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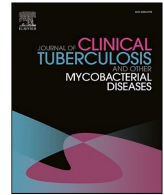

Case Report

Intensive reprocessing of reusable bronchoscopes can reduce the false positive rate of Xpert MTB/RIF caused by nucleic acid residue

Xingxing Jin [a,1], Qianfang Hu [a,1], Yishi Li [a], Xia Zhang [a], Wan Tao [a], Houyu Zhong [a], Qinghai Zhao [b,*]

[a] Department of Respiratory and Critical Care Medicine, The First Affiliated Hospital of Chongqing Medical University, Chongqing Medical University, Chongqing, China
[b] Department of Nursing, The First Affiliated Hospital of Chongqing Medical University, Chongqing Medical University, Chongqing, China

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ABSTRACT

Background/Purpose: Tuberculosis remains a leading cause of infectious death worldwide, The potential for nucleic acid residue on bronchoscopes to cause false positive results in molecular diagnostic methods and subsequently lead to tuberculosis misdiagnosis has long perplexed clinical.

Methods: We utilized Xpert MTB/RIF to analyze the liquid collected after bronchoscope washing, employed by patients either with or without active pulmonary tuberculosis, and subjected to standard reprocessing (SR) or intensive reprocessing (IR) procedures. The IR procedure included specialized training and the provision of patient information to cleaning staff before the SR procedure, and repeated washing and suction of the bronchoscope with sterilized water post SR procedure.

Results: 55 participants enrolled in the study were divided into three groups: SR group (n = 28), IR group (n = 14), and the control group(n = 13). Among the 55 enrolled patients, neither Mycobacterium tuberculosis nor contamination was detected by MIGT 960 liquid culture in the washing liquid. The positive rate of MTB/RIF in the SR group (12/28) was significantly higher than that in the IR group (1/14), with a statistically significant difference observed between them (42.86 % vs. 7.14 %, P=0.018).

Conclusions: Nucleic acid residue on reusable bronchoscopes cleaned via the SR procedure was found to potentially cause false positives in MTB/RIF tests. Reprocessing bronchoscopes via the IR procedure was effective in significantly reducing nucleic acid residue, although complete elimination was not achieved.

1. Introduction

Tuberculosis (TB) ranks as the second leading infectious cause of death globally, following COVID-19, and stands as the 13th leading cause of death overall. It represents the primary cause of mortality among individuals with HIV and is a significant contributor to deaths attributed to antimicrobial resistance. In 2022, an estimated 1.3 million deaths worldwide were attributed to TB, with a prevalence incidence of 10.6 million cases, of which only 7.5 million were diagnosed [1]. Patient identification plays a pivotal role in the WHO's goal to end the TB epidemic by 2035 and is crucial in the treatment of the disease. Presently, standard TB diagnostic methods, such as acid-fast bacillus (AFB) smear microscopy and bacilli culture, remain time-consuming with moderate accuracy and sensitivity [2]. Immunological techniques, such as the tuberculin skin test and the γ-interferon release assay, serve as auxiliary tools in TB diagnosis. However, they are unable to differentiate between active TB and latent TB infection, thus limiting their clinical utility [3]. Nucleic acid-based molecular diagnostic methods have gained widespread acceptance in clinical practice due to their high sensitivity and specificity. Techniques such as conventional PCR, Xpert MTB/RIF, tuberculosis loop-mediated isothermal amplification assay, *meta*-genomics next-generation sequencing (mNGS), and droplet digital PCR (ddPCR) have been utilized. mNGS and ddPCR, in particular, boast a limit of detection (LOD) that can reach as low as 1 reads/ml [4–7].

In 2011, the World Health Organization (WHO) endorsed the Xpert MTB/RIF assay (Cepheid, CA) for diagnosing pulmonary tuberculosis (PTB) and detecting rifampicin resistance [8]. The Xpert MTB/RIF assay is an automated, single-cartridge-based nucleic acid amplification test

* Corresponding author at: Department of Nursing, The First Affiliated Hospital of Chongqing Medical University, Chongqing Medical University, 1 You yi Road, Yuzhong District, Chongqing 400016, China.
E-mail address: qh20063@163.com (Q. Zhao).

[1] Contributed equally.

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capable of concurrently detecting M. tuberculosis and rifampicin resistance within 2 to 3 h [9]. The LOD of MTB/RIF was approximately 113 colony forming units per milliliter (CFU/ml), for its second-generation product, the Xpert MTB/RIF Ultra assay, the LOD was 15.6 CFU/ml of sputum [10]. Currently, Xpert MTB/RIF frequently employs sputum samples for PTB diagnosis. Meta-analysis indicates that as an initial test replacing smear microscopy, Xpert MTB/RIF exhibited a pooled sensitivity of 89 % and pooled specificity of 99 % [7]. Nonetheless, a significant proportion of PTB patients present with sputum smear-negative and sputum-scarce PTB in clinical practice [11]. Interestingly, bronchoscopy has been proposed as a valuable approach for diagnosing sputum smear-negative and sputum-scarce PTB, as it yields high-quality biological samples such as bronchoalveolar lavage fluid (BALF) and transbronchial biopsy [12]. Compared to culture methods, the pooled sensitivity and specificity of Xpert MTB/RIF using BALF were 87 % and 92 %, respectively [13]. However, the bronchoscope is a reusable medical device, and existing cleaning quality control standards do not mandate the removal of nucleic acid residues [14,15]. Consequently, it has not been explored whether samples obtained via flexible bronchoscopy may yield false positives in molecular biological detection. In this study, MTB/RIF was employed to detect the ropB gene fragment of Mycobacterium tuberculosis (MTB) following thorough cleaning and disinfection of reusable bronchoscopes. The aim was to investigate the impact of nucleic acid residues on molecular biological diagnostic methods and strategies for mitigating such influence.

2. Methods and materials

2.1. Study design

Patient enrollment with pulmonary clinical symptoms and chest imaging findings was conducted at the First Affiliated Hospital of Chongqing Medical University from November 2019 to May 2022. This study was approved by the Ethics committee of The First Affiliated Hospital of Chongqing Medical University (approval no. 20188501). The guidelines outlined in the Declaration of Helsinki were followed.

The inclusion criteria for the patients are as follows: 1. Persistent cough and expectoration for more than two weeks, or accompanied by symptoms such as fever, night sweats, and weight loss; 2. Chest CT indicating pulmonary shadow changes suggestive of tuberculosis; 3. The need for bronchoscopy to confirm the diagnosis, with no contraindications for bronchoscopic examination; 4. BALF AFB-positive and BALF Xpert MTB/RIF positive. Patients who were BALF AFB-negative or BALF Xpert MTB/RIF-negative or −positive (very low) were excluded.

Subjects were prospectively enrolled, and the flexible bronchoscopes used on them were cleaned using the standard reprocessing (SR) procedure, forming the SR group. At the same day, a bronchoscope used on a non-tuberculosis patient was selected for the SR procedure, designated as the control group. Following the completion of enrollment for both the control and SR groups, cleaning personnel received professional training. They were informed about the patients highly suspected of having aPTB prior to cleaning the bronchoscopes. After the SR procedure, the bronchoscopes were subjected to the intensive reprocessing (IR) procedure, involving repeated suctioning and rinsing with sterile water at least 20 times. We used the semi-quantitative bacterial load results from L-J culture of bronchoalveolar lavage fluid as a matching factor, adopting a matching ratio of 1:2 for IR group and SR group. This set of subjects was classified as the IR group.as shown in Fig. 1.

2.2. Bronchoscope reprocessing procedure

Subsequent to bronchoscopy, bronchoscopes underwent reprocessing via either a SR procedure or an IR procedure, The SR procedure include cleaning the external and internal surfaces, including brushing and flushing of the working channel with a solution of water, leak detection, use of enzymatic detergent, and rinsing with water, then immersing the bronchoscope for at least 5 min in 5 % *ortho*-phthaladehyde (Johnson & Johnson, Canada). Inner channels were irrigated

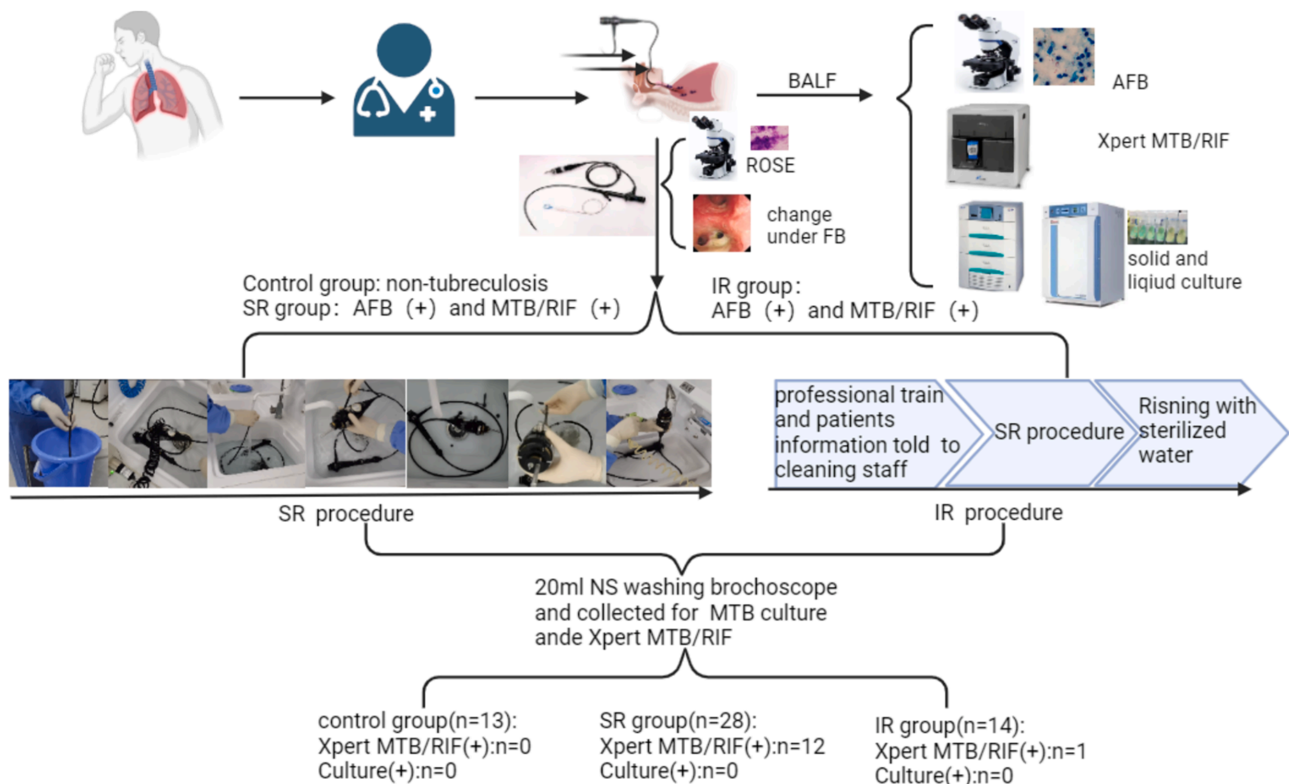

Fig. 1. The workflow of this study. Abbreviation: FB, flexible bronchoscope, BALF, bronchoalveolar lavage fluid; AFB, acid-fast bacillus; MTB, Mycobacterium tuberculosis; ROSE, rapid onsite evaluation; SR, standard reprocessing, IR, intensive reprocessing.

manually with the disinfectant. Once disinfected, the equipment was rinsed with sterile water and medical air was forced through to dry the endoscopes without alcohol rising. The detailed steps of SR procedure refer to Regulation for cleaning and disinfection technique of flexible endoscope,[15] The IR procedure involves the professional training of cleaning staff and telling him on clinical information regarding patients who are highly suspected of having aPTB prior to the SR procedure, the repeated flushing and suctioning of the bronchoscope exceeded 20 times with a substantial volume of sterilized water after the SR procedure. This step carried out by the cleaning technician under the supervision of the same research staff member. The bronchoscopes used in this study were all cleaned by the same dedicated individual. Following either SR procedure or IR procedure, the bronchoscope undergoes repeated washing and suctioning more than 20 times with 20 ml of normal saline (NS). Subsequently, 20 ml of NS is collected for MTB culture and Xpert MTB/RIF assay analysis, as shown in Fig. 1.

2.3. Smear, culture and Xpert MTB/RIF for BALF

Each BALF sample should contain 5–10 ml of BALF and be equally divided into three samples. One sample underwent smear preparation using the sandwich cup method as per the manufacturer's protocol, was stained with Ziehl-Neelsen (ZN) AFB staining solution (Hunan Tianqi New Technology Co., China), and examined under optical microscopy (OLYMPUS, Japan). Solid culture using Löwenstein-Jensen (L-J) medium was performed in accordance with guidelines from the Chinese Anti-TB Association [16], and the Mycobacteria Growth Indicator Tube (MGIT) 960 culture adhered to the manufacturer's protocol. Another sample was liquefied and decontaminated using the sodium hydroxide-sodium citrate-N-acetyl-L-cysteine method. Subsequently, 0.5 ml of BALF sediment was inoculated onto an L-J slant and incubated at 37 °C for up to 8 weeks. Furthermore, 0.5 ml was inoculated into a 7-ml MGIT tube and incubated in the BACTEC MGIT 960 system (BD, USA). Positive cultures in the MGIT960 were confirmed by identifying AFB in ZN-stained smears. The last sample was processed using the Xpert MTB/RIF assay according to the manufacturer's protocol. The process entailed mixing the sample with double the amount of buffer, incubating at room temperature for 15 min, then transferring the sample into a cartridge for analysis in the Xpert machine. Results were interpreted as either detecting Mycobacterium tuberculosis (categorized as very low, low, medium, high) with or without rifampicin resistance, or no target detected.

2.4. MTB culture and Xpert MTB/RIF for washing liquid

Approximately 20 ml of NS used for bronchoscope washing was collected and centrifuged for 20 min at 3500g and 4 °C. The supernatant was discarded, and the pellet was resuspended in 1 ml of NS. Then, 0.5 ml of this resuspension solution was inoculated into a 7-ml MGIT and incubated in the BACTEC MGIT 960 system. The remaining resuspension was combined with a pretreatment solution and subsequently analyzed using the Xpert MTB/RIF assay, as described previously.

2.5. Statistical analysis

All statistical analyses in this study were carried out using SPSS software, version 23.0. Continuous variables are presented as mean values along with their ranges, while categorical variables are expressed as percentages (%). Differences between the groups under study were considered statistically significant if the P values were less than 0.05.

3. Results

3.1. Study population

A total of 55 participants were enrolled in the study. These participants were divided into three groups: 28 subjects in the SR group, 14 subjects in the IR group, and 13 subjects in the control group. The average ages of the SR, IR, and control groups were 42.75 (range 19–80), 45 (range 21–75), and 56 (range 25–80) years, respectively. The majority of participants were female, except in the control group. In other words, the number of females in both the IR and SR groups, who were assigned to different groups based on the reprocessing procedure for reusable flexible bronchoscopes, significantly outnumbered the males, whereas the control group showed the opposite trend. No statistical differences in age or sex were observed between the SR and IR groups. In the SR group, 85 % (23/28) of the patients exhibited greyish-white ulcerated necrotic material attached to the bronchial surface under bronchoscopy, a phenomenon observed in 100 % (14/14) of the IR group, though the difference was not statistically significant (P = 0.285). Tuberculosis-specific pathological changes, such as granulomatous inflammation or caseous necrosis, were observed during ROSE in 35.71 % (10/28) of the SR group and 28.57 % (4/14) of the IR group, respectively; again, the difference was not statistically significant (P = 0.459). In the semi-quantitative bacterial load measured by L-J solid culture, there was no statistical difference in the bacterial load between the SR group and the IR group. Neither greyish-white ulcerated necrotic material attached to the bronchial surface under bronchoscopy nor granulomatous inflammation or caseous necrosis in ROSE was observed in the control group, as shown in Table 1.

3.2. Detected results of MTB culture and Xpert MTB/RIF for washing liquid

Among the 55 patients enrolled in the study, neither MTB nor contamination was detected by the MIGT 960 liquid culture in 20 ml of NS collected after washing the bronchoscope, which had been cleaned by either SR procedure or IR procedure. In the SR group (n = 28), 14 samples were detected as positive for MTB/RIF, significantly higher than in the IR group (n = 14), with a statistically significant difference observed between them (42.86 % vs 7.14 %, P = 0.018). However, on the day when subjects were successfully enrolled in the SR group, no MTB nucleic acid residue was detected in the bronchoscope rinse solution used by non-tuberculosis patients in the control group. In the SR group, of the 12 MTB-positive samples, 8 tested low, 1 medium, and 3 very low in concentration. In contrast, the IR group had only one sample testing very low. This indicated that the amount of nucleic acid residue in the SR group was significantly higher than that in the IR group, as shown in Table 2.

4. Discussion

To the best of our knowledge, this study is the first to describe the impact of nucleic acid residues following bronchoscope reprocessing on molecular biological diagnostic methods and how to mitigate this effect using straightforward techniques. Our research demonstrated that a bronchoscope used on patients with a high MTB load, after undergoing SR, resulted in a high detection rate (42.86 %) with the nucleic acid amplification-based Xpert MTB/RIF assay. Among these, 9 samples were positive (1 medium and 8 low) in the Xpert MTB/RIF test, indicating the presence of a lot of nucleic acid residues from MTB in those samples. However, when 55 samples of 20 ml NS collected after flexible bronchoscopy washing were directly cultured for MTB after centrifugation, neither positive results nor contamination were detected, which confirmed that both reprocessing procedures met the existing quality control standards [15]. Due to the fact that the detection target for Xpert MTB/RIF is the ropB gene fragment of MTB,a positive result suggests the presence of ropB nucleic acid fragments in the sample, but it does not confirm the presence of viable MTB. Culturing methods, on the other hand, are capable of detecting only living bacteria. This study suggests that even if the cleaning criteria for flexible bronchoscopy are met, a high proportion of nucleic acid residues is unavoidable. Ofstead, Cori L.,

Table 1
Demographic characteristics of participants included in the study.

Patient characteristics	Total (n = 55)	Control group (n = 13)	SR group (n = 28)	IR group (n = 14)	*P* value*
Age (years, range)	46.47 (25 ~ 80)	56.00 (25 ~ 80)	42.75 (19 ~ 80)	45.00 (21 ~ 75)	0.309
Sex (n,%)					
Male	18(32.72)	8 (61.54)	5(17.86)	5(35.72)	0.259
Female	37(67.27)	5 (38.46)	23(82.14)	9(64.28)	
L-J Culture positive (bacterial load)					1.000
4+	26(47.27)	0(0.00)	17(60.71)	9(64.28)	
3+	11(20.00)	0(0.00)	8(28.57)	3 (21.43)	
2+	5(9.09)	0(0.00)	3(10.72)	2(14.29)	
Morphology under bronchoscopy					
Greyish-white ulcerated necrotic matter (Yes, %)	39(70.91)	0 (0.00)	25 (89.28)	14 (100.00)	0.285
Rapid On Site Evaluation (ROSE)					
Granulomatous inflammation or caseous necrosis (Yes, %)	14(25.45)	0 (0.00 %)	10(35.71)	4(28.57)	0.459

*P value was counted between SR Group and IR Group, because control group was regarded as a blank group in this study. Abbreviations: SR, standard reprocessing; IR, intensive reprocessing; L-J: Löwenstein-Jensen,

Table 2
MTB culture and Xpert MTB/RIF for 20 ml normal saline collected after reprocessing bronchoscope.

Classification	Total (n = 55)	Control group (n = 13)	SR group (n = 28)	IR group (n = 14)	*P* value[a]
MTB culture (n, %)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	
Xpert MTB/RIF (n)					
Medium	1	0	1	0	0.018
Low	8	0	8	0	
Very low	4	0	3	1	
Positve rate for XpertMTB/RIF (n,%)	13 (23.63)	0(0.00)	12(42.86)	1(7.14)	

[a]P value was counted between SR Group and IR Group. Abbreviations: SR group: standard reprocessing group, IR group: intensive reprocessing group; MTB, mycobacterium tuberculosis.

et al. also observed that 100 % of bronchoscopes had residual contamination after manual cleaning [17]. Informing the professional cleaning staff about the clinical status of bronchoscopy patients with a high suspicion of aPTB before the SR process, and repeated washing and suction of the bronchoscope with a large volume of sterilized water more than 20 times after the SR procedure can significantly reduce the amount of nucleic acid residue, thereby lowering the detection rate of MTB/RIF for MTB from 42.86 % to 7.1 %. Furthermore, a study has shown that high-level disinfection reprocessing cannot eliminate a 3 % risk of bronchoscope contamination by potentially pathogenic microorganisms, and that additional manual flushing with 70 % ethyl alcohol after each disinfection cycle effectively reduces this risk [18]. Therefore, additional manual flushing proves to be an effective method for reducing contamination or residue.

For over 150 years, bronchoscopy, especially flexible bronchoscopy, has been a mainstay for airway inspection, the diagnosis of airway lesions, therapeutic aspiration of airway secretions, and transbronchial biopsy to diagnose parenchymal lung disorders [19]. Molecular diagnosis of pathogenic microorganisms following bronchoscopy sampling presents a viable alternative for patients with scant or absent sputum [20]. This study verified that the residue of nucleic acids remaining after the cleaning of bronchoscopes can lead to false positive outcomes in such diagnostic methods, thereby increasing the misdiagnosis rate among patients. This consequence is particularly detrimental to the physical and mental health of patients, especially in cases of infectious diseases such as tuberculosis, deep pulmonary fungal infections, and rare pathogen infections. Notably, the treatment duration for TB patients is six months; however, if misdiagnosed with drug-resistant tuberculosis, the treatment period extends, and the adverse effects are significantly magnified [21].

With the advancements in molecular biology, increasingly sensitive techniques like mNGS and ddPCR are being progressively incorporated into clinical settings. These methods enhance the detection and identification of pathogenic microorganisms, offering higher accuracy and sensitivity compared to traditional diagnostic approaches. Their application in clinical practice marks a significant step forward in the precise diagnosis and effective treatment of various infectious diseases [22,23]. Consequently, the likelihood of nucleic acid residues inducing false-positive outcomes with such techniques has risen. Although the results of this study indicate that the implementation of IRP for bronchoscopes can significantly reduce the residual amount of nucleic acids, complete removal is not yet achievable. Consequently, future research is necessary to investigate methods for eliminating nucleic acid residues or to consider the use of disposable bronchoscopy to prevent such false positives. Disposable bronchoscopes, characterized by their complete sterility and the absence of the need for cleaning, mitigate the risk of cross-contamination due to improper cleaning, disinfection, or flushing procedures, as well as inadequate leak detection or drying [24]. Since the outbreak of the coronavirus disease 2019 (COVID-19), the utilization and proliferation of disposable bronchoscopes have surged, particularly following the American Association for Bronchology and Interventional Pulmonology's (AABIP) endorsement of the use of "disposable bronchoscopes" for patients suspected or confirmed to have COVID-19. [25] However, the application of disposable bronchoscopes in the context of tuberculosis patients, in terms of preventing cross-contamination or reducing the impact of nucleic acid residue, has not yet been evaluated. This study discovered that the residual nucleic acids from reusable fiberoptic bronchoscopes can lead to false-positive results in highly sensitive molecular diagnostic methods. However, the use of disposable fiberoptic bronchoscopes, which are not intended for use in subsequent patients, theoretically eliminates this impact.

However, our study had some limitations. Firstly, our study was limited by a relatively small sample size, which might have introduced bias in our interpretation of the data, and further studies to evaluate the effect of nucleic acid residue in reprocessing bronchoscopy are required. Secondly, the study was conducted in a single center, and the bronchoscope cleaning was performed manually. This approach does not eliminate the potential impact of environmental and personnel factors on the study outcomes. Thirdly, the MTB/RIF method employed in our study cannot detect or quantify trace amounts of nucleic acid residues, which may result in underestimation of these residues.

After bronchoscopy procedures in patients with high-burden pathogenic bacteria, the SRP of bronchoscopes can still lead to false positive results in molecular biological diagnostic methods for subsequent samples. The IRP, including professional training for cleaning staff and briefing on patient information prior to the SRP, coupled with suction and washing with sterile water at least 20 times post-cleaning, can significantly reduce nucleic acid residue. However, these measures

cannot completely eliminate the residue.

Author contributions

QFH: contributed to the data analysis, wrote the manuscript and made research protocol. XXJ: made research protocol, collected sample and data. YSL: recruited patients XZ: collected sample and data. TW and HYZ: laboratory test sample and collected data, QHZ: made research protocol and edit manuscript. All authors contributed to the article and approved the submitted version.

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CRediT authorship contribution statement

Xingxing Jin: Visualization, Supervision, Investigation, Data curation, Conceptualization. **Qianfang Hu:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Yishi Li:** Supervision, Investigation, Conceptualization. **Xia Zhang:** Methodology, Investigation, Data curation. **Wan Tao:** . **Houyu Zhong:** Data curation. **Qinghai Zhao:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

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.

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