is presumed to be responsible for the high prevalence of *pvl* in foodborne MRSA strains.

2.2. Bacteria strains

PSR assays were developed and evaluated on a total of 37 strains, including 5 MRSA strains, 14 methicillin sensitive *S. aureus* (MSSA) strains, and 18 non-Staphylococcus strains (Table 1). All strains used in this study were phenotypically (culturing and identification) and genotypically (PCR and Sanger sequencing) identified to the species level, followed by PCR detection on *sea*, *seb*, *sec*, *see* and *pvl* genes and Sanger sequencing (for positive samples) on their PCR products.

2.3. Template DNA extraction

The bacterial whole DNA extraction kit (Dongsheng Biotech Co. Ltd., Guangzhou, China) was used to extract template DNA from MRSA, MSSA, and 18 non-Staphylococcus strains. At 260 and 280 nm, the concentration and purity were measured respectively (Nano Drop 2000, Thermo Fisher Scientific Inc, Waltham, MA, USA).

2.4. Development of PSR assays

Primer Premier 5 was used to design primers for each of the seven targets for PSR application (Table 2). PSR assays were carried out in 25 μL (for electrophoresis determination) or 26 μL (for color change determination) volume containing: 20.0 mM Tris-HCl, 10.0 mM KCl, 10.0 mM $(NH4)_2SO_4$, 8.0 mM, $MgSO_4$, 0.7 M betaine (Sigma, USA), 1.4 mM dNTP mix, 0.1% Tween 20, 8 U *Bst* DNA polymerase large fragment (NEB, USA), 0.8 μM (each) of the primers IF and IB, 1.6 μM (each) of the primers Ft and Bt, 1 μL mixture chromogenic agent ($MgCl_2$ and calcein, for color change determination only), 2 μL template DNA, nuclease free water was used to increase the volume to 25 μL or 26 μL. PSR reaction mixtures were incubated at 65 °C for 60 min before being heated to 80 °C for 2 min to complete the reaction. The negative control is a mixture without DNA template. Electrophoresis on 1.5% agarose gels with ethidium bromide staining were used to detect the amplified products.

Table 1
Bacterial strains used for PSR assays.

Reference strains	No.	PSR/PCR assays						
		mecA	femA	sea	seb	sec	see	pvl
Staphylococcus aureus (MRSA) 0314030635	1	+	+	−	−	−	+	−
Staphylococcus aureus (MRSA) 971311004	1	+	+	−	−	−	−	−
Staphylococcus aureus (MRSA) 0313113664	1	+	+	−	−	−	−	−
Staphylococcus aureus (MRSA) 0314030668	1	+	+	−	−	−	−	−
Staphylococcus aureus (MRSA) 10071	1	+	+	−	−	−	−	+
Staphylococcus aureus (MSSA) 132115, 0315022822, 0613120003, 0314020129	4	−	+	+	+	+	−	−
Staphylococcus aureus (MSSA) 0315011480	1	−	+	−	+	−	−	−
Staphylococcus aureus (MSSA) 130149	1	−	+	−	−	+	−	−
Staphylococcus aureus (MSSA) 132113, 0314030635, 971310004, 132112	4	−	+	−	−	−	+	−
Staphylococcus aureus (MSSA) 0315040330	1	−	+	−	−	−	−	+
Staphylococcus aureus (MSSA) 0713100037	1	−	+	−	−	−	−	+
Staphylococcus aureus (MSSA) 0314020556	1	−	+	−	−	−	−	+
Staphylococcus aureus (MSSA) 0315011480	1	−	+	−	−	−	−	+
Escherichia coli O157 ATCC43895	1	−	−	−	−	−	−	−
Escherichia coli O157 E019, E020, E043, E044	4	−	−	−	−	−	−	−
Salmonella ATCC29629, ATCC19585, ATCC14028, ATCC13076,	4	−	−	−	−	−	−	−
Listeria monocytogenes ATCC19113/4/5/6/8, ATCC15313	6	−	−	−	−	−	−	−
Vibrio parahaemolyticus ATCC27969, ATCC17802	2	−	−	−	−	−	−	−
Lactobacillus casei	1	−	−	−	−	−	−	−

Table 2
List of oligonucleotide primer sequence.

Target gene	Primer	Sequences (5′-3′)
femA	Ft	AGGTATAGACTTCGATGTTTCAAATCGCGGTCCAGTG
	Bt	TTGTAGCTTCAGATATGGAAACCAATCATTACCAGCA
mecA	Ft	CCAATAACTGCATCATCTGCGACTTCACATCTATTAGG
	Bt	TCTACTACGTCAATAACCGACACGATAGCCATCTTCA
Sea	Ft	GTCTAGCCATAAATTGATTGGGTGGTACACCAAACAAAACA
	Bt	GGTTAGTTAAATACCGATCTGGCTTGAAGATCCAACTCCTG
Seb	Ft	TAGCTCCAAACACATCTACGGGACACTAAGTTAGGGAATT
	Bt	GCATCTACACAAACCTCGATTACACCACCATACATACAAG
Sec	Ft	CATAGTAATTTGATCCATACGAAGATTTAGCAAAGAAG
	Bt	CATACCTAGTTTAATGATACCATACAAGTTTTACCACC
See	Ft	AATCATAACTTACCGTGGACGGTAAGGTGCAAAGAGGC
	Bt	CAGGTGCCATTCAATACTAACAAATCAATATGGAGGTTC
Pvl	Ft	AGTTCTATAGCTTTCTTGTTAACGGCTTATCAGGT
	Bt	TGTTCTTTCGATATCTTGATGTGCTTCAACATCCC

2.5. Optimization and specificity of PSR assays

To test primer specificity, genomic DNA from five targeted strains (MRSA strains carrying the *sea, seb, sec, see,* and *pvl* genes) was amplified. The concentration ratios of calcein and Mn^{2+} were measured in the range of 1:20 to 1:2. Six concentrations of betaine were measured as it is needed for DNA melting: 0.4 M, 0.5 M, 0.6 M, 0.7 M, 0.8 M, and 0.9 M.

2.6. Limit of detection (LOD) of PSR assays

The LOD of the PSR assays was evaluated using 10-fold serial dilutions of genomic DNA and 1.5% agarose gel electrophoresis. All experiments were performed in triplicate.

2.7. Artificial contamination of MRSA in food samples

Three types of rice and flour food products including mantou, radish pastry and Cantonese cake (Guangzhou Restaurant Group Co., Ltd., Guangzhou) were used. For sample processing, 25 g of rice and flour products were applied to 225 mL saline, tested strains were incubated in TSB (Huankai Biology Co., Ltd., Guangzhou, China) overnight (~10^8 CFU/mL), followed by artificial contamination in food samples to concentrations ranging from 10-10^8 CFU/mL (Ou et al., 2021). A rapid processing method was used to extract DNA from artificially contaminated food samples, following by PSR reaction. The LOD was tested using five MRSA strains and a 10-fold serial dilution, as mentioned above. Each PSR assay was performed with a starting concentration of 10^7 CFU/mL on all of the strains tested.

3. Results

3.1. Development and optimization of PSR assays

PSR assays for the seven targets were developed, and amplified products were detected using agarose gel electrophoresis. PSR reactions generate different size amplicons consisting of alternately inverted repeats of each target, resulting in typical ladder band patterns (Fig. 1). In addition to electrophoresis, color observation was performed for the PSR system using 1 μL mixture chromogenic agent ($MgCl_2$ and calcein), where the dye color changed from orange (negative) to green (positive) (Fig. 1). In terms of optimization, the optimal ratio of calcein to Mn^{2+} was discovered to be 1:4. The rate of false positives increased when the ratio reached 1:2. (Fig. 2). Also, the optimum concentration of betaine was discovered to be 0.7 M, and as the color change resulted in a clear solution, when the betaine concentration was higher than 0.6 M (Fig. 3).

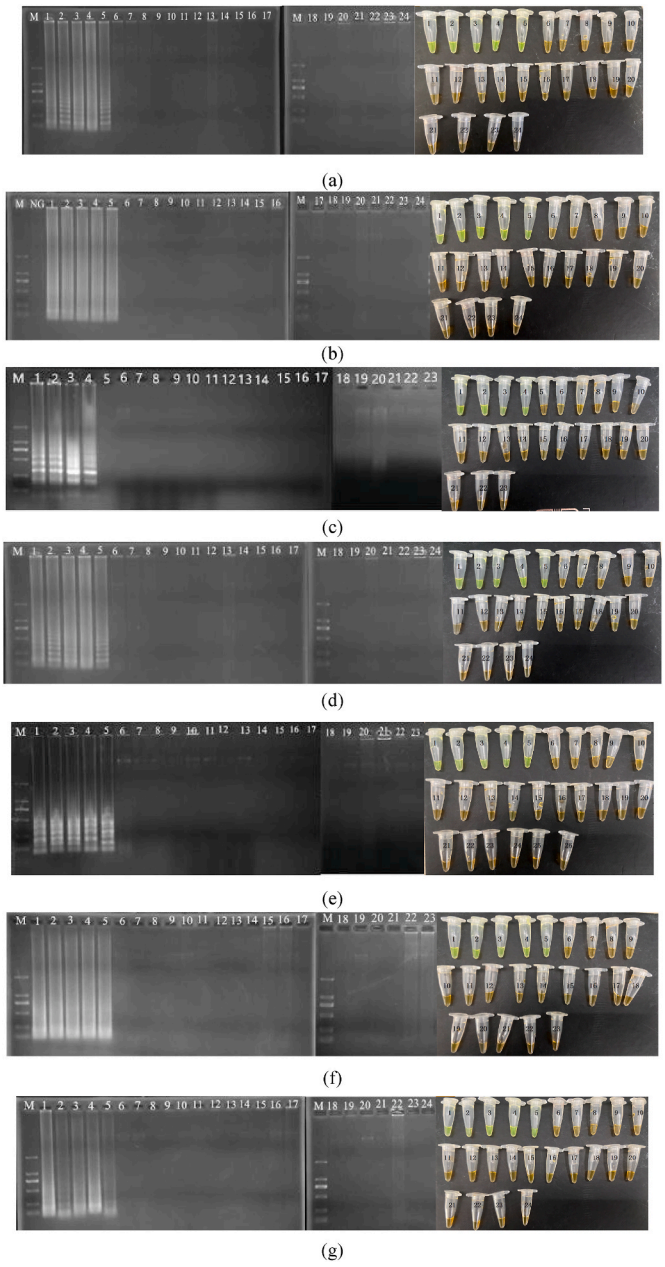

Fig. 1. Specificity of PSR detection for different strains by 1.5% agarose gel electrophoresis and mixed chromogenic agent.
For *femA* (a) and *mecA* (b)genes, lanes/tubes 1–5, *Staphylococcus aureus* 0314030635, 971311004, 0313113664, 0314030668, 10071; lanes/tubes 6–24; non-MRSA strains, negative control.
For *sea* (c) genes, lanes/tubes 1–4, *Staphylococcus aureus* 132115, 0315022822, 0613120003, 0314020129; lanes/tubes 5–23, non-*sea* strains, negative control.
For *seb* (d) genes, lanes/tubes 1–5 *Staphylococcus aureus* 132115, 0315022822, 0613120003, 0314020129, 0315011480, lanes/tubes 5–24, non-*seb* strains, negative control.
For *sec* (e) genes, lanes/tubes 1–5, 132115, 0315022822, 0613120003, 0314020129, 130149; lanes/tubes 6–23, non-*sec* strains, negative control.
For *see* (f) genes, lanes/tubes 1–5, 0315040330, 132113, 0314030635, 971310004, 132112; lanes/tubes 6–23, non-*see* strains, negative control.
For *pvl* (g) genes, lanes/tubes 1–5, 10071, 0713100037, 0315011480, 0315040330, 0314020556. lanes/tubes 6–24, non-*pvl* strains, negative control.

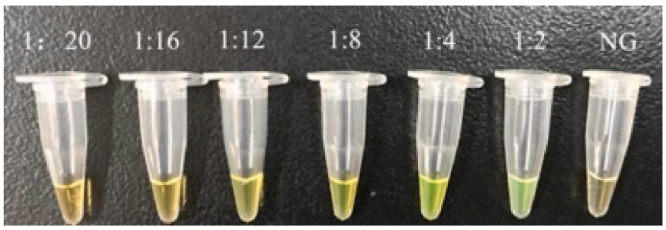

Fig. 2. Optimization of the concentration ratio of calcein and Mn^{2+} in the PSR assay, the optimal ratio of calcein and Mn^{2+} was found to be 1:4. However, when the ratio reached 1:2, the rate of false positive increased.

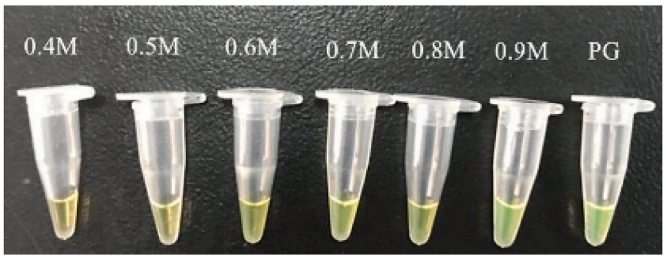

Fig. 3. Optimization of betaine concentration in the PSR assay, PG-positive control, the optimal concentration of betaine was found to be 0.7 M, as the color change resulted in a clear solution, when the betaine concentration was higher than 0.6 M. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.2. Specificity and LOD of PSR assays

Negative amplification was obtained when any single primer for each target was omitted, suggesting that each primer was needed for the PSR reaction. All PSR assays were subjected to all tested strains for detection specificity, and no cross-reactions were detected, implying that the designed PSR assays have a high specificity (Fig. 4). As sensitivity was concerned, LOD for genomic DNA was 4.2 pg/μL for *femA*, 0.42 pg/μL for *mecA*, 630 pg/μL for *sea*, 63 pg/μL for *seb*, 12.3 pg/μL for *sec*, 1.015 pg/μL for *see* and 53 pg/μL for *pvl*, respectively. The results determined by color change were consistent to those from agarose gel electrophoresis, indicating the applicability of. Comparing with regular PCR, PSR exhibited 10–1000 higher LOD, meaning the significantly higher sensitivity of PSR than PCR.

3.3. LOD and application of PSR assays in artificially contaminated food samples

In terms of the LOD of each PSR assay in artificially contaminated food samples, similar LODs were obtained for each target in various rice and flour products. In detail, LOD was found to be for 10^4 CFU/mL *femA*, 10^3 CFU/mL for *mecA*, 10^3 CFU/mL for *sea*, 10^3 CFU/mL for *seb*, 10^3 CFU/mL for *sec*, 10^3 CFU/mL for *see* and 10^3 CFU/mL for *pvl*, respectively (Fig. 5). No false positive amplification was detected in the specificity test, suggesting that each PSR assay had high specificity and LOD. In addition, as compared to regular PCR, PSR had a 10–1000 higher LOD, suggesting that PSR has a significantly higher sensitivity than PCR.

4. Discussion

MRSA is one of the most successful modern pathogens (Turner et al., 2019). The widespread and rapid growth of community-associated MRSA and livestock-associated MRSA have raised the concern of MRSA in food industry (Wendlandt et al., 2013). As a result of the prevalence of MRSA in multiple food samples, a timely and accurate

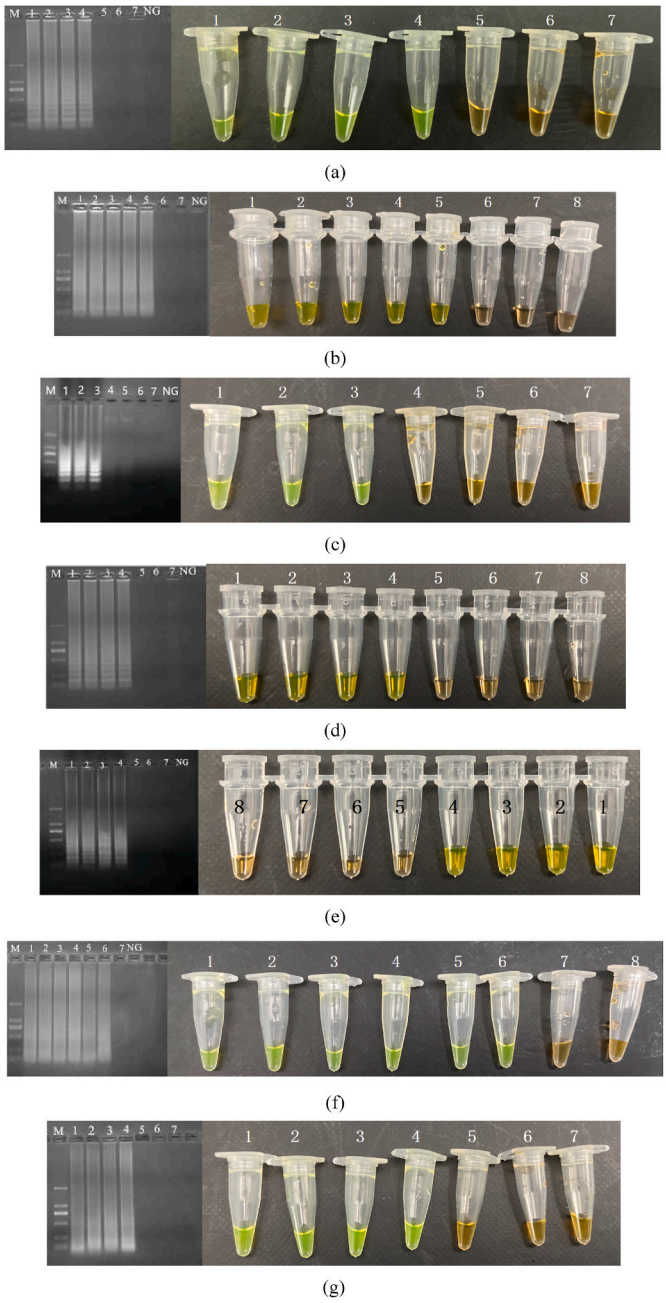

Fig. 4. Sensitivity of the PSR assay in genomic DNA by 1.5% agarose gel electrophoresis and mixed chromogenic agent.
Sensitivity from 10071 of *femA* (a) and *mecA* (b) genes. M, DNA marker; lanes/tubes 1–7, 53 ng/μL, 5.3 pg/μL, 530 pg/μL, 53 pg/μL, 5.3 pg/μL, 530 fg/μL, 53 fg/μL.
Sensitivity from 132115 of *sea* (c) and *seb*(d) genes by 1.5% agarose gel electrophoresis. lanes/tubes 1–7, 63 ng/μL, 6.3 pg/μL, 630 pg/μL, 63 pg/μL, 6.3 pg/μL, 630 fg/μL, 63 fg/μL.
Sensitivity from 0313110664 of *sec* (e) genes. lanes/tubes 1–7, 12.3 ng/μL, 1.23 ng/μL, 123 pg/μL, 12.3 pg/μL, 1.23 pg/μL, 123 fg/μL, 12.3 fg/μL.
Sensitivity from 0314030635 of *see* (f) genes, lanes/tubes 1–7, 101.5 ng/μL, 10.05 ng/μL, 1.005 ng/μL, 101.5 pg/μL, 10.15 pg/μL, 1.015 pg/μL, 101.5 fg/μL.
Sensitivity from 10071 of *pvl* (g) genes, lanes/tubes 1–7, 53 ng/μL, 5.3 ng/μL, 530 pg/μL, 53 pg/μL, 5.3 pg/μL, 530 fg//μL, 53 fg/μL. NG, Negative control.

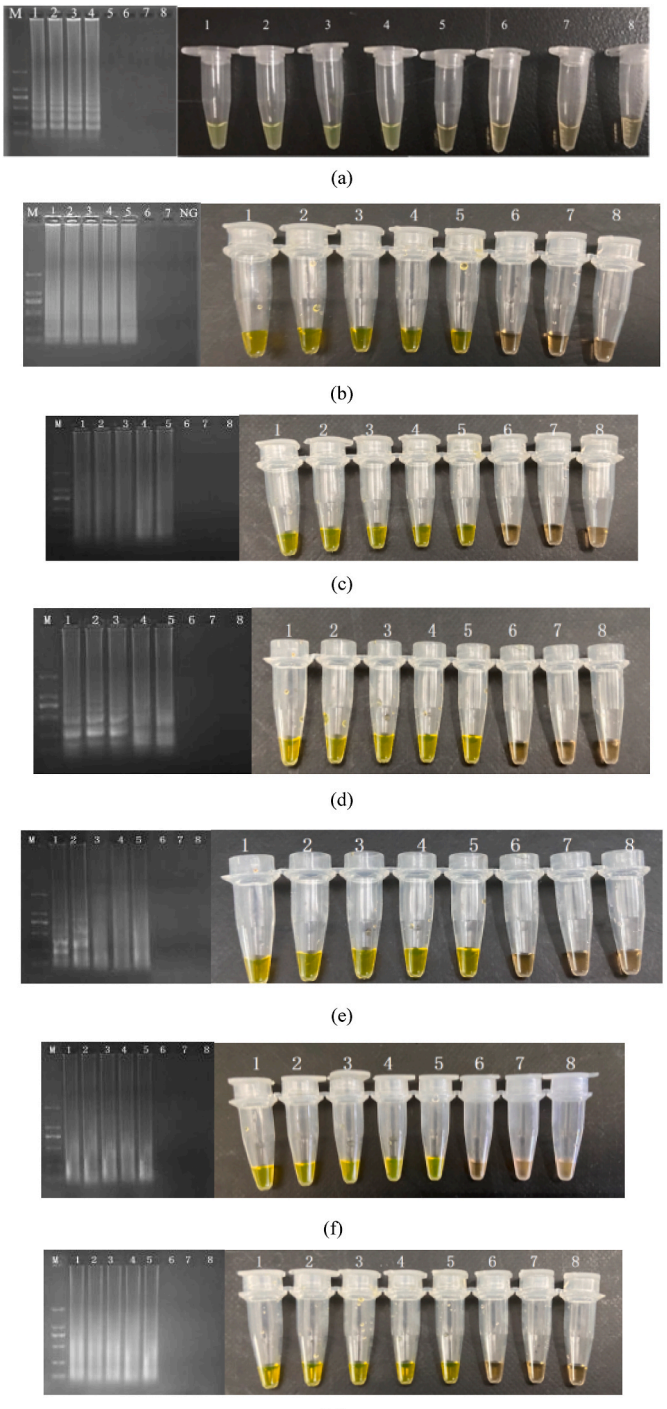

Fig. 5. Sensitivity of the PSR by 1.5% agarose gel electrophoresis and mixed chromogenic agent in food samples of *femA* (a), *mecA* (b), *sea* (c), *seb* (d), *sec* (e), *see* (f), *pvl* (g). M, DNA marker; lanes/tubes 1–7, artificially contaminated MRSA concentration at 10^7 CFU/mL, 10^6 CFU/mL, 10^5 CFU/mL, 10^4 CFU/mL, 10^3 CFU/mL, 10^2 CFU/mL, 10^1 CFU/mL, 1 CFU/mL.

detection method is in need (Crago et al., 2012). The standard method for microbe detection in food industry is CFU counting. However, it is time consuming and unable to identify specific strain types, including MRSA. In addition, capability to produce toxins including staphylococcal enterotoxins and Panton-Valentine leukocidin enables MRSA causing SFP, representing a potential hazard for consumers (Hennekinne et al., 2012). Nowadays, PCR is the most widely used method to detect strains in species specific level and their carriage of different genes (Graveland et al., 2011). Thus, in this study, in comparison with PCR assay, a detection strategy was designed targeting methicillin-resistance determinant *mecA*, *S. aureus*-specific *femA*, and five virulence genes (*sea*, *seb*, *sec*, *see* and *pvl*) to aid in the detection MRSA and its pathogenic factors in food settings.

One point limited the application of PCR-based assays in food industry especially on-site detection is the requirement of highly qualified equipment (precise thermal cycler) and staff. In 2015, a novel PSR method combining the advantage of PCR in which only one pair of primers is needed and isothermal amplification techniques such as RCR and 3SR was developed (Sharma et al., 2022). Such technique has facilitated on-site detection as precise thermal cycler is not required in isothermal nucleic acid amplification reaction. Upon the development of PSR targeting $bla_{\text{NDM-1}}$ gene for potential application in clinical setting, it had been applied in the rapid detection of multiple species including *Mycobacterium tuberculosis* (Liu et al., 2018), *Salmonella* (Momin et al., 2020; Xu et al., 2019), *Clostridium perfringens* (Milton et al., 2021), *Trypanosoma evansi* (Sharma et al., 2022), *Actinobacillus pleuropneumoniae* (Sarkar et al., 2022), *Candida albicans* (Jiang et al., 2016), *Pseudomonas aeruginosa* (Dong et al., 2015), *Brucella* (Das et al., 2018), *Vibrio parahaemolyticus* (He et al., 2020), and *Listeria monocytogenes* (Chen et al., 2022). However, the application of PSR on the detection of MRSA had not been reported. In this research, visual PSR assays targeting seven genes were used to establish a new method for accurately detecting MRSA and its pathogenicity. The developed PSR assay could detect MRSA and its carriage of virulence genes within 60 min. The whole experiment could be performed in a water bath over a wide temperature range (Ou et al., 2021; Wu et al., 2019). The PSR assay requires only one pair of primers for amplification, thus saving time, effort, and money in the process of designing and upgrading primers (Wu et al., 2019). Also, the PSR results are visible by color change (orange to green), avoiding open lid thus reducing false positive detection rate. Most importantly, the specificity the developed PSR assay was 100% and the sensitivity was 10–1000 times higher than that of PCR method.

In addition, concerning on-site detection, the efficiency and sensitivity of detection method can be influenced by environmental factors, especially complicated composition in food samples. The PSR technique applied to date had been rarely applied in food samples, except the detection of *Salmonella* (Momin et al., 2020; Xu et al., 2019) and *L. monocytogenes* (Chen et al., 2022). In this study, artificially contaminated food samples were used to test the applicability of the PSR assay to detect MRSA from various rice and flour products, without pretreatment including sample enrichment and DNA isolation. Sensitivity was determined by using food samples contaminated with different concentration of MRSA cells. No false positive amplification and a 10–1000 higher LOD than PCR, suggesting that the PSR assay has high efficiency and sensitivity to detect MRSA and its virulence genes in food samples.

5. Conclusion

In conclusion, this is the first study on the development of PSR assay for rapid and visual detection of MRSA and its virulence genes especially in rice and flour products. The limit of detection of PSR assay for *mecA*, *femA*, *sea*, *seb*, *sec*, *see* and *pvl* genes was 4.2 pg/μL, 0.42 pg/μL, 630 pg/μL, 63 pg/μL, 12.3 pg/μL, 1.015 pg/μL and 53 pg/μL, respectively, which was 10–1000 times higher than the sensitivity of PCR method. In rice and flour products, the detection limit for *mecA*, *femA*, *sea*, *seb*, *sec*, *see* and *pvl* genes was 10^4 CFU/mL, 10^3 CFU/mL, 10^3 CFU/mL, 10^3 CFU/mL, 10^3 CFU/mL, 10^3 CFU/mL and 10^3 CFU/mL, respectively. Within 60 min, the target genes were successfully amplified at 65°C. Thus, the PSR assays can be useful and efficient tools for detecting MRSA and its pathogenicity rapidly. The advantages of PSR assay over traditional methods make it an ideal tool for food-borne pathogen diagnosis in both industrial and clinical settings. With such benefits, the developed PSR assay could be a valuable addition to current approaches for detecting

MRSA in foodstuffs during surveillance programs and outbreak investigations.

Funding information

This work was supported by the National Natural Science Foundation of China (32202199), Guangdong Major Project of Basic and Applied Basic Research (2020B0301030005), Guangdong International S&T Cooperation Programme (2022A0505070001),Guangdong Basic and Applied Basic Research Foundation Natural Science Foundation (Zhenbo Xu), Young S&T Talent Training Program of Guangdong Provincial Association for S&T, China (SKXRC202207), Young Talent Support Project of Guangzhou Association for Science and Technology (QT20220101076), Guangdong Province Key Laboratory for Green Processing of Natural Products and Product Safety (202207), Guangzhou Basic and Applied Basic Research Project (Junyan Liu), Guangdong Provincial Key Laboratory of Lingnan Specialty Food Science and Technology (No. 2021B1212040013), Guangdong Province Key Construction Discipline Research Ability Improvement Project (2021ZDJS005), Guangdong Provincial Agricultural Science and Technology Innovation and Extension Project in 2021 (No. 2021KJ101).

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

Not applicable.

CRediT authorship contribution statement

Junyan Liu: Validation, Funding acquisition, Investigation, Writing - review & editing. **Tengyi Huang:** Investigation, Methodology, Writing – original draft. **Yuzhu Mao:** Investigation, Methodology, Writing – original draft. **Thanapop Soteyome:** Software, Visualization. **Gongliang Liu:** Supervision. **Gamini Seneviratne:** Visualization, Supervision. **Birthe V. Kjellerup:** Supervision. **Zhenbo Xu:** Conceptualization, Funding acquisition, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Chen, M., Huang, T., Du, M., Bai, X., Soteyome, T., Yuan, L., Bai, C., Lan, H., Hong, W., Peng, F., Fu, X., Peng, G., Liu, L., Kjellerup, B. V., & Xu, Z. (2022). Establishment and application of a rapid visual detection method for Listeria monocytogenes based on polymerase spiral reaction (PSR). *Bioengineered, 13*(3), 7860–7867. https://doi.org/10.1080/21655979.2022.2044262

Corrente, M., Normanno, G., Martella, V., Bellacicco, A. L., Quaglia, N. C., Dambrosio, A., Buonavoglia, D., D'Abramo, M., & Buonavoglia, C. (2007). Comparison of methods for the detection of methicillin resistance in Staphylococcus aureus isolates from food products. *Letters in Applied Microbiology, 45*(5), 535–539. https://doi.org/10.1111/J.1472-765X.2007.02226.X

Crago, B., Ferrato, C., Drews, S. J., Svenson, L. W., Tyrrell, G., & Louie, M. (2012). Prevalence of Staphylococcus aureus and methicillin-resistant S. aureus (MRSA) in food samples associated with foodborne illness in Alberta, Canada from 2007 to 2010. *Food Microbiology, 32*(1), 202–205. https://doi.org/10.1016/J.FM.2012.04.012

Das, A., Kumar, B., Chakravarti, S., Prakash, C., Singh, R. P., Gupta, V., Singh, K. P., Agrawal, R. K., Chaturvedi, V. K., Abhishek, & Shrinet, G. (2018). Rapid visual isothermal nucleic acid-based detection assay of Brucella species by polymerase spiral reaction. *Journal of Applied Microbiology, 125*(3), 646–654. https://doi.org/10.1111/JAM.13882

Dean, F. B., Nelson, J. R., Giesler, T. L., & Lasken, R. S. (2001). Rapid amplification of plasmid and phage DNA using Phi29 DNA polymerase and multiply-primed rolling circle amplification. *Genome Research, 11*(6), 1095–1099. https://doi.org/10.1101/GR.180501

Dong, D., Zou, D., Liu, H., Yang, Z., Huang, S., Liu, N., He, X., Liu, W., & Huang, L. (2015). Rapid detection of Pseudomonas aeruginosa targeting the toxA gene in intensive care unit patients from Beijing, China. *Frontiers in Microbiology, 6*(OCT). https://doi.org/10.3389/FMICB.2015.01100/FULL

Euler, M., Wang, Y., Otto, P., Tomaso, H., Escudero, R., Anda, P., Hufert, F. T., & Weidmann, M. (2012). Recombinase polymerase amplification assay for rapid detection of Francisella tularensis. *Journal of Clinical Microbiology, 50*(7), 2234–2238. https://doi.org/10.1128/JCM.06504-11

Gao, X., Li, C., He, R., Zhang, Y., Wang, B., Zhang, Z., & Ho, C. (2022). Research advances on biogenic amines in traditional fermented foods: Emphasis on formation mechanism, detection and control methods. *Food Chemistry, 405*, Article 134911. https://doi.org/10.1016/j.foodchem.2022.134911

Gizaw, Z. (2019). Public health risks related to food safety issues in the food market: A systematic literature review. *Environmental Health and Preventive Medicine, 24*(1). https://doi.org/10.1186/S12199-019-0825-5

Graveland, H., Wagenaar, J. A., Bergs, K., Heesterbeek, H., & Heederik, D. (2011). Persistence of livestock associated MRSA CC398 in humans is dependent on intensity of animal contact. *PLoS One, 6*(2), Article e16830.

He, S., Jang, H., Zhao, C., Xu, K., Wang, J., Pang, B., Si, X., Jin, M., Song, X., & Li, J. (2020). Rapid visualized isothermal nucleic acid testing of Vibrio parahaemolyticus by polymerase spiral reaction. *Analytical and Bioanalytical Chemistry, 412*(1), 93–101. https://doi.org/10.1007/S00216-019-02209-Y

Hennekinne, J. A., De Buyser, M. L., & Dragacci, S. (2012). Staphylococcus aureus and its food poisoning toxins: Characterization and outbreak investigation. *FEMS Microbiology Reviews, 36*(4), 815–836. https://doi.org/10.1111/J.1574-6976.2011.00311.X

Jiang, X., Dong, D., Bian, L., Zou, D., He, X., Ao, D., Yang, Z., Huang, S., Liu, N., Liu, W., & Huang, L. (2016). Rapid detection of Candida albicans by polymerase spiral reaction assay in clinical blood samples. *Frontiers in Microbiology, 7*(JUN). https://doi.org/10.3389/FMICB.2016.00916/FULL

Kadariya, J., Smith, T. C., & Thapaliya, D. (2014). *Staphylococcus aureus and staphylococcal food-borne disease: An ongoing challenge in public health*. BioMed Research International. https://doi.org/10.1155/2014/827965, 2014.

Köck, R., Loth, B., Köksal, M., Schulte-Wülwer, J., Harlizius, J., & Friedrich, A. W. (2012). Persistence of nasal colonization with livestock-associated methicillin-resistant staphylococcus aureus in pig farmers after holidays from pig exposure. *Applied and Environmental Microbiology, 78*(11), 4046–4047. https://doi.org/10.1128/AEM.00212-12

Lindsay, J. A. (2009). For CA-MRSA, how much PVL is too much? *Microbiology, 155*(Pt 11), 3473–3474. https://doi.org/10.1099/MIC.0.034041-0

Liu, W., Dong, D., Yang, Z., Zou, D., Chen, Z., Yuan, J., & Huang, L. (2015). Polymerase spiral reaction (PSR): A novel isothermal nucleic acid amplification method. *Scientific Reports, 1*(5), 1–8. https://doi.org/10.1038/srep12723, 2015 5:1.

Liu, W., Zou, D., He, X., Ao, D., Su, Y., Yang, Z., Huang, S., Zhao, Q., Tang, Y., Ma, W., Lu, Y., Wang, J., Wang, X., & Huang, L. (2018). Development and application of a rapid Mycobacterium tuberculosis detection technique using polymerase spiral reaction. *Scientific Reports, 1*(8), 1–8. https://doi.org/10.1038/s41598-018-21376-z, 2018 8:1.

Milton, A. A. P., Momin, K. M., Ghatak, S., Priya, G. B., Angappan, M., Das, S., Puro, K., Sanjukta, R. K., Shakuntala, I., Sen, A., & Kandpal, B. K. (2021). Development of a novel polymerase spiral reaction (PSR) assay for rapid and visual detection of Clostridium perfringens in meat. *Heliyon, 7*(1), Article e05941. https://doi.org/10.1016/J.HELIYON.2021.E05941

Momin, K. M., Milton, A. A. P., Ghatak, S., Thomas, S. C., Priya, G. B., Das, S., Shakuntala, I., Sanjukta, R., Puro, K.u., & Sen, A. (2020). Development of a novel and rapid polymerase spiral reaction (PSR) assay to detect Salmonella in pork and pork products. *Molecular and Cellular Probes, 50*, Article 101510. https://doi.org/10.1016/J.MCP.2020.101510

Monteiro, L., Bonnemaison, D., Vekris, A., Petry, K. G., Bonnet, J., Vidal, R., Cabrita, J., & Mégraud, F. (1997). Complex polysaccharides as PCR inhibitors in feces: Helicobacter pylori model. *Journal of Clinical Microbiology, 35*(4), 995–998. https://doi.org/10.1128/JCM.35.4.995-998.1997

Ou, A., Wang, K., Mao, Y., Yuan, L., Ye, Y., Chen, L., Zou, Y., & Huang, T. (2021). First report on the rapid detection and identification of methicillin-resistant Staphylococcus aureus (MRSA) in viable but non-culturable (VBNC) under food storage conditions. *Frontiers in Microbiology, 11*. https://doi.org/10.3389/FMICB.2020.615875/FULL

Palavecino, E. L. (2020). Rapid methods for detection of MRSA in clinical specimens. *Methods in Molecular Biology*, 29–45. https://doi.org/10.1007/978-1-4939-9849-4_2, 2069.

Radovanovic, R. (2011). Food Safety: The global problem as a challenge for future initiatives and activities. *NATO Science for Peace and Security Series A: Chemistry & Biology*, 27–48. https://doi.org/10.1007/978-94-007-1100-6_3

Sarkar, R., Roychoudhury, P., Kumar, S., Dutta, S., Konwar, N., Subudhi, P. K., & Dutta, T. K. (2022). Rapid detection of Actinobacillus pleuropneumoniae targeting the apxIVA gene for diagnosis of contagious porcine pleuropneumonia in pigs by polymerase spiral reaction. *Letters in Applied Microbiology, 75*(2), 442–449. https://doi.org/10.1111/LAM.13749

Sharma, D., Gupta, S., Sethi, K., Kumar, S., & Kumar, R. (2022). Polymerase Spiral Reaction (PSR) as a novel rapid colorimetric isothermal point of care assay for detection of Trypanosoma evansi genomic DNA. *Veterinary Parasitology, 302*, Article 109644. https://doi.org/10.1016/J.VETPAR.2021.109644

Su, J., Liu, X., Cui, H., Li, Y., Chen, D., Li, Y., & Yu, G. (2014). Rapid and simple detection of methicillin-resistance staphylococcus aureus by orfX loop-mediated isothermal amplification assay. *BMC Biotechnology, 14*. https://doi.org/10.1186/1472-6750-14-8

Tacconelli, E., De Angelis, G., de Waure, C., Cataldo, M. A., Torre, G. La, & Cauda, R. (2009). Rapid screening tests for meticillin-resistant Staphylococcus aureus at hospital admission: Systematic review and meta-analysis. *The Lancet Infectious Diseases, 9*(9), 546–554. https://doi.org/10.1016/S1473-3099(09)70150-1

Tomita, N., Mori, Y., Kanda, H., & Notomi, T. (2008). Loop-mediated isothermal amplification (LAMP) of gene sequences and simple visual detection of products. *Nature Protocols, 3*(5), 877–882. https://doi.org/10.1038/nprot.2008.57, 2008 3:5.

Turner, N. A., Sharma-Kuinkel, B. K., Maskarinec, S. A., Eichenberger, E. M., Shah, P. P., Carugati, M., Holland, T. L., & Fowler, V. G. (2019). Methicillin-resistant Staphylococcus aureus: An overview of basic and clinical research. *Nature Reviews Microbiology, 17*(4), 203–218. https://doi.org/10.1038/s41579-018-0147-4, 2019 17:4.

Voss, A., Loeffen, F., Bakker, J., Klaassen, C., & Wulf, M. (2005). Methicillin-resistant Staphylococcus aureus in pig farming. *Emerging Infectious Diseases, 11*(12), 1965. https://doi.org/10.3201/EID1112.050428

Wendlandt, S., Schwarz, S., & Silley, P. (2013). Methicillin-resistant staphylococcus aureus: A food-borne pathogen? *Annual Review of Food Science and Technology, 4*(1), 117–139. https://doi.org/10.1146/ANNUREV-FOOD-030212-182653

Wu, Q., Xu, X., Chen, Q., Zuo, K., Zhou, Y., Zhang, Z., Kan, Y., Yao, L., Ji, J., Bi, Y., & Xie, Q. (2019). Rapid and visible detection of Mycoplasma synoviae using a novel polymerase spiral reaction assay. *Poultry Science, 98*(11), 5355–5360. https://doi.org/10.3382/PS/PEZ356

Xu, W., Gao, J., Zheng, H., Yuan, C., Hou, J., Zhang, L., & Wang, G. (2019). Establishment and application of polymerase spiral reaction amplification for Salmonella detection in food. *Journal of Microbiology and Biotechnology, 29*(10), 1543–1552. https://doi.org/10.4014/JMB.1906.06027