

Applications of polymerase chain reaction-based methods for the diagnosis of plague (Review)

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Abstract. Plague is an acute bacterial infection caused by *Yersinia pestis*. The three major clinical forms of plague are bubonic, pneumonic and septicemic, which have high case-mortality rates. Therefore, rapid and reliable diagnostic tools are crucial. Currently, bacteriological means and traditional serological assays are used for detecting infection with *Y. pestis*. However, such methods have their limitations. Polymerase chain reaction (PCR) is one of the most useful tools for rapid diagnosis of plague. The present review introduced the main PCR techniques and their applications for detecting and confirmation of *Y. pestis*. The advantages and disadvantages of the different PCR methods were also summarized.

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1. Introduction

Plague is a zoonotic infection disease having a high mortality rate without treatment. It may present three distinct clinical forms: bubonic, septicemic and pneumonic (1). *Yersinia pestis* (*Y. pestis*), a member of the genus *Yersinia* which belongs to the *Enterobacteriaceae* family, is the etiological agent of plague (2). *Y. pestis* is a highly pathogenic gram-negative coccobacillus, which are nonmotile, non-spore-forming, oxidase-negative, catalase-positive and lactose-negative, exhibiting bipolar staining with Giemsa, Wright's and Wayson stains (3). It grows at temperatures ranging from 4–40°C and the optimal temperature for growth is 28–30°C (4). At present, four biotypes of *Y. pestis* are recognized, including *Antiqua*, *Orientalis*, *Mediavalis* and *Microtus*, on the basis of their ability to ferment glycerol and form nitrite from nitrate (5,6). Among them, three classic biotypes (*Antiqua*, *Orientalis* and *Mediavalis*) of *Y. pestis* demonstrate no difference in their pathology in animals or humans (7). By contrast, *Microtus* is nonpathogenic for humans (8). *Y. pestis* has a complex infectious cycle, which starts within an insect vector (fleas) followed by transmission to a mammalian host (rodents and humans) (9) (Fig. 1).

The bacterial pathogen, *Y. pestis*, has caused at least three pandemics in human history. The first historically documented pandemic started with the Justinianic Plague (AD 541–544) in Pelusium, Egypt (10), which caused tens of millions of mortalities throughout North Africa, Europe, central and southern Asia and Arabia. The second plague pandemic (14–18th centuries) started with the Black Death (1347–1353). This pandemic persisted for over 400 years and devastated Europe and the nearby regions (11). The third plague pandemic originated from Yunnan province of China in the 1850s and spread globally at the end of the 19th century (12).

Early diagnosis and treatment can effectively reduce the mortality of bubonic plague and septicemic plague (13,14). Polymerase chain reaction (PCR)-based methods have enabled the rapid identification of cultured or uncultured bacteria (15).

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Abbreviations: *cafI*, 60-Md plasmid-located gene; DELFIA, dissociation-enhanced lanthanide fluorescent immunoassays; DOT-ELISA, dot enzyme-immunosorbent assay; ELISA, enzyme-linked immunosorbent assay; *inv*, invasins protein gene; Polymerase chain reaction; PHA, passive hemagglutination; *pla*, plasminogen activator gene

Key words: polymerase chain reaction techniques, plague, *Yersinia pestis*, molecular diagnosis

Previous reviews describing microbiological and molecular aspect, molecular typing and molecular diagnostic techniques of *Y. pestis*, are available (16-20). The present review focused on the applications of PCR-based methods for detection of *Y. pestis* and attempt to compile and update technical aspects of PCR strategies in diagnosis of *Y. pestis* infection.

Laboratory diagnosis of plague. At present, there are various laboratory tests for diagnosis of plague, such as bacterial culture, staining techniques, serological evidence, phage tests, DNA hybridization and PCR analysis (21). Isolation and identification of pathogen in the laboratory is gold standard for plague diagnosis (22). Clinical specimens for analysis can include blood, bubo aspirates, sputum, or cerebrospinal fluid. *Y. pestis* can be cultivated on culture media, such as brain heart infusion broth, MacConkey agar and sheep blood agar. Isolation of *Y. pestis* should be performed under biosafety level 3 conditions. However, bacteriological evidence is time consuming due to the low growth rate of *Y. pestis*. Serological tests are often used to diagnosis plague, including the agar-gel precipitin inhibition, the complement fixation, passive hemagglutination (PHA) test (23), immunochromatography test (24), enzyme-linked immunosorbent assay (ELISA) (25), dot enzyme-immunosorbent assay (DOT-ELISA) (26) and the dissociation-enhanced lanthanide fluorescent immunoassays (DELFA) (27). Serological tests seem to be more effective but are expensive and labor intensive. Moreover, it can be unspecific due to serological cross-reactivity with other enteropathogenic bacteria (24). DNA hybridization using *Y. pestis*-specific DNA probe may be used for plague diagnosis (28). The minimum detection limits of this method are $\sim 10^5$ bacteria, which limits its clinical application. PCR is well suited molecular biology tool for diagnosis of pathogens. At present, confirmation of plague is performed using reverse transcription PCR targeting a plasminogen activator gene (*pla*) and 60-Md plasmid-located gene (*cafI*) and in the case of discordant or uncertain results, a PCR targeting *pla*, *cafI* and an invasin protein gene (*inv*) is performed (Fig. 2).

PCR-based methods for diagnosis of plague. The rapid identification of the *Y. pestis* is crucial, so that more specific therapy can be initiated. PCR is a key technique for accurate detection of *Y. pestis* due to its higher sensitivity and specificity within several hours and without any cultivation. *Y. pestis* genome has a size of $4,380 \pm 135$ kb with a 46 to 47 mol% G+C content. The advances concerning the structure of the *Y. pestis* genome led to the development of specific PCR assays for plague diagnosis. The first PCR-based test for identification of *Y. pestis* was introduced by Bulat *et al* (29) in 1991. They performed gene typing with PCR assay to identify six *Yersinia* species (*Y. pestis*, *Y. pseudotuberculosis*, *Y. enterocolitica*, *Y. kirstensenii*, *Y. frederiksenii* and *Y. intermedia*). Previous studies further report the determination of the molecular typing and the genetic variations of *Y. pestis* using PCR methods (30-35). For example, the PCR-based O-genotyping proves useful to type *Y. pseudotuberculosis* and *Y. pestis* (36). PCR is also a useful tool for analysis of genomic polymorphism of typical and atypical strains of the *Y. pestis* (37). Additionally, the developed approach based on PCR allows for an effective differentiation of *Y. pestis* strains of various subspecies (38-40).

Some studies elucidated a mechanism by which *Y. pestis* may be transmitted between host species using PCR (41,42). Researchers also developed a standard curve-based competitive PCR to quantitate *Y. pestis* in individual fleas, which is more reliable than colony count (43). The PCR method is used to determine bacterial susceptibility to antibiotics by the quantification of differentially expressed marker genes (44-47). A number of studies present the rapid diagnosis of plague and the detection of prominent virulence markers of *Y. pestis* strains using this technique (48,49). So far, PCR has proven useful in application as a diagnostic method for routine plague surveillance and outbreak investigations (50-55).

2. Standard PCR

Standard PCR is replacing the more traditional microbiological assays in the detection of *Y. pestis*. This approach requires development of highly specific oligonucleotide primers unique to *Y. pestis*. Primer pairs include the primers for sequences of *cafI*, *pla*, *inv*, a *Y. pestis*-specific region of a *yopM* gene, 23S ribosomal DNA interspace region and insertion sequence (56-60). Table I gives the different primers for standard PCR.

Standard PCR is a cost-effective approach for the rapid detection of *Y. pestis* (61). Zasada *et al* (62) present an application of this assay for detection and identification of *Y. pestis*, which takes <50 min and is cheaper than reverse transcription PCR. Studies also showed that this approach showed high specificity when compared with ELISA and the culture of *Y. pestis* (56). Additionally, standard PCR seems to be relatively rapid and sensitive when compared to the conventional culture-based method. Hinnebusch and Schwan (15) report that this assay may detect as few as 10 cells of *Y. pestis*. Singh *et al* (63) developed a standard PCR assay coupled with lateral flow strips. The analytical sensitivity of assay is 1 pg genomic DNA of *Y. pestis* and 500 copies of target DNA sequence harbored in a recombinant plasmid. However, Rahalison *et al* (56) reveal that the sensitivity of this technique is 50% relative to the results of culture and 35.2% relative to the results of the ELISA due to suboptimal field conditions and the volumes of samples. Therefore, it is worth noting that sample volume used and efficient DNA extraction protocol direct influence the sensitivity of standard PCR. Moreover, standard PCR may be used for the effective differentiation of typical and atypical plague pathogen strains. A rapid and sensitive one-step PCR assay has been developed to identify and discriminate pathogenic *Y. enterocolitica* from other members of this genus using a set of species-specific primers (64,65). Zhou *et al* (66) also identified 28 signature genes of *Y. pestis*. PCR amplification of these signature sequences is ideal for rapid and specific characterization of pathogens without cross-reaction with the closely related *Y. pseudotuberculosis*.

By using specific probe for the amplicon detection, standard PCR is considered sensitive and specific. However, it cannot be monitored in real time and requires the performance of any postreaction processing, such as the electrophoresis gel. Moreover, standard PCR method is relatively poor in detecting the low numbers of pathogens in the biopsy sample. So far, there have been numerous modifications of the PCR technology for increasing the sensitivity of detection.

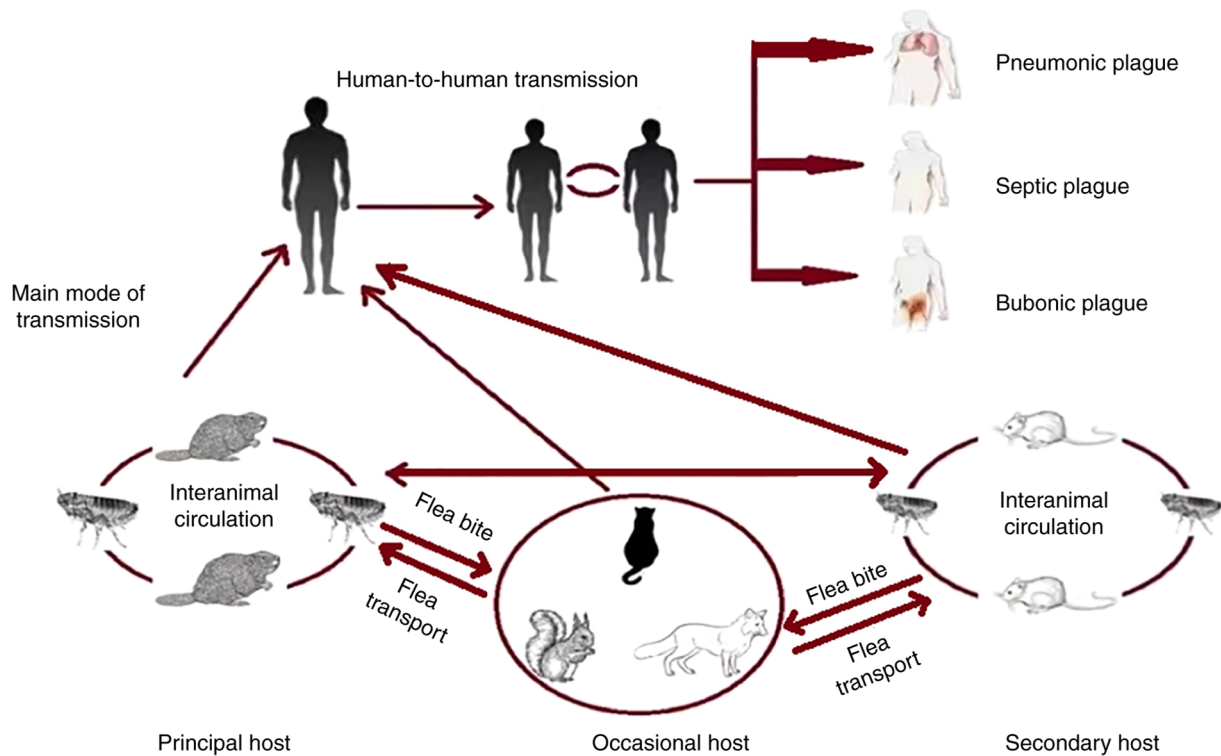


Figure 1. Transmission routes of plague. Rodents act as hosts and reservoirs for *Y. pestis*, vectored by fleas. Mammals, which are not the natural hosts of *Y. pestis*, may become infected via the bite of an infected flea. Infection may be transmitted to humans through flea bites or through direct contact with infected animals.

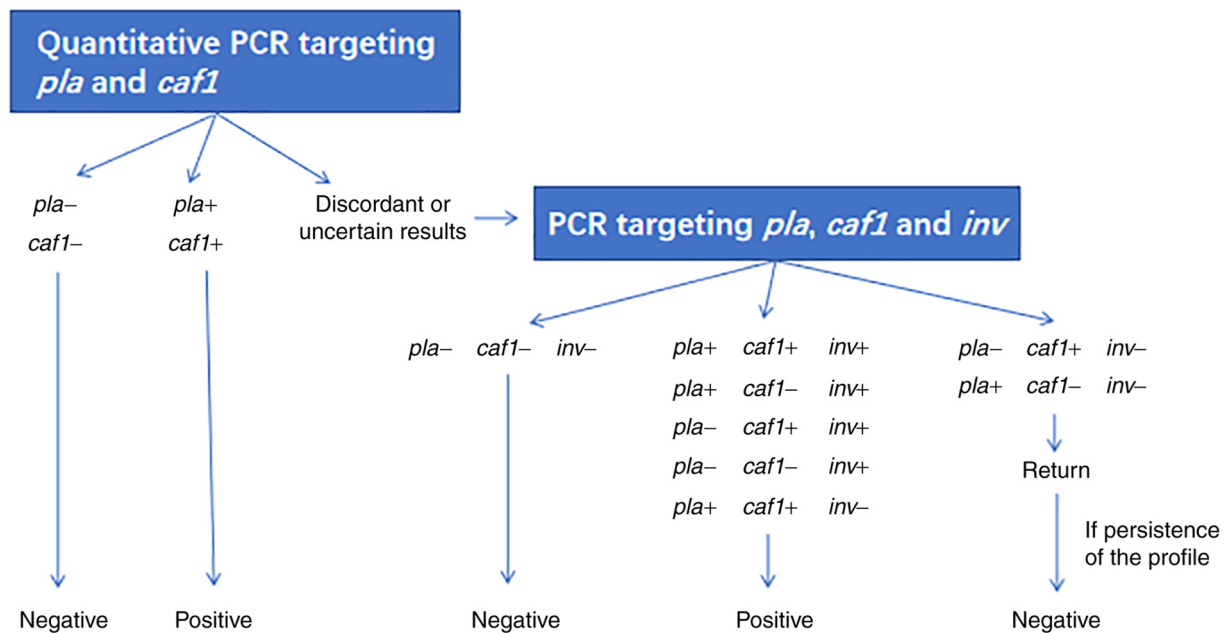


Figure 2. Algorithm for the molecular biology tests of plague. DNA confirmation on the presence of *Y. pestis* in human specimens is performed using quantitative PCR targeting *pla* and *caf1* and in the case of discordant or uncertain results, a conventional PCR targeting *pla*, *caf1* and *inv* is performed. *pla*, plasminogen activator gene; *caf1*, 60-Md plasmid-located gene; *inv*, invasin protein gene.

3. Reverse transcription PCR

Compared to conventional PCR, reverse transcription PCR has several advantages, including speed, simplicity, reproducibility, quantitative capability and low risk of contamination (67-70).

Reverse transcription PCR for the rapid detection and differentiation of *Y. pestis* has been developed, targeting *caf1*, *Ymt*, *pla*, hemin storage genes (*hmsH*, *hmsF* and *hmsR*) and *irp2* iron-regulating gene (71,72). Table II gives the different primers and probes for the reverse transcription PCR.

Table I. Sequences of primers for the standard PCR.

| Author, year | Gene | Forward primer | Reverse primer | Product, bp | (Refs.) |
|-------------------------------|--------------------|--------------------------------|----------------------------------|-------------|---------|
| Rahalison <i>et al</i> , 2000 | <i>cafI</i> | CAGTTCCGTTATCG CCATTGC | TATTGGTTAGATACG GTTACGGT | 501 | (56) |
| Nyirenda <i>et al</i> , 2018 | <i>pla</i> | ATCTTACTTTCCGTG AGAAG | CTTGGATGTTGAGCT TCCTA | 480 | (57) |
| Tsukano <i>et al</i> , 1996 | <i>inv</i> | TAAGGGTACTATCGC GGCGGA | CGTGAAATTAACCGT CACACT | 295 | (59) |
| | <i>yopM</i> | ATAACTCATCGGGGG CAAAAT | GCG TTA TTT ATC CGA ATT TAG C | 565 | |
| | <i>cafI</i> | CAGGAACCACTAGC ACATC | CCCCACAAGGTTC TCAC | 171 | |
| Radnedge <i>et al</i> , 2001 | Insertion sequence | TGTAGCCGCTAAGCA CTACCATCC | GGCAACAGCTCAACAC CTTTGG | 276 | (58) |
| | Insertion sequence | GCATGACCGAAACGT CATCCTG | GGATACTTCGCGCATATC TTGCC | 332 | |
| | Insertion sequence | GGATAACGTTGCAG CAGCTTCG | CCTTCGCCACCTTCAC CTGC | 250 | |
| | Insertion sequence | TCCAAAATCGGAGA ATTACTATGGGC | CGTTGTTGATGCCGT CA CTTTG | 226 | |
| | 23S rRNA | CTACCTTAGGACC GTTATAGTTAC | GAAGGAACTAGGCAAA ATGGT | | |
| | <i>JS</i> | GCAGCTTAGGCTGTC ATCG | CTATCGCCTGATTGGA GAGG | 223 | |

cafI, capsular antigen fraction 1; *pla*, plasminogen activator; *inv*, invasins; *yopM*, *Yersinia* outer protein M.

Reverse transcription PCR is proposed as a timely, cost-effective and accurate diagnostic assay (73,74). The reliability of this method was evaluated in 1,050 clinical specimens and high values of specificity were obtained (75). An autonomous pathogen detection system was developed by coupling reverse transcription TaqMan assay, which generate extremely low false positive rate (76). Woubit *et al* (77) also identify the genomic targets of *Y. pestis* to design the primers. Primer sets are used to specifically detect pathogen with reverse transcription PCR assays and this assay is found to be sensitive. A 5' nuclease PCR assay for detection of the *Y. pestis* has been developed with a detection threshold of 1.6 pg of total cell DNA (78). Tomaso *et al* (79) established a reverse transcription PCR assay for the specific detection of *Y. pestis*. The lower limit of detection is ~0.1 genome equivalent. Skottman *et al* (80) report the development of reverse transcription PCR assays for detection of *Y. pestis* with a sensitivity of 1 fg of total DNA in the PCR tube. In addition, some researchers develop and validate reverse transcription PCR for the differentiation and quantification of *Y. pestis*. Comer *et al* (81) report reverse transcription PCR assays to determine absolute bacterial numbers in flea vector and mammalian host tissues. A quadruplex reverse transcription PCR assay proved to be successful in differentiating *Y. pestis* from *Y. pseudotuberculosis* (82). Chase *et al* (83) also designed reverse transcription PCR assays to discriminate *Y. pestis* DNA from all other *Yersinia* species tested and from the closely related *Y. pseudotuberculosis*. Moreover, reverse transcription

PCR assays have been developed for simultaneous detection of various organisms. Liu *et al* (75) developed a reverse transcription PCR-based TaqMan array card that can simultaneously detect 26 organisms, including *Y. pestis*. Notably, reverse transcription PCR allows the detection of only live *Y. pestis* using amplification of plague diagnostic bacteriophages (84). It is therefore a useful method for the differentiation among inactive and active states of *Y. pestis*.

Some researchers develop reverse transcription PCR for the specific detection and quantification of *Y. pestis* from various samples, such as complex food, synthetic building debris and leachate and spleen samples of animals (85-89). Hennebique *et al* (90) also report the development of a reverse transcription PCR assay for the detection of *Y. pestis* in various types of samples and demonstrate good performances.

Some researchers have compared reverse transcription PCR assay performance across various platforms. Christensen *et al* (91) detect *Y. pestis* by reverse transcription PCR on the R.A.P.I.D., the LightCycler and the Smart Cycler platforms. They find that the tested assays have comparable sensitivity and specificity on these rapid cycling instruments. Matero *et al* (92) also compare this assay performance between the Applied Biosystems 7300/7500 and the RAZOR instruments for detection of *Y. pestis*. Although no notable differences between two platforms were observed in analytical sensitivity or specificity, the duration of thermocycling with the RAZOR instrument was significantly shorter (40 min vs. 100 min with ABI 7300/7500). Mölsä *et al* (93) compare

Table II. Sequences of primers and probes for the reverse transcription PCR.

| Author, year | Gene | Primer/probe sequences (5'-3') | Product, bp | (Refs.) |
|----------------------------|-----------------|--|-------------|---------|
| Bai <i>et al</i> , 2020 | <i>pst</i> | Forward: GCGAAGCAAACAGGATTTATTG Reverse: GAGGTGCTGTTCTCACTTTATC Probe: FAM-AGCCTCCTTCCCTCGAAGCAT ATAATACCC-BHQ1 | 116 | (40) |
| | <i>ypo2088</i> | Forward: TCGGCAACAGCTCAACACCT Reverse: ATGCATTGGACGGCATCACG Probe: CALRD610-CGCCCTCGAATCGCT GGCCAACTGC-BHQ2 | 107 | |
| | <i>opgG</i> | Forward: ACGTGGGCGTGAATTCTCTCAA Reverse: GCCGTTGGGATCTCCACCAA Probe: QUAS670-CCTGCGCCCAAGCGCG TGGG-BHQ2 | 126 | |
| | <i>18S rRNA</i> | Forward: CAGATACCGCCCTAGTTCTAA Reverse: GTT TCA GCT TTG CAA CCA TAC Probe: HEX-TCATCGGAGGAACCTTCGGC GGATC-BHQ1 | 153 | |
| Riehm <i>et al</i> , 2011 | <i>pst</i> | Forward: TACGTTACGGTTACAGCAT Reverse: GGTGATCCCATGTACTTAACA Probe: 6FAM-ACCTGCTGCAAGTTTACC GCCTTTGG-BBQ | | (72) |
| | <i>Ymt</i> | Forward: AGGACCTAATATGGAGCATGAC Reverse: CTAACAAAGCCTCAATAATCCA Probe: 6FAM-TCCAAGCACTCACGAGA TCTTGCTAA-BBQ | | |
| Liu <i>et al</i> , 2016 | <i>cafI</i> | Forward: CCACTGCAACGGCAACTCTT Reverse: TGTAATTGGAGCGCCTTCCT Probe: QUAS705-TTGAACCAGCCCGCAT CACTCTTACA-BHQ3 | 71 | (75) |
| Woron <i>et al</i> , 2006 | <i>cafI</i> | Forward: GCAACTGCTAATGCGGCAGAT Reverse: CCTGTTTTATAGCCGCCAAGAG Probe: TAMRA-TGCAAGCACCCTGC AACGGCAAC-BHQ1 | 176 | (98) |
| | <i>pla</i> | Forward: GCTTTATGACGCAGAAACAGGA Reverse: AACCAGCCTTTCACATTGAGGT Probe: JOE-TGGACGTCTCTGGCTTCC GGTC-BHQ2 | 270 | |
| | <i>entF3</i> | Forward: AACGACGGCATTACGGTA Reverse: TGGTGATGAGTTGGACGTTAGG Probe: ROX-CGGCCAAAATGGCGTGAT AAATACCTT-BHQ1 | 122 | |
| Tomaso <i>et al</i> , 2003 | <i>pla</i> | Forward: GTAATAGGTTATAACCAGCGCTT Reverse: AGACTTTGGCATTAGGTGTG Probe: HEX-ATGCCATATATTGGAATTG CAGGCCAGT-BHQ1 | 232 | (79) |

cafI, capsular antigen fraction 1; *pla*, plasminogen activator.

the performance of a novel portable reverse transcription PCR thermocycler PikoReal to ABI 7300 for the detection of *Y. pestis*. The PikoReal system may be a more efficient alternative to detect biothreat agents under field conditions.

When compared to other PCR based methods used for detection of *Y. pestis*, reverse transcription PCR is a sensitive method that quantifies the number of *Y. pestis* in biopsy specimens through the quantification of bacterial DNA in

real time. However, it may not be as sensitive as nested PCR. Additionally, the application of reverse transcription PCR is usually based on the commercial kits, so it will be more expensive, especially when two genes are targeted.

4. Multiplex PCR

Multiplex PCR is a type of PCR technique which amplifies more than one target DNA in one reaction system at one time. Elsholz *et al* (94) designed a multiplex PCR method for the parallel detection of a panel of the pathogens, including *B. anthracis*, *Y. pestis*, *F. tularensis* and *ortho pox* viruses (genus). Stenkova *et al* (95) show that the multiplex PCR provides an improved method for detection of the *Yersinia* genus with identification of pathogenic species (*Y. pestis*, *Y. pseudotuberculosis*, *Y. enterocolitica*). Stevenson *et al* (96) further detect flea-associated microorganisms, such as *Bartonella* strains and *Y. pestis*, in prairie dogs and their fleas using multiplex PCR. Additionally, the multiplex PCR can be used to detect and identify *Y. pestis* using multiplex primers, including *cafI*, *yopM*, *pla* and *inv* genes (97). Woron *et al* also reported the 4-target multiplex reverse transcription PCR assay for *Y. pestis* (98).

The multiplex PCR assay can successfully identify *Y. pestis* with high sensitivity (99). Vanlalhmuka *et al* (100) developed a multiplex PCR-based reverse line blot macroarray for simultaneous detection and characterization of four pathogens, including *B. anthracis*, *Y. pestis*, *B. melitensis* and *B. pseudomallei*. This assay is able to detect 8×10^2 cfu/ml for *Y. pestis*. Similarly, Batra *et al* (101) describe a sensitive and specific multiplex PCR assay for the simultaneous detection of *B. anthracis*, *Y. pestis*, *B. pseudomallei* and *Brucella* species. The sensitivity in spiked blood samples was 50 colony forming units (cfus)/25 μ l reaction for the detection of *Y. pestis*.

Multiplex PCR demonstrates high specificity and reliability (102). Wilson *et al* (103) developed a multiplexed PCR-coupled liquid bead array for the detection of *Y. pestis*. The assay correctly identified the presence of pathogen with low material costs. Tran *et al* (104) detected *Y. pestis* DNA in dental pulp specimens collected from graves with high throughput multiplex PCR, confirmed the outbreaks of plague in medieval Venice. Melo *et al* (105) show that the multiplex-PCR technique is a valuable tool for the plague control programme. A multiplex oligonucleotide ligation-PCR has also been developed for the detection of *Y. pestis*, representing considerable potential in the field of diagnostics and surveillance (106). A previous study also showed that there was no significant difference in detection rates between blood culture, singleplex PCR and multiplex PCR within the *Y. pestis* model (107).

Multiplex PCR can be a powerful tool for the simultaneous quantification of more than one pathogen in a single reaction by combination of primers and probes. The advantages of this method include ease of sample collection, improvement in amplification efficiency and reduction of laboratory time. This technique is more suitable for screening of pathogenic bacteria.

5. Nested and semi-nested PCR

The nested and semi-nested PCR assays have advantages of high sensitivity and easy applicability for the detection of

Y. pestis in various samples. Trebesius *et al* (108) present the semi-nested PCR approach based on 16S and 23S rDNAs with respect to diagnosis of plague. A single-tube nested-PCR technique targeting the *cafI* gene was evaluated for plague diagnosis, which showed more sensitive than conventional PCR (109). Glukhov *et al* (110) develop a nested PCR method to distinguish the culture of *Y. pestis* from cultures of other microorganism, demonstrating a higher sensitivity and specificity than standard PCR.

6. Other PCR-based assays

A microchip PCR array instrument was developed for rapid detection of *Y. pestis* with the detection limits of 10^5 - 10^7 organisms/L (111). Pingle *et al* (112) developed a PCR-ligase detection reaction-capillary electrophoresis assay for the identification of pathogens, including *Y. pestis*. Jacob *et al* (113) describe the identification of highly pathogenic bacteria using an assay coupling biothreat group-specific PCR with electrospray ionization mass spectrometry. Song *et al* (114) also developed a SNP-based multiplexed oligonucleotide ligation-PCR for rapid *Y. pestis* detection and antibiotic resistance characterization. Souza *et al* (115) developed a method to differentiate *Yersinia* species using high-resolution melting analysis. Jeng *et al* (116) further reported a reverse transcription-PCR-electrospray ionization mass spectrometry assay for distinguishing biothreat agents, including *B. anthracis*, *Y. pestis*, *F. tularensis*, *Brucella* spp., *Burkholderia* spp. and *R. prowazekii*. Other PCR-based assays have been used for detection of *Y. pestis*, such as ligation-mediated PCR, suicide PCR, immuno-PCR and viability PCR (117-123).

7. Sampling and sample treatment

The sensitivity limit of PCR depends on the method used for preparing the sample (124) and the presence of PCR inhibitors that are often found in biological samples (125). A previous study showed that some components in the tissues can inhibit PCR (126). Leal *et al* (127) found that the spleen suspension of animals experimentally infected with *Y. pestis* can be used as PCR amplification template without DNA extraction. The sensitivity and specificity were enhanced by amplification after the second-round PCR. Afanas'ev *et al* (128) treated the samples of plague-infected fleas with an affine sorbent prior to PCR analysis. They found that the use of magnoimmunosorbent prevents the inhibitory effect of flea tissues and makes it possible to have a specific concentration of plague microbial DNA. The high-quality DNA before PCR gene amplification is essential for the diagnostic of pathogenic bacteria. Coyne *et al* (129) evaluate the Schleicher and Schuell IsoCode Stix DNA isolation device and the Qiagen QIAamp DNA Mini kit for isolating *Y. pestis* DNA from serum and whole-blood samples. They find that the two methods achieve comparable detection limits. Dauphin *et al* (130) evaluate five commercially available DNA extraction kits. TaqMan reverse transcription PCR analysis revealed that the MasterPure kit was best extraction method for *Y. pestis* suspensions and spiked environmental samples. Gilbert *et al* (131) show that various methods of tooth manipulation can influence the PCR-based detection of *Y. pestis* DNA in human teeth from European

excavations of putative plague victims. They use a novel contamination-minimizing embedding technique to reduce the levels of environmental bacterial DNA presented in DNA extracts. Hong-Geller *et al* (132) evaluate the sample recovery efficiencies of two collection methods (swabs and wipes) for *Y. pestis* from nonporous surfaces. They found that collection efficiency was surface-dependent, indicating the importance of surface interactions in pathogen detection.

8. Perspective and challenge

The developed approach based on PCR is applicable for identifying and confirming *Y. pestis* (133,134). This system also allows for effective differentiation of *Yersinia* strains of various subspecies. In addition, the PCR assay is able to determine bacterial susceptibility to antibiotics and prominent virulence markers of *Y. pestis*. Compared with traditional techniques, PCR-based is simple, rapid, highly sensitive and specific and it has proven useful in application as a diagnostic strategy for routine plague surveillance of epidemics. However, the PCR inhibitors may be present in samples. The suboptimal field conditions, sample recovery efficiency and DNA extraction quality directly influence the sensitivity and specificity of most PCR-based methods. Therefore, future studies should focus on the standardization of sample processing.

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Authors' contributions

YZ contributed to the acquisition, analysis and systematization of data and manuscript writing. ZW and WW contributed to the acquisition and analysis of data. HY and MJ contributed to the systematization of data and critical revision. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patients consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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