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Detection of pathogens from resected heart valves of patients with infective endocarditis by next-generation sequencing

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ABSTRACT

Objectives: Identification of the underlying pathogens of infective endocarditis (IE) is critical for precision therapy.
Methods: We evaluated a metagenomic method with next-generation sequencing (NGS) for the direct detection of pathogens from the resected valves of 44 IE patients and seven rejected IE patients according to the modified Duke criteria.
Results: NGS displayed sensitivity, specificity, positive predictive values and negative predictive values of 97.6%, 85.7%, 97.6%, and 85.7% compared with 46.2%, 100%, 100%, and 12.5% for blood culture and 17.1%, 100%, 100%, and 17.1% for valve culture and 51.4%, 100%, 100%, and 26.1% for valve Gram staining, respectively.
Conclusions: NGS technique had superior sensitivity and shorter turnaround time compared with culture-based methods for identifying causative pathogens of IE. The NGS technology should be considered an essential supplement to culture-based methods, particularly for unculturable or difficult-to-culture microorganisms.

Introduction

Infective endocarditis (IE) is associated with high morbidity, with 30 to 100 episodes per million patients per year (Thuny et al., 2012; Hoen et al., 2005) and high mortality, with more than a third of patients dying within the first year if they do not receive proper treatment (Cabell et al., 2002; Thuny et al., 2005). The prognosis for patients with IE is strongly dependent on early and optimized antibiotic therapy. Therefore, identification of the causative pathogens is crucial and currently, therapeutic schedules mainly rely on blood culture followed by identification and susceptibility testing of the isolate (Maneg et al., 2016). However, antibiotic treatment starting prior to blood culture or IE caused by fastidious or yet uncultured microorganisms may lead to negative culture results (Houpikian and Raoult, 2005; Poesen et al., 2014; Peeters et al., 2016). These microorganisms comprise typically *Abiotrophia* spp., *Bartonella* spp., *Brucella* spp., *Coxiella burnetii, Listeria monocytogenes, Mycoplasma* spp., *Legionella* spp., *Tropheryma whipplei, Cardiobacterium* spp., *Eikenella corrodens, Kingella* spp. and members of the HACEK group (Moter et al., 2010). To isolate these organisms, specialized media are required and even then growth is slow.

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Previous studies showed that blood cultures were negative in 2.5% to 31% of suspected IE cases despite improvements in laboratory procedures (Lamas and Eykyn, 2003; Hoen et al., 1995; Brouqui and Raoult, 2006). In such cases, misdiagnosis and inaccurate treatment may occur which may also have a serious negative impact on patient outcomes. Over the past few decades, molecular techniques such as PCR targeting the 16S rRNA gene have been developed to detect pathogens from resected heart valves and such approaches have proven superior to traditional culture-based techniques (Marsch et al., 2015; Shrestha et al., 2015; Kumar et al., 2013; Madico and Rice, 2008). Recently, whole genome sequencing has become a routine tool for detecting clinical microbiology (Capobianchi et al., 2013) and metagenomic analysis using next-generation sequencing (NGS) has been used to detect pathogens directly from clinical samples, without the need of culture (Hasman et al., 2014). In theory, NGS can identify all types of pathogens from a clinical sample (Naccache et al., 2014; Guan et al., 2016). Yuto et al. published a case report in which NGS was applied for pathogens detection from resected valves, but only a small number of cases were included (Yuto et al., 2015). Recently, Oberbach et al. published an eight-case study aimed at identifying the potential biodiversity among bacterial species from IE patients (Oberbach et al., 2017). To our knowledge, there are very few reports of using metagenomic analysis for detecting pathogens directly from excised heart tissues in IE patients and the performance of this new method needs to be elevated. The aim of this study was to elevate the performance of NGS for detecting pathogens directly from the resected heart valves of IE patients who had undergone surgery.

Methods

Sample collection and information

The resected valves were collected randomly from the Center of Cardiac Surgery in Fuwai Hospital, National Center for Cardiovascular Diseases (Beijing, China), from April 2017 to September 2018. The study was approved by the ethics committee of the hospital. All patients involved in this study had provided their written informed consent, and samples were used for research only. In our study, we included 51 patients (including 37 men and 14 women, Table S1). 41 patients were diagnosed with definite IE and three patients with possible IE according to the modified Duke's criteria. Another seven patients also underwent cardiac surgery for non-infectious reasons and were treated as negative controls in this study. The specimens were cut into two equal pieces using sterile scissors in a biosafety cabinet. One piece of tissue was randomly selected for immediate culturing, while the other was snap-frozen at −80°C for metagenome sequencing and Sanger validation.

Valve culture (VC) and blood culture (BC)

The specimens were physically ground into particles using a sterile grinder, then placed in sterile tubes containing 5 ml of brain heart infusion broth and incubated in a CO_2 enriched atmosphere (5%) at 35 °C for 7 days. Growth was evaluated daily. After 7 days of incubation, all samples were subcultured onto blood agar plates (Oxoid, Beijing, China), chocolate agar plates (Oxoid), and MacConkey agar plates (Oxoid), regardless of whether or not growth was suspected. An average of three sets of blood samples was drawn by peripheral venous puncture prior to antibiotic use. Blood samples (10 ml for adults, 13 ml for children) were injected into aerobic and anaerobic blood culture bottles (Becton Dickinson, Sparks, MD, USA). Blood culture bottles were loaded into an automated continuous monitoring system (BD BACTEC™ FX400, USA) within an hour of being drawn and were incubated at 35 °C for 7 days. If the subculture of the blood or valves showed bacterial growth, identification was carried out by VITEK MALDI-TOF mass spectrometry (bioMérieux, Marcy l'Étoile, France) and antibiotic susceptibility testing was performed subsequently with VITEK 2 COMPACT (bioMérieux, Marcy l'Étoile, France).

NGS

The frozen valves were thawed at room temperature for 30 min and were then cut into pieces as small as possible with sterile scissors. Approximately 25 mg of tissue was treated with proteinase K before DNA extraction. Total DNA was extracted using a TIANamp Micro DNA kit (DP316, TIANGEN BIOTECH, Beijing, China) according to the manufacturer's recommendation. The extracted DNA was fragmented with a Bioruptor (ThermoFisher Scientific, Waltham, MA, USA)) instrument to generate 200–300 bp fragments. The libraries were then prepared as follows: firstly, the DNA fragments were subjected to end-repair and A-tailing; secondly, the resulting DNA was ligated with bubble-adapters that contained a barcode sequence, and then amplified with PCR. Quality control was carried out with an Agilent 2100 (Agilent Technologies, Santa Clara, CA, USA) to assess the fragment size and using a Qubit dsDNA HS Assay kit (Thermo Fisher Scientific Inc,Waltham, MA, USA) to measure the DNA libraries concentrations. Qualified libraries were pooled together to form single stranded DNA (ssDNA) circles and then DNA nanoballs were generated with rolling circle replication. The final DNA nanoballs were loaded onto a sequencing chip and were sequenced with a HiSeq platform (Illumina, Ca, USA). High-quality sequencing data were generated by removing low-quality reads, adapter contamination, and duplicated reads and short reads (length < 50 bp). Human sequence data were excluded and mapped to a human reference (GRCh38) using a powerful alignment tool called Burrows-Wheeler Alignment (BWA). After removing human sequences, the remaining sequencing data were aligned to NCBI nt database by SNAP. The mapped data were processed for advanced data analysis with in-house scripts, including: taxonomy annotation, genome coverage/depth calculation and abundance calculation. All the NGS positive results were validated by Sanger sequencing.

PCR and Sanger validation

For NGS or VC or BC positive findings, the extracted DNA of resected valves was validated by specific PCR using the primers 27 F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The PCR reaction was performed as follows: 96 °C for 150 s; (96 °C, 30 s; 55 °C, 30 s; and 72 °C, 90 s) ×30 cycles, and 72 °C for 7 min, ending at 4 °C. PCR products were detected by agarose gel electrophoresis and purified with a gel extraction kit (DC3511-02, Biomiga Inc., SanDiego, CA). Sanger sequencing was performed with an ABI PRISM 3730 DNA Sequencer (Applied Biosystems, Foster City, CA, USA) to validate the sequencing results. Finally, the sequences were aligned to the NT database with NCBI Blast online software (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM5blastn&PAGE_TYPE5BlastSearch&LINK_LOC5blasthome).

Statistical methods

The sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) were calculated by MedCalc software.

Results

Clinical characteristics

51 patients were included in this study. Table 1 outlines the key patient characteristics. Most of the IE patients were male (n = 31,

Table 1
Baseline characteristics of the 51 study cases.

Characteristic[a]	IE Patients (N = 44)[b]	Rejected (control) N = 7
Mean (±SD) age-yr[c]	42 ± 17.0	55 ± 7.1
Male sex no. (%)	31 (70.5%)	6 (85.7%)
LVEF (%)	61 ± 5.8	57 ± 8.3
Main impaired valve (s) no. (%)		
AV + MV	7 (15.9%)	None
AV	25 (56.8%)	4 (57.1%)
MV	10 (22.7%)	1 (14.3%)
TV	2 (4.5%)	None
Other tissue	None	2 (28.6%)
Vegetation no. (%)		
AV + MV	6 (13.6%)	None
AV + TV	1 (2.3%)	None
AV	23 (52.3%)	None
MV	10 (22.7%)	None
TV	2 (4.5%)	None
Not found	2 (4.5%)	7 (100.0%)
Antibiotics used before admission no. (%)		
Yes	35 (79.5%)	None
No	2 (4.5%)	7 (100.0%)
Not clear	7 (15.9%)	None
Laboratory data		
CRP (mg/L)	24.5 ± 26.9	4.3 ± 4.8
ESR (mm/h)	32.7 ± 25.4	4 ± 3.6

[a] AV, aortic valve; MV, mitral valve; TV, tricuspid valve; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; LVEF: Left ventricular ejection fraction.
[b] 41 definite IE patients and three possible IE patients were included in the table.
[c] IE patients consisted of 41 adults and three children.

70.5%), with a mean age of 42.0 (including three children). According to the modified Duke's criteria, 41 patients (65.0%) were diagnosed with definite IE and another three patients were classified as possible IE. The remaining seven patients were not IE patients and were treated as controls. Among the 44 IE patients (including definite and possible IE patients), 35 (79.5%) had taken antibiotics themselves at home or in primary hospitals before being admitted to our hospital (the details of antibiotic use in provided in Table S1).

Valve Gram staining

18 IE patients (47.4%) had positive valve Gram staining results and six patients had no staining data (Table 2). One Gram-negative bacillus (GNB) and 17 Gram-positive cocci (GPC) were observed among the 41 definite IE and three possible IE patients.

VC and BC results

19 IE patients (30.8%) had positive BC results (six in our hospital and 13 in primary hospitals) with the causative pathogens comprising one *Pseudomonas aeruginosa,* one *Staphylococcus cohnii*, one *Enterococcus faecalis*, two *Staphylococcus aureus* and 14 *Streptococcus viridians* (Table 2). Eight strains were cultured in the resected valve tissue in the 44 IE patients including one *P. aeruginosa,* one *filamentous fungus*, one *E. faecalis*, one *S. aureus*, two *Coagulase-negative Staphylococcus* and two *Streptococcus viridians*.

NGS results

Among the 41 definite IE patients, causative pathogens were detected in 40 (97.6%) (Table 2). In addition, *Coxiella burnetii* was detected in a possible IE patient who had negative valve Gram staining and culture-based results. In 18 blood-positive IE patients, the NGS results were 100% concordant with the BC results in genus level and 83.3% in species level. Of all of the NGS positive results, Sanger sequencing verification revealed genus concordance of 100% and species concordance of 87.5% (NGS detected four isolates of *Streptococcus oralis and one Streptococcus constellatus*, whereas the Sanger sequencing results detected *S. viridians* spp. and *Streptococcus* sp., respectively, Table 2).

False-positive and false-negative results

A *filamentous fungus* was cultured in the resected valve in case A5 (Table 2), while *Streptococcus gordonii* was detected with NGS, and Gram-positive cocci were observed with valve Gram staining. *S. cohnii* was cultured in case A16 which was positive in a sole aerobic bottle of the four sets of BC. The aforementioned two strains were likely to be contaminants and were considered to be false-positive results. In case A9 (Table 2), definite IE was confirmed with echocardiography and histopathologic examination whereas valve Gram staining, culture-based methods, and NGS gave negative results (Figure S1). This was treated as a false-negative result for all test modalities. In case A45, *C. burnetii* was detected with NGS, while a classification of rejected IE (rheumatic valvular disease) was assigned. This was treated as a false-positive result (Figure S2).

The performance of different test modalities

Table 3 outlines the sensitivity, specificity, PPV and NPV of the four test modalities for diagnosing IE. The sensitivity for detecting the causative pathogens in 41 definite IE patients was 97.6% (95% confidence interval [CI], 87.1%–99.9%) for NGS, 51.4% (95% CI, 34.0%–68.6%) for valve Gram staining, 46.2% (95% CI, 30.1%–62.8%) for BC, and 17.1% (95% CI, 7.2%–32.1%) for VC. The specificity calculated in seven rejected IE patients was 85.7% (95% CI, 42.1%–99.6%) for NGS, 100% for valve Gram staining, BC, and VC. The PPV and NPV were 97.6% and 85.7% for NGS, 100% and 26.1% for valve Gram staining, 100% and 12.5% for BC, 100% and 17.1% for VC, respectively.

Discussion

In our study, we evaluated the performance of NGS for detecting causative pathogens from resected valves compared with culture-based methods in IE patients. And also we confirmed the ability of NGS for identifying various microorganisms directly from heart tissues, including Gram-negative bacilli, Gram-positive cocci, *Bartonella* spp., and *C. burnetii.*

By reviewing medical records and telephone follow-ups, we found that 79.5% of patients had taken antibiotics at home or in the primary hospital (Table 1). Since the symptoms of early IE closely resemble those of a cold, lots of IE patients had self-prescribed antibiotics or had been treated in rural hospitals with antibiotics. Some IE patients were diagnosed accurately in city or provincial hospitals and had received sufficient antibiotic therapy before being admitted to our hospital for surgery. This was reflected by the low BC positive rate (12.8% in house and 46.2% in total, Table 3) in our hospital, which was much lower than previously published reports (Marín et al., 2007; Vondracek et al., 2011). This study showed that NGS has advantages in countries where antibiotics are widely used because it can detect microorganisms theoretically as long as the microbial nucleic acids remain in the sample, which overcomes the limitation of low sensitivity in conventional culture-based methods (Yuto et al., 2015; Imai et al., 2014). Interestingly, we found that microbial nucleic acids were enriched in resected valves compared with sterile samples such as blood, hydrothorax, cerebrospinal fluid, and urine. The number of unique reads for NGS in our study usually reached the thousand levels or

Table 2
Clinical diagnosis and main laboratory results.

Case no.	Duke's criteria	Valve Gram staining[a]	Blood culture	Valve culture	NGS results	Sanger sequencing verification
A1	Definite	Negative	Negative	Negative	*B. quintana*	*B. quintana*
A2	Definite	Negative	Negative	Negative	*C. burnetii*	*C. burnetii*
A3	Definite	GNB	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*	*P. aeruginosa*
A4	Definite	GPC	*S. anginosus*[c]	Negative	*S. anginosus*	*S. anginosus*
A5	Definite	GPC	Negative	*Filamentous fungi*[b]	*S. gordonii*	*S. gordonii*
A6	Definite	GPC	No data	Negative	*S. mutans*	*S. mutans*
A7	Definite	GPC	Negative	negative	*S. oralis*	*S. viridans* spp.
A8	Definite	GPC	Negative	Negative	*S. oralis*	*S. viridans* spp.
A9	Definite	Negative	Negative	Negative	Not detected	Not detected
A10	Definite	Negative	*S. oralis*[c]	Negative	*S. oralis*	*S. viridans* spp.
A11	Definite	GPC	*S. sanguis*[c]	Negative	*S. sanguis*	*S. sanguis*
A12	Definite	No data	Negative	Negative	*S. sanguis*	*S. sanguis*
A13	Definite	GPC	Negative	Negative	*S. sanguis*	*S. sanguis*
A14	Definite	No data	*S. mutans*[c]	Negative	*S. mutans*	*S. mutans*
A15	Definite	GPC	*S. gallolyticus*[c]	*S. gallolyticus*	*S. gallolyticus*	*S. gallolyticus*
A16	Definite	GPC	*S. cohnii*[b]	Negative	*A. defectiva*	*A. defectiva*
A17	Definite	GPC	*S. viridans* spp.[c]	Negative	*A. defectiva*	*A. defectiva*
A18	Definite	GPC	Negative	Negative	*S. oralis*	*S. oralis*
A19	Definite	No data	Negative	*S. hominis*	*S. pneumoniae*	*S. pneumoniae*
A20	Definite	Negative	Negative	Negative	*S. constellatus*	*Streptococcus* sp.
A21	Definite	Negative	Negative	Negative	*H. parainfluenzae*	*H. parainfluenzae*
A22	Definite	GPC	*S. aureus*[c]	Negative	*S. aureus*	*S. aureus*
A23	Definite	Negative	No data	Negative	*C. burnetii*	*C. burnetii*
A24	Definite	GPC	*S. oralis*[c]	Negative	*S. oralis*	*S. oralis*
A25	Definite	GPC	*S. gordonii*	Negative	*S. gordonii*	*S. gordonii*
A26	Definite	Negative	*S. gordonii*	Negative	*S. gordonii*	*S. gordonii*
A27	Definite	No data	Negative	Negative	*S. gordonii*	*S. gordonii*
A28	Definite	No data	*S. sanguinis*	Negative	*S. sanguinis*	*S. sanguinis*
A29	Definite	GPC	Negative	Negative	*S. oralis*	*Streptococcus* sp.
A30	Definite	Negative	Negative	*S.haemolyticus*	*S. oralis*	*S. oralis*
A31	Definite	No data	*S. aureus*	*S. aureus*	*S. aureus*	*S. aureus*
A32	Definite	Negative	*S. gordonii*[c]	*S. gordonii*	*S. gordonii*	*S. gordonii*
A33	Definite	GPC	*E. faecalis*[c]	*E. faecalis*	*E. faecalis*	*E. faecalis*
A34	Definite	Negative	*S. viridans* spp.[c]	Negative	*S. sanguinis*	*S. sanguinis*
A35	Definite	Negative	*S. anginosus*[c]	Negative	*S. anginosus*	*S. anginosus*
A36	Definite	Negative	Negative	Negative	*S. mitis*	*S. mitis*
A37	Definite	GPC	Negative	Negative	*S. gallolyticus*	*S. gallolyticus*
A38	Definite	Negative	Negative	Negative	*S. sanguinis*	*S. sanguinis*
A39	Definite	Negative	*S. viridans* spp.[c]	Negative	*S. gordonii*	*S. gordonii*
A40	Definite	Negative	Negative	Negative	*C. burnetii*	*C. burnetii*
A41	Definite	Negative	Negative	Negative	*C. burnetii*	*C. burnetii*
A42	Possible	Negative	Negative	Negative	*Not detected*	*Not detected*
A43	Possible	Negative	No data	Negative	*C. burnetii*	*C. burnetii*
A44	Possible	Negative	Negative	Negative	Not detected	Not detected
A45	Rejected	Negative	No data	Negative	*C. burnetii*	*C. burnetii*
A46	Rejected	Negative	No data	Negative	Not detected	Not detected
A47	Rejected	Negative	Negative	Negative	Not detected	Not detected
A48	Rejected	Negative	No data	Negative	Not detected	Not detected
A49	Rejected	Negative	No data	Negative	Not detected	Not detected
A50	Rejected	No data	Negative	Negative	Not detected	Not detected
A51	Rejected	Negative	Negative	Negative	Not detected	Not detected

[a] GNB, Gram negative bacilli; GPC, Gram positive coccus.
[b] This result was considered to be contamination.
[c] The blood culture was positive in primary hospitals but negative in our hospital.

Table 3
Sensitivity, specificity, PPV and NPV of different methods for diagnosis of IE.[a]

Methods modality	Available[b]	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Valve Gram staining	41	51.4 (34.0–68.6)	100.0 (54.1–100.0)	100.0 (81.5–100.0)	26.1 (10.2–48.4)
Blood culture	42	46.2 (30.1–62.8)	100.0 (29.2–100.0)	100.0 (81.5–100.0)	12.5 (2.7–32.4)
Valve culture	48	17.1 (7.2–32.1)	100.0 (59.0–100.0)	100.0 (59.0–100.0)	17.1 (7.2–32.1)
NGS	48	97.6 (87.1–99.9)	85.7 (42.1–99.6)	97.6 (87.1–99.9)	85.7 (42.1–99.6)

[a] PPV, positive predictive value; NPV, negative predictive value.
[b] Case A5 and A16 (Table 2) was highly suspected to be a contamination and was treated as a negative result.

even higher (Table S2), whereas this number was in the dozens for blood samples (Long et al., 2016).

In a BC-positive IE patient (case A16, Table 2), *S. cohnii* was cultured from one aerobic bottle of the four sets (eight bottles in all) while *Abiotrophia defectiva* was detected by NGS. Compared with *S. cohnii*, it is clear that *A. defectiva* is a member of nutritionally variant streptococci (NVS), which are typical pathogens of IE and *S. cohnii* was likely to be contamination in this case. In one VC-positive IE patient, *S. gordonii* was detected with NGS and valve Gram staining revealed a Gram-positive coccus, whereas a *filamentous fungus* was cultured (case A5, Table 2). This VC result was considered to be contamination during broth enrichment or subculture because both histopathologic examination and the clinical characteristics did not support the results and *S. gordonii* was thought to the causative pathogen. In the other VC-positive IE case, *Streptococcus pneumoniae* was identified with NGS, while a strain of *Staphylococcus hominis* was cultured and the valve Gram stain was GPC (case A19, Table 2). This result therefore did not conclusively reveal the causative pathogen. As *S. hominis* is part of the normal flora colonizing the skin surface, it is more likely to be a contaminant acquired during the operation, unlike *S. pneumoniae.* However, we cannot exclude the possibility that the cultured *S. hominis* was part of a mixed infection, as *S. hominis* has been reported to be a cause of IE (Brinkman et al., 2013).

One strain of *Bartonella quintana* and six strains of *C. burnetii* were detected with NGS in our study. The above-mentioned strains are intracellular bacteria and cannot be cultured with routine methods. Although they can also be tested by serologic methods in the routine laboratory, these methods cannot be applied effectively when faced with unknown pathogens. NGS detected another strain of *Haemophilus parainfluenzae* in case A21, which is one of the HACEK group organisms, associated with endocarditis.

NGS, BC and VC were all negative for one definite IE case in our study, which was confirmed with histopathologic examination (case A9, Table 2). This may have been due to the fact that the patient was recovering from inflammation or that the location from which the samples were drawn was inappropriate. The other patient diagnosed with rheumatic valvular disease gave negative BC and VC results but positive NGS results (case A45, Table 2). A mild inflammatory reaction was observed on histopathology and IE could not be excluded in this case (Figure S2). It was therefore treated as a false-positive result, despite potentially being a true positive result due to the possibility of a missed diagnosis or misdiagnosis. If it was a true positive result, the specificity of NGS would increase from 85.7% to 100% for our study sample. Of course, the number of nonendocarditis samples was too small to evaluate specificity in this study.

In this study, we also tried to detect the antimicrobial resistance (AMR) gene in the identified species of NGS data. The case A31 was selected for AMR gene analysis because it was a confirmed methicillin-resistant Staphylococcus aureus (MRSA) strain validated by VITEK 2 COMPACT antibiotic susceptibility analyzer and Sanger sequencing. Nine structured antibiotic resistance genes were detected in the identified species according to the CARD (Table S3).The mecA gene (the marker of MRSA) was successfully detected with abundance of 372.3701359, which revealed this MRSA strain was resistant to all Beta-lactam antibiotics. Although this case showed NGS may have great potentiality to find AMR genes, challenges remain. Firstly, due to the limited host-extracted sequences reads, we failed to detect allelic variants AMR; secondly, short Illumina data reads cannot give context to the position of resistance genes, failing to distinguish between plasmid-borne AMR genes and chromosomal-borne AMR genes; lastly, even if an AMR gene being detected, it may fail to cause resistance, owing to poor expression, silencing or inactivation. In future studies, we will focus on the AMR gene analysis of NGS.

In general, NGS has several advantages for detecting pathogens involved in IE compared with traditional methods. Firstly, NGS technique has superior sensitivity compared with culture-based methods for identifying the causative pathogens of IE. In our study, NGS displayed sensitivity of 97.6% compared with 46.2% for BC and 17.1% for VC. NGS technique can detect fastidious or uncultured microorganisms, as long as microbial nucleic acids remain in the clinical samples. Secondly, NGS technique is faster than culture-based methods (Hasman et al., 2014; Yuto et al., 2015). NGS technique takes approximately 2 days to identify a microorganism, whereas culture-based methods take 3–7 days or longer. Thirdly, NGS technique can detect all potential microorganisms, including fungi and viruses (Imai et al., 2014; Capobianchi et al., 2013) while targeted PCR such as 16S rRNA gene can only be used to screen bacteria and is not applicable to fungi and viruses. Previous research showed that 2% of IE cases are caused by fungi (Murdoch et al., 2009). Lastly, NGS is an unbiased method that can amplify and sequence the entire DNA content of a sample without use of primers or probes. However, targeted amplicon sequencing such as 16S rRNA sequencing can induce bias due to unequal amplification of the 16S rRNA genes from certain species (Shah et al., 2011). Currently, NGS has also been reported with several limitations. Firstly, like all sequencing approaches, the main weakness of NGS is the inability to distinguish between living and dead microorganisms. Secondly, due to different manufacturers and different software of bioinformatics analysis, it is difficult to standardize this technology, which may lead to selected bias. Thirdly, the cost of NGS is 20–30 times more expensive than culture-based methods currently, which is doomed to be difficult to carry out widely before NGS is included in medical insurance, especially in areas with low economic level. Our study had several limitations. The cases selected were subject to selection bias, since only patients undergoing valve surgery were included in our study. The analysis of consecutive specimens is necessary to evaluate the clinical performance of these method modalities in future studies. In addition, fungi were not included among the pathogens in this work. Verification of the ability to detect fungi with NGS is therefore needed in future studies. Finally, the number of negative control samples is too small to evaluate the specificity of NGS. The ability of NGS to diagnose IE requires larger sample size studies in future research.

Conclusion

The NGS technology has superior sensitivity and shorter turnaround times compared with culture-based methods for identifying causative pathogens, particularly for unculturable or difficult-to-culture microorganisms. The application of NGS technology can identify all types of microorganisms simultaneously and detect AMR gene in the identified species, which can not only help the diagnosis of IE, but also guide the postoperative antibiotic therapy and prevent the recurrence of IE. This technology should be considered an essential supplement to BC and VC, especially in patients with culture-negative IE.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2019.03.007.

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