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An integrated microfluidic system using mannose-binding lectin for bacteria isolation and biofilm-related gene detection

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Abstract

Molecular diagnosis of biofilm-related genes (BRGs) in common bacteria that cause periprosthetic joint infections may provide crucial information for clinicians. In this study, several BRGs, including *ica*, *fnbA*, and *fnbB*, were rapidly detected (within 1 h) with a new integrated microfluidic system. Mannose-binding lectin (MBL)-coated magnetic beads were used to isolate these bacteria, and on-chip nucleic acid amplification (polymerase chain reaction, PCR) was then performed to detect BRGs. Both eukaryotic and prokaryotic MBLs were able to isolate common bacterial strains, regardless of their antibiotic resistance, and limits of detection were as low as 3 and 9 CFU for methicillin-resistant *Staphylococcus aureus* and *Escherichia coli*, respectively, when using a universal 16S rRNA PCR assay for bacterial identification. It is worth noting that the entire process including bacteria isolation by using MBL-coated beads for sample pre-treatment, on-chip PCR, and fluorescent signal detection could be completed on an integrated microfluidic system within 1 h. This is the first time that an integrated microfluidic system capable of detecting BRGs by using MBL as a universal capturing probe was reported. This integrated microfluidic system might therefore prove useful for monitoring profiles of BRGs and give clinicians more clues for their clinical judgments in the near future.

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Abbreviations

16S rRNA	16S ribosomal ribonucleic acid
AB	*Acinetobacter baumannii*
BRGs	Biofilm-related genes
CFU	Colony-forming unit
CNS	Coagulase-negative *Staphylococcus*
E. coli	*Escherichia coli*
fnbA	Fibronectin binding protein A
fnbB	Fibronectin binding protein B
ica	Intercellular adhesion
LOD	Limit of detection
MBL	Mannose-binding lectin
MOI	Multiplicity of infection
MRSA	Methicillin-resistant *Staphylococcus aureus*
OD	Optical density
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PJIs	Periprosthetic joint infections
PS	*Pseudomonas syringae*
rMBL	Recombinant mannose-binding lectin
SA	*Staphylococcus aureus*

SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
Sf21	*Spodoptera frugiperda* cell line
VRE	Vancomycin-resistant enterococci

1 Introduction

Staphylococcal including methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common bacteria associated with periprosthetic joint infections (PJIs; Tsuneda et al. 2003; Sawhney and Berry 2009). Bacteria that are capable of producing biofilms are more difficult to treat because bulky metallic implants are present in PJIs. The biofilms can form on the surface and interfaces of prosthesis and render an environment suitable for bacteria proliferation and resistant to host immunity and antibiotic therapy. It is generally believed that in PJIs caused by biofilm-producing bacteria, a procedure of debridement antibiotics with implant retention (DAIR) has a high chance of failure particularly if the infection is established for more than 3 weeks (Parvizi et al. 2011). Ideally, rapid identification of pathogens could help surgeons make decisions as to whether the prosthesis should be removed or retained, as well as which antibiotic would serve best. Once patients are tested positive for PJIs, either a DAIR or a re-implantation protocol (one-stage or two-stage) will be chosen to eradicate the infection and to restore the joint functions (Rao et al. 2011). Theoretically, the sooner the identification of causing bacteria and their capacity in producing biofilms, the better the clinical decisions in choosing antibiotic(s) and deciding implant removal.

Recently, molecular diagnostics based on nucleic acid amplification techniques, such as polymerase chain reaction (PCR), have been developed for rapid detection of MRSA. Otherwise, the isolation of a single strain requires a culture step and the time from colony isolation to identification of pathogens takes approximately 72 h. These methods are relatively time-consuming and labor-intensive (Safdar et al. 2003; Warren et al. 2004). For instance, the timeline to get a complete bacterial report by using traditional bacterial cultivation usually takes about 3–7 days. The joint samples from patients will be first tested with aerobic (1 day) and anaerobic (2 days) bacterial culture operation in certain conditions to evaluate different types of microorganisms. In practice, the report for negative samples usually takes at least 3 days. It takes much more time (up to 7 days) to further confirm and culture for positive infection samples. Therefore, there is a gap in clinical practice that in acute PJIs, when urgent surgery is mandatory but the causing bacteria is unknown such that no reliable guidelines or references can be used for a DAIR or a staged procedure.

Mannose-binding lectin (MBL) is a liver-derived serum protein that can recognize carbohydrate patterns on a broad range of pathogens, including Gram-positive and Gram-negative bacteria, yeasts, parasites, mycobacteria, and even viruses (Fraser et al. 1998). The intercellular adhesion (*ica*) locus mediates biosynthesis of polysaccharide-induced intercellular adhesion as well as cell–cell adhesion. The locus features four main major genes, including *icaA*, *icaB*, *icaC*, and *icaD*. The *ica* genes drive biofilm formation in many bacterial species, including MRSA (McKenney et al. 1998; Solati et al. 2015). Furthermore, the molecular detection of biofilm-related genes (BRGs) revealed that fibronectin genes (*fnbA* and *fnbB*) were present with high frequencies in MRSA isolates, especially from orthopedic surgical infections (Ando et al. 2004; Arciola et al. 2005; Gowrishankar et al. 2016). Therefore, identification of these BRGs may provide insight into the proper treatment options for those with PJIs. In this study, the BRGs of MRSA including *icaA*, *fnbA* and *fnbB* were investigated by using a new integrated microfluidic system. MBL-coated magnetic beads will be used to isolate these bacteria from complicate samples.

Recently, integrated microfluidic systems were reported to detect bacteria via vancomycin-coated magnetic beads which served as a universal bacteria probe. The on-chip PCR was subsequently used in developed microfluidic systems to amplify the 16S ribosomal RNA (16S rRNA) gene (Chang et al. 2014). Others have instead used nanogold particles as probes for on-chip bacteria detection (Chang et al. 2015). The limits of detection (LODs) for these two integrated microfluidic systems were about 100 colony-forming units (CFUs) per reaction. However, vancomycin-resistant enterococci (VRE) cannot be detected by these systems because VRE lacks the cell wall peptides that bind vancomycin-coated beads (Cetinkaya et al. 2000). In addition, these systems were not designed to detect BRGs. Given these issues, as well as the aforementioned need for a rapid diagnostic system for identifying BRGs, especially those diagnostic of MRSA, we sought herein to develop an integrated microfluidic system capable of rapidly identifying key BRGs.

Therefore, an integrated microfluidic system using MBL as a universal capture probe to isolate bacteria from complicate samples and on-chip PCR processes to detect BRGs is proposed in this study. The experimental results showed that both eukaryotic MBL and prokaryotic MBL can isolate commonly seen bacteria no matter their antibiotics-resistant properties with LODs as low as 3 CFU for *MRSA* and 9 CFU/reaction for *Escherichia coli (E. coli)* when using 16S rRNA PCR for bacterial identification. It is worth noting that the entire process including bacteria isolation by using MBL-coated beads for sample pre-treatment, on-chip PCR, and fluorescent signal detection could be completed on an integrated microfluidic system within 1 h. This is the first time that an integrated microfluidic system capable

of detecting BRGs by using MBL as a universal capturing probe was reported. It might be useful for monitoring profiles of biofilm-related genes and give clinicians more clues for their clinical judgments in the near future.

2 Materials and methods

2.1 Experimental procedures

To summarize the protocol for on-chip detection of BRGs, bacterial samples were first incubated with MBL-coated magnetic beads for 10 min (Fig. 1a). Details of the magnetic-bead coating process are described in Sect. 2.3. Then, a magnet in the integrated control system (Chang et al. 2014) was placed at the bottom of the microfluidic chip to collect bacteria–bead complexes for bacteria isolation (Fig. 1b). After the magnetic isolation process, distilled water was transported from washing buffer reservoir to sample chamber (in the middle of chip) to wash out unwanted materials (Fig. 1c). Then, 20 μL of PCR reagents (described in Sect. 2.4 and listed in Table 1) was transported to re-suspend the bacteria–bead complexes for the following PCR step (Fig. 1d). PCR thermocycling (Fig. 1e) was then carried out by a thermal control module in the integrated control system (Chang et al. 2014). Finally, a photomultiplier tube collected fluorescence signals, and software was used to analyze the results. A photograph and an illustration of the integrated microfluidic chip are shown in Fig. 1g. The dimensions of the integrated microfluidic chip were measured to be 75 mm × 59 mm. Details of the microfluidic chip design can be found in our prior works (Chang et al. 2013, 2014). Briefly, it was equipped with micropumps, microvalves, and micromixers (Fig. 1g) such that the bacteria isolation step, wash step, PCR, and fluorescent signal collection and analysis could be completed automatically within an hour.

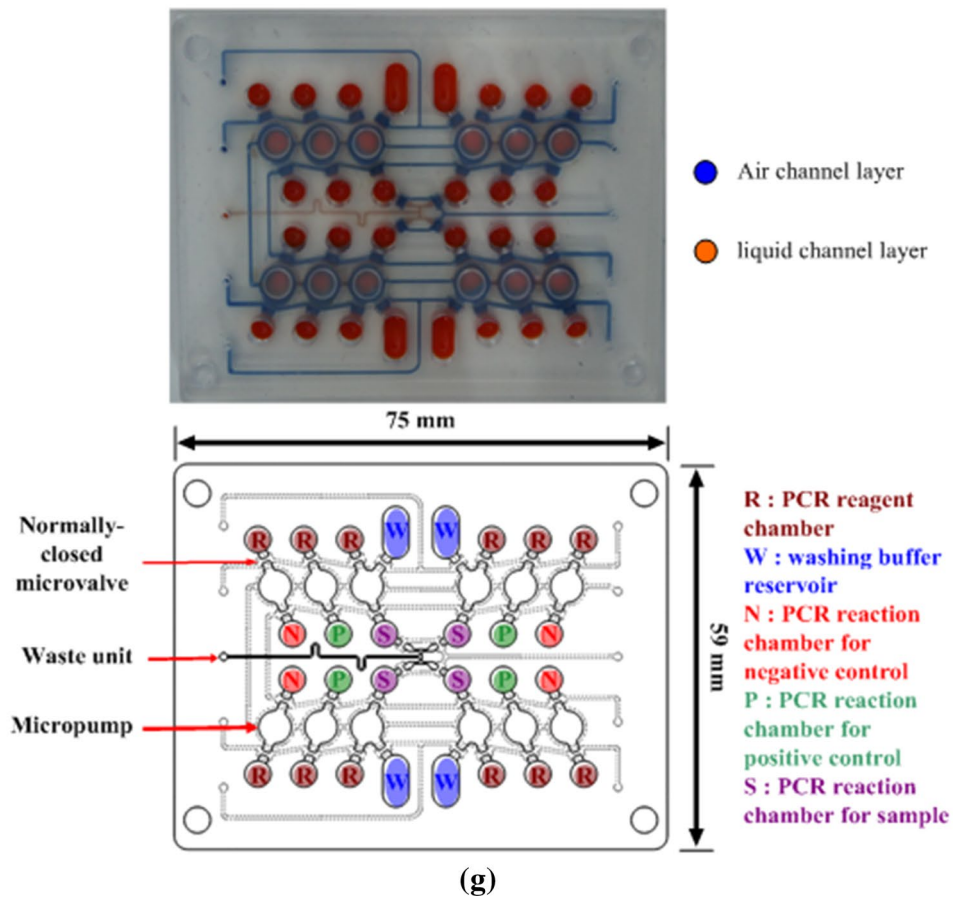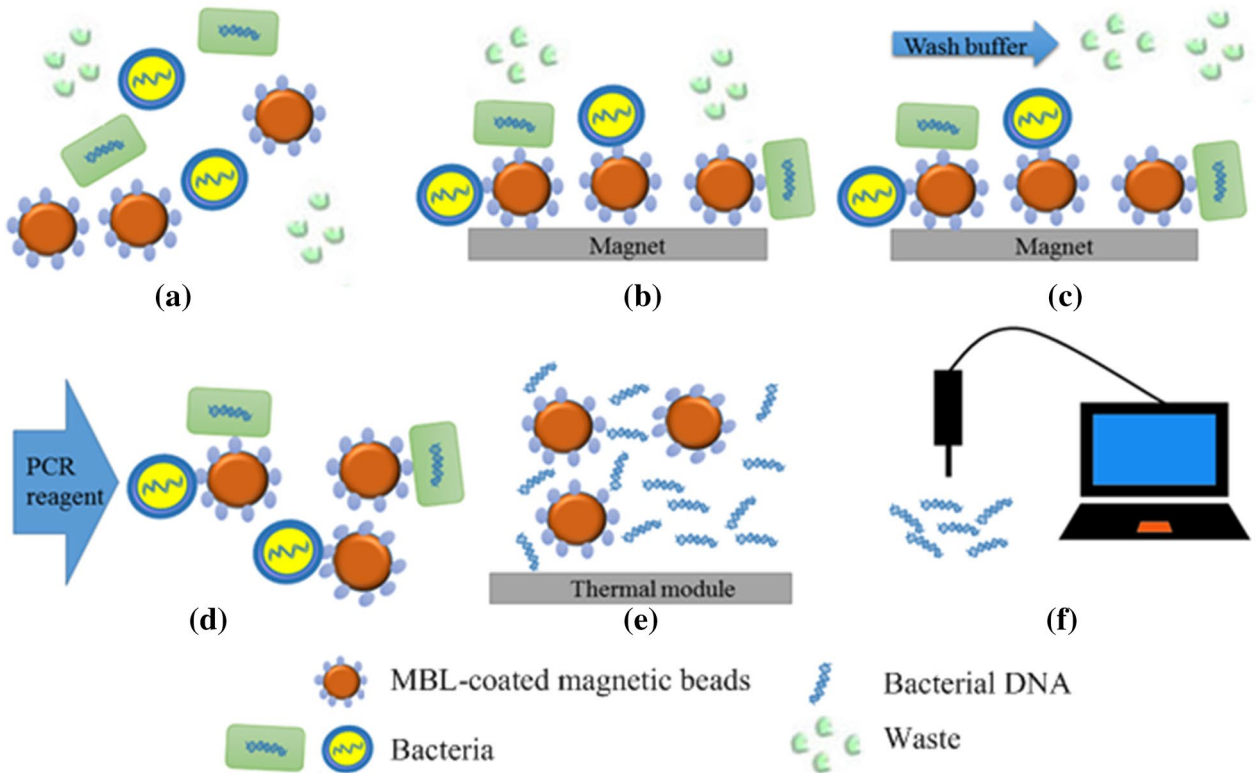### 2.2 Expression and purification of eukaryotic and prokaryotic MBLs

2.2.1 Eukaryotic system for MBL expression and purification

The *Spodoptera frugiperda* 21 (Sf21) cell line was cultured at 28 °C in TNM-FH Insect Medium (Sigma, USA) containing 10% fetal bovine serum for generation of recombinant baculoviruses. Sf21 cells were maintained at 28 °C in Sf-900II serum-free media (Gibco, USA) for protein expression. Generation of the recombinant virus, vAcGP6-MBL-Rhir-E, was performed with Sf21 transfected with pFast-BacGP67-MBL-Rhir-E recombinant bacmid DNA (3 μg) with the aid of cellfectin (3 μL, Invitrogen, USA). The recombinant viruses were used to infect Sf21 cells,

and green fluorescence plaques were counted under an inverted fluorescence microscope at day 4 after transfection. The titer of the recombinant viruses was based on the end-point dilution with the 50% tissue culture infectious dose method in a 96-well plate. To amplify the viruses, Sf21 cells (2×10^5 cells) were seeded on 24-well plates and infected with vAcGP67-MBL-Rhir-E viruses at a multiplicity of infection (MOI) of 1.0. Cells were harvested 4 days post-infection, and enhanced green fluorescent protein (EGFP) expression was observed under the fluorescence microscope (Eclipse TE300, Nikon, Japan). The harvested supernatants (500 μL) were centrifuged at 3000g for 15 min to remove cell debris, and the virus solution was filtered through a syringe filter (0.22 μm) at 4 °C. To overexpress the recombinant MBL in insect cells, Sf21 cells (2×10^7 cells) were first seeded on T75 flasks and maintained at 28 °C in Sf-900II serum-free media. Then they were infected with vAcGP67-MBL-Rhir-E viruses at MOI of 1.0 while continuously shaking the flasks at 100 rpm. Cells were harvested 4 days post-infection, and the supernatants were harvested 3 days post-infection and centrifuged at 3000g for 15 min. The proteins in the supernatants were precipitated with ammonium sulfate to a final concentration of 100% (w/v) followed by gentle stirring at 4 °C overnight. Then, 1 mL of phosphate-buffered saline (PBS) was used to dissolve the protein pellets, which were subsequently dialyzed against 5 L of PBS at 4 °C overnight. The suspension was centrifuged at 12,000g for 10 min to remove insoluble impurities, and the supernatant was subsequently incubated overnight with HIS-select nickel affinity gel (BIOMAN, Taiwan) at 4 °C. The resin was loaded onto a column and washed with 20 column volumes of a washing buffer (50 mM Tris–HCl, 300 mM NaCl, 10% glycerol and 20 mM imidazole, pH 7.4). The 6× His-tagged recombinant MBL proteins were then eluted in elution buffer (50 mM Tris–HCl, 300 mM NaCl, 10% glycerol, and 200 mM imidazole, pH 7.4).

2.2.2 Prokaryotic system for MBL expression and purification

The bovine MBL proteins with its mature full length (amino acid from 21 to 249 with an exact gene size of 687 base pairs (bp); NCBI accession number: NP_776532.1) were cloned into the pET21b vector with a 6-His tag and the codon optimization approach (Puigbò et al. 2007) and were used to improve protein expression efficiency in the *E. coli* BL21(DE3) cell (Sigma, USA). The transformed *E. coli* cells were cultured in 2.4 L of LB broth (UniRegion Bio-Tech, Spain) containing 50 mg/L ampicillin in the final concentration at 37 °C. When the cell density reached an optical density (OD) of 0.4–0.5 at 600 nm, protein expression was induced with 0.5 mM isopropyl-β-D-thiogalactoside for 16 h at 25 °C. The cell pellets were

(a) (b) (c)

(d) (e) (f)

- ● MBL-coated magnetic beads
- ⟋ Bacterial DNA
- ▭ ◉ Bacteria
- ℮ Waste

- ● Air channel layer
- ● liquid channel layer

75 mm

59 mm

Normally-closed microvalve

Waste unit

Micropump

R : PCR reagent chamber
W : washing buffer reservoir
N : PCR reaction chamber for negative control
P : PCR reaction chamber for positive control
S : PCR reaction chamber for sample

(g)

◄**Fig. 1** Experimental procedures performed on the integrated microfluidic system, including **a** sample incubation with mannose-binding lectin (MBL)-coated beads, **b** bacteria isolation via application of an external magnet to attract bacteria–bead complexes, **c** washing away unbound materials, **d** PCR reagent transport/re-suspension of bacteria–bead complexes, **e** PCR, and **f** fluorescence detection and analysis. **g** A photograph and an illustration of the microfluidic chip

harvested by centrifugation at 6000g and solubilized in Tris buffer (50 mM Tris–HCl, 50 mM NaCl, 2 mM MgCl$_2$, 14 µM β-mercaptoethanol, 0.2 mM phenylmethylsulfonyl fluoride, pH 7.8). Cells were sonicated, and the supernatant was separated by ultracentrifugation (100,000g) for 1 h at 4 °C. The supernatant was treated with the Ni Sepharose 6 Fast Flow kit (GE Healthcare, England) containing the Tris buffer (including 20 mM imidazole), and the loaded resins were used to fill the column by gravity with slow rotation overnight. The purified MBL protein was eluted in Tris buffer (including 250 mM imidazole). The target protein was then dialyzed against Tris buffer with the Amicon kit (Merck Millipore, USA) to remove excessive imidazole for long-term storage.

2.2.3 Identification of eukaryotic and prokaryotic MBL proteins by SDS-PAGE and Western blot

The recombinant eukaryotic and prokaryotic MBL proteins were analyzed by 10% SDS-PAGE with a voltage of 100 V for 30 min, and the gel was stained with Brilliant Blue R solution (Sigma, USA) and agitated for 1 h. The gel was destained in 50 mL of destain solution (30% methanol, 10% acetic acid and 60% H$_2$O), until the background was clear.

The recombinant eukaryotic and prokaryotic MBL proteins were subjected to Western blot analysis by mixed with Laemmli buffer (0.25 M Tris–HCl, 2% SDS, 5% β-mercaptoethanol, 10% glycerol, and 0.002% bromophenol blue, pH 6.8) and then separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with a voltage of 100 V for 30 min, along with a broad range of prestained protein molecular standard (Bio-Rad, UK). The proteins were electrotransferred (Bio-Rad) onto polyvinylidene fluoride membranes (Bio-Rad, UK) with a voltage of 100 V for 2 h at 4 °C. Then the membrane was blocked in 5% non-fat milk and incubated with 1:4000-diluted anti-HIS (Bio-Rad, UK) primary antibodies overnight at 4 °C. The membranes were then washed with the buffer containing 1× PBS with 0.1% Tween-20 and incubated with 1:10,000-diluted secondary antibody (anti-mouse immunoglobulin G antibody conjugated with horseradish peroxidase (Cell Signaling Technology, USA). After extensive washing, the membranes were incubated with the enhanced chemiluminescence substrates (PerkinElmer, USA) and exposed to an X-ray film.

2.3 Preparation of eukaryotic and prokaryotic MBL-coated magnetic beads

Eukaryotic and prokaryotic MBL proteins (2.5 µg in 30 µL) were added to separate 1.5-mL microcentrifuge tubes and incubated with 950 µL of tenfold diluted magnetic beads (diameter 1.05 µm, 10 mg/mL, Dynabeads® MyOne™ Carboxylic Acid, Thermo Fisher Scientific, USA). Then, 20 µL of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (120 mg/mL, Invitrogen, USA) was added, and the beads/proteins mixtures were incubated at room temperature in the dark for 18 h. The unbound MBL was washed out by using 1 mL of 0.02% Tween-20 (Sigma, USA) and 1 mL of 0.1% sodium dodecyl sulfate (Sigma, USA), and 1 mL of 0.1 M of ethanolamine (Sigma, USA) was used to block the surface of the magnetic beads at room temperature for 1 h to avoid non-specific capturing. Finally, 1 mL of PBS was used to wash away excessive ethanolamine, and the MBL-coated magnetic beads were suspended in another 1 mL of PBS. Please note that the MBL-coated magnetic beads may lose their function upon freezing; they were therefore stored at 4 °C. However, 4 °C is not suitable for long-term storage of MBLs. Thus, MBL-coated magnetic beads should be used as soon as possible after the coating procedure.

2.4 Capture ability of eukaryotic and prokaryotic MBL-coated magnetic beads

The capture ability of the eukaryotic and prokaryotic MBL-coated magnetic beads was investigated by capturing the target bacteria and then amplifying the universal 16S rRNA gene with PCR. Ninety-five microliter (~ 10^9/mL) of seven common bacteria, including coagulase-negative *Staphylococcus* (CNS), *Pseudomonas syringae* (PS), *Staphylococcus aureus* (SA), MRSA, *Acinetobacter baumannii* (AB), *E. coli*, and VRE that had been cultured overnight in LB broth at 37 °C, was incubated with 5 µL of eukaryotic or prokaryotic MBL-coated magnetic beads (1 mg/mL) for 10 min. Then, 100 µL of PBS was used to wash away unbound bacteria. After the washing step, the bacteria–bead complexes were suspended in 20 µL of the PCR reagents and subjected to PCR with the universal 16S rRNA primers (Bergin et al. 2010).

2.5 PCR and slab-gel electrophoresis

A PCR primer set (Forward: 5′tttttttttttattagataccctggtagtccacgcc, Reverse: 5′tttttttttttcgtcatccccaccttcctcc) designed for 16S rRNA was adopted from a previous study (Bergin et al. 2010). The PCR primer sets designed for three BRGs to be found within the genomes of bacteria associated with PJIs are as follows: 5′-GAGGTAAAGCCA ACGCACTC and 5′-CCTGTAACCGCACCAAGTTT for

Table 1 Constituents of PCR reagents

PCR	Volume (μL)		
	Negative PCR reagents	Positive PCR reagents	Sample PCR reagents
ddH$_2$O	6.2	5.2	6.2
Primer F (10 μM)	0.4	0.4	0.4
Primer F (10 μM)	0.4	0.4	0.4
KAPA Fast	10	10	10
SYBR Green	3	3	3
Genomic DNA for positive control	0	1	0
Total	20	20	20

KAPA Fast: KAPA Biosystems Inc., USA; SYBR Green: AppliChem GmbH, German

icaA (intercellular adhesion gene); 5′-AAATTGGGAGCA GCATCAGT and 5′-GCAGCTGAATTCCCATTTTC for *fnbA* (fibronectin binding protein A) (Atshan et al. 2013); 5′-GGAGAAGCTAAGGCGACAGG and 5′-AATTCCTTC ACCGAATTTCCATTT for *fnbB* (fibronectin binding protein B); the PCR primer sets for the antibiotic-resistant gene (*mecA*) are as follows: 5′-TATGGCTCAGGTACT GCTATCC and 5′-TGAGTTGAACCTGGTGAAGTTG. The constituents of the PCR reagents are listed in Table 1. Thermocycling was conducted at 95 °C for 5 min, followed by 25 cycles of 95 °C for 15 s and 60 °C for 30 s. In addition to detecting fluorescent signals with our integrated microfluidic control system (Chang et al. 2014), PCR products were also analyzed by slab-gel electrophoresis to confirm the performance of the entire automatic

procedure. Five microliter of the PCR products with 1 μL of 6x DNA loading dye (Promega, USA) was loaded into a 2% agarose-TBE (Sigma, USA) gel, and 100 V was applied to separate the DNA fragments for 30 min. Ten microliter of ethidium bromide (50 mg/mL, Sigma, USA) was dissolved in 1000 mL of TBE to stain the gels so that the PCR products could be observed at a wavelength of 302 nm under the UV™ transilluminator (UVP, Canada) for 2 s. DNA ladders (100 bp, 108 ng/μL, 5 μL, DM003-R500, GeneDireX, USA) were used to determine PCR product size.

3 Results and discussion

3.1 Capture ability of eukaryotic and prokaryotic MBL-coated magnetic beads

Purities of 85 and 95% were achieved for the prokaryotic (Fig. 2a; 27 kDa) and eukaryotic (Fig. 2b; 47 kDa) expression systems, respectively. Due to the post-translational modifications in insect cells (Geisler et al. 2015), we hypothesized that the latter would more effectively capture MRSA and other biofilm-associated bacteria, though both eukaryotic (Fig. 3a) and prokaryotic MBLs (Fig. 3b) were able to successfully capture all seven target bacteria, including antibiotic-resistant ones (i.e., MRSA and VRE). In contrast, vancomycin-coated beads may not bind these bacteria because vancomycin binding occurs via the D-Ala-D-Ala chain on the bacterial cell walls (Cetinkaya et al. 2000). Once the D-Ala-D-Ala chain mutates, vancomycin does not effectively bind it; this likely makes MBL a better universal bacterial probe.
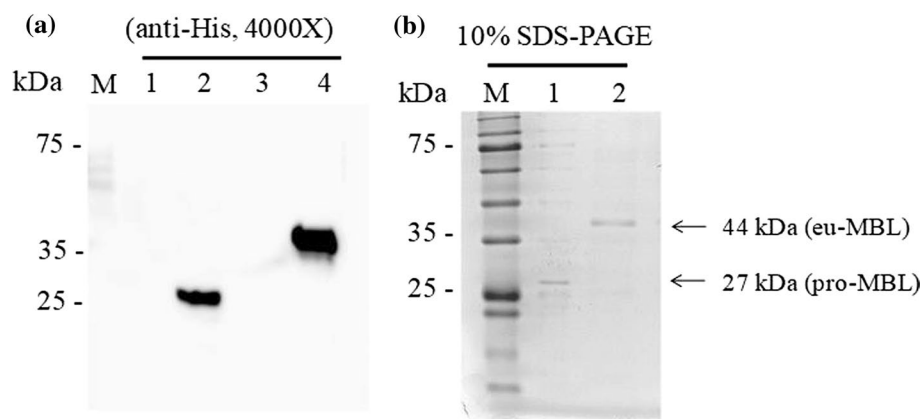
Fig. 2 Expression of mannose-binding lectin (MBL) proteins in eukaryotic and prokaryotic systems. **a** Anti-HIS electropherogram for MBL; lane 1: serum-free media (extracellular negative control); lane 2: recombinant mannose-binding lectin (rMBL) expression from *E.* *coli*; lane 3: Sf21 cell lysate (intracellular negative control); lane 4: rMBL expression from Sf21 cells. **b** The over-expressed eukaryotic (lane 1) and prokaryotic (lane 2) rMBL were analyzed in 10% SDS-PAGE with coomassie brilliant blue staining
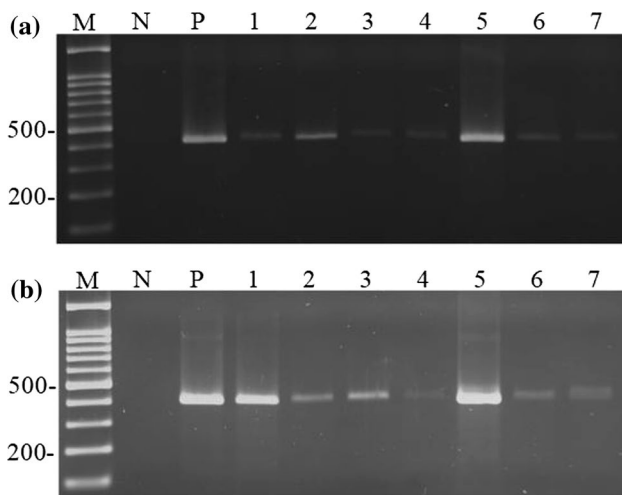
Fig. 3 Specificity tests of mannose-binding lectin (MBL)-coated beads. **a** Bacteria capture ability of eukaryotic MBL-coated beads. M: 100-bp ladder, lanes 1–7 = CNS, SA, MRSA, *E. coli*, PS, AB, and VRE, respectively. **b** Bacteria capture ability of prokaryotic MBL-coated beads. Lanes 1–7 = MRSA, SA, CNS, *E. coli*, PS, AB, and VRE, respectively. In both panels "M," "P," and "N" correspond to 100-bp ladders, positive controls (genomic DNA, which was extract from MRSA), and negative controls (water), respectively

3.2 LOD for eukaryotic and prokaryotic MBL-coated magnetic beads verified via PCR with universal 16S rRNA

After confirming the capture ability of eukaryotic and prokaryotic MBL-coated magnetic beads, LODs of eukaryotic (Fig. 4a) and prokaryotic (Fig. 4b) MBL-coated magnetic beads were investigated while 3×10^8 CFU/ reaction of *MRSA* was used as the target template. The LODs were experimentally found to be 3 CFU/reaction for both MBL-coated magnetic beads. However, the signal intensity for eukaryotic MBL-coated magnetic beads was much stronger than that of prokaryotic MBL-coated magnetic beads. This might be because *E. coli* post-translationally modifies proteins to a less extent so that the recombinant MBL proteins may not have been correctly modified or folded. For instance, glycosylation might stabilize the intact protein structure in active forms, thereby increasing the binding efficiency of the eukaryotic MBL proteins to pathogen cell wall surfaces (Khow and Suntrarachun 2012). Therefore, eukaryotic MBL-coated magnetic beads were chosen for all subsequent tests. With gram-negative *E. coli* (9×10^8 CFU/reaction; Fig. 4c), the LOD was experimentally found to be 9 CFU/reaction; this is a suitable LOD for severe infections such as those in the bloodstream or root canals.
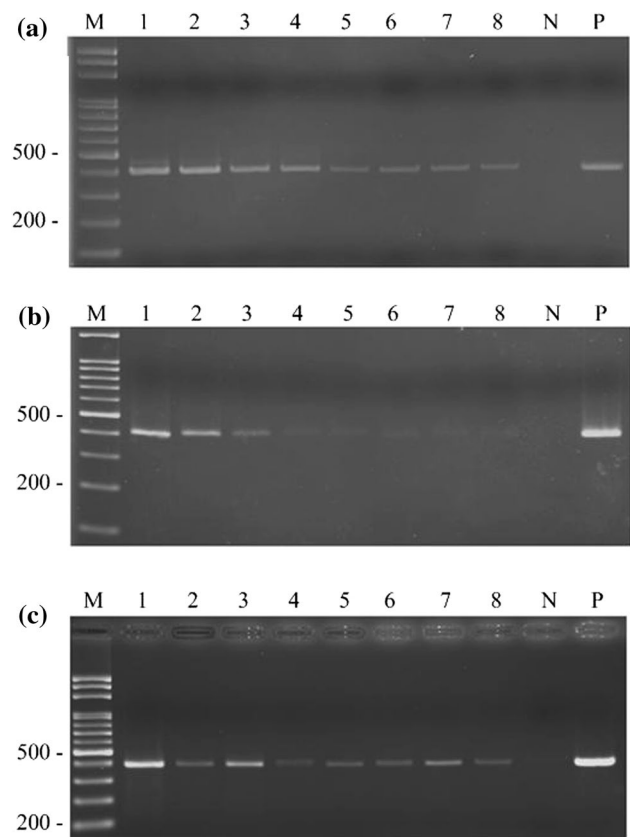
Fig. 4 Limits of detection (LODs) for methicillin-resistant *Staphylococcus aureus* (MRSA) and *E. coli* using eukaryotic and/or prokaryotic mannose-binding lectin (MBL)-coated magnetic beads followed by PCR with universal 16S rRNA primers. **a** LODs of MRSA using eukaryotic and prokaryotic MBL-coated magnetic beads followed by PCR with universal 16S rRNA primers. Lanes 1–8: tenfold serial dilutions of *MRSA* with an initial concentration of 3×10^8 CFU/ reaction. P: positive control (genomic DNA, 10 ng/μL), N: negative control (distilled water). **b** LODs for MRSA using prokaryotic MBL-coated magnetic beads followed by PCR with universal 16S rRNA primers. Lanes 1–8: tenfold serial dilutions of *MRSA* with an initial concentration of 3×10^8 CFU/reaction. **c** LODs of *E. coli* using eukaryotic and prokaryotic MBL-coated magnetic beads followed by PCR with universal 16S rRNA primers. Lanes 1–8: tenfold serial dilutions of *E. coli* with an initial concentration of 9×10^8 CFU/reaction. In all panels, "M," "P," and "N" correspond to 100-bp ladders, positive controls (genomic DNA, 10 ng/μL), and negative controls (distilled water), respectively

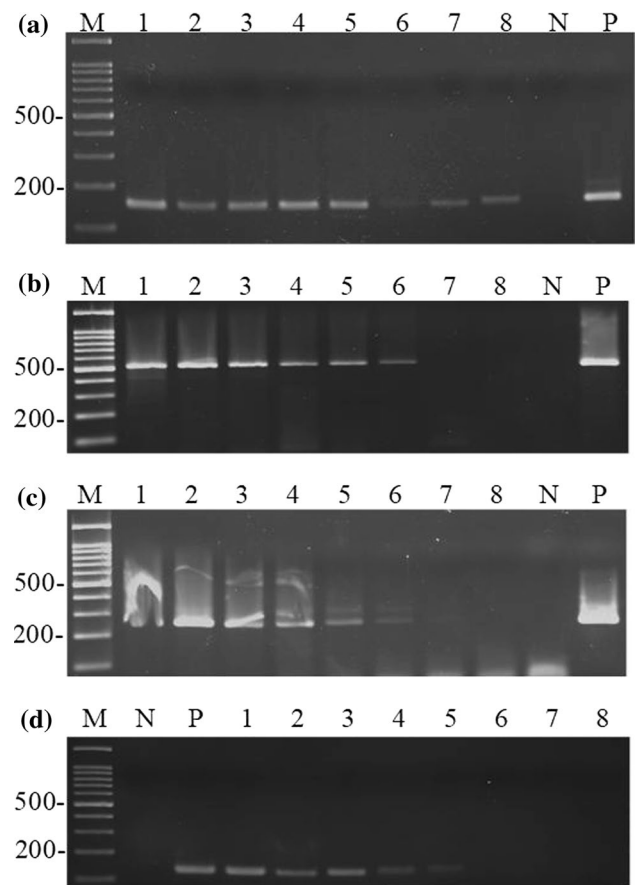## 3.3 Specificity tests for detection of BRGs

The specificity of the developed assay was explored by using seven bacterial strains including MRSA, SA, CNS, *E. coli*, PS, AB, and VRE (Fig. 5). As expected, *icaA* and *fnbA* can only be detected in SA and MRSA and *mecA* and *fnbB* can only be detected in MRSA but not in other bacteria, indicating that the proposed procedure has high specificity to identify four bacteria.
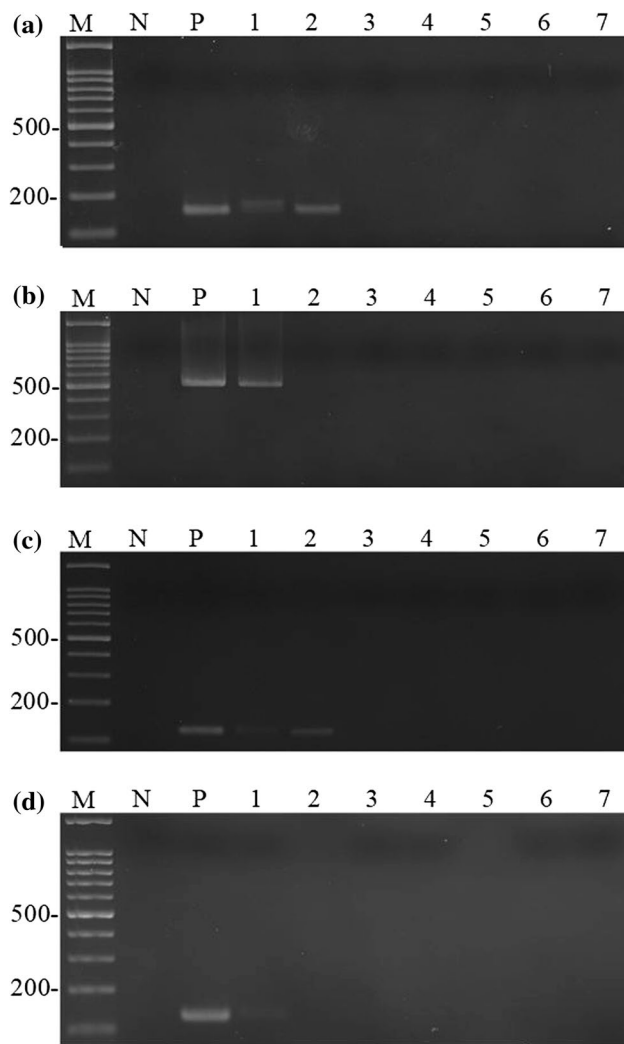
Fig. 5 Specificity test for the detection of one antibiotic-resistant and three biofilm-related genes using PCR assay. The PCR assays were performed in seven bacteria species for **a** *icaA* gene, **b** *mecA* gene, **c** *fnbA* gene, and **d** *fnbB* gene. In all panels, M = 100-bp ladder, lanes 1–7 = *MRSA*, *SA*, *CNS*, *E. coli*, *PS*, *AB*, and *VRE*, respectively. In all panels, "M," "P," and "N" correspond to 100-bp ladders, positive controls (genomic DNA, 10 ng/μL), and negative controls (distilled water), respectively

Fig. 6 Limits of detection (LODs) of methicillin-resistant *Staphylococcus aureus* (MRSA) biofilm-associated genes using mannose-binding lectin (MBL)-coated beads followed by PCR on-bench. **a** LOD for the *icaA* gene. Lanes 1–8: tenfold serial dilutions of *MRSA* with an initial concentration of 3.2×10^9 CFU/mL (6.4×10^7 CFU/reaction). **b** LOD for the *mecA* gene. Lanes 1–8: tenfold serial dilutions of *MRSA* with an initial concentration of 1.8×10^9 CFU/mL (3.6×10^7 CFU/reaction). **c** LOD for the *fnbA* gene. Lanes 1–8: tenfold serial dilutions of MRSA with an initial concentration of 3.3×10^9 CFU/mL (6.6×10^7 CFU/reaction). **d** LOD of the *fnbB* gene. Lanes 1–8: tenfold serial dilutions of *MRSA* with an initial concentration of 5.1×10^9 CFU/mL (10^8 CFU/reaction). In all panels, "M," "P," and "N" correspond to 100-bp ladders, positive controls (genomic DNA, 10 ng/μL), and negative controls (distilled water), respectively

### 3.4 LOD of BRGs by utilizing MBL-coated beads with PCR on-bench and on-chip

The LODs documented upon using eukaryotic MBL-coated magnetic beads to detect BRGs and the antibiotic-resistant gene were further investigated with starting bacteria concentration of ~ 10^9 CFU/mL. The LODs for the antibiotic-resistant gene (*mecA*) and BRGs (*icaA, fnbA and fnbB*) were experimentally found to be 360, 6, 66, and 900 CFU/reaction on-bench, respectively (Fig. 6). The LODs for *icaA*, *mecA*, and *fnbA* are superior to those reported by Chang et al. (2014), indicating that MBLs can be used to isolate

and identify genes from biofilm-associated bacteria. Further, the LODs for the *mecA* antibiotics-resistant gene and the three BRGs (*icaA*, *fnbA*, and *fnbB*) were 640, 3200, 3200, and 11,400 CFU/reaction when performed on the integrated microfluidic systems, respectively, with the prokaryotic MBL-coated magnetic beads (Fig. 7). It is worth noting that we tested the LOD by using 10X serial dilutions from pure overnight bacteria culture. Then, the CFU was counted by using culture plates. It is the reason why the CFU numbers counted for LOD varied between bacteria and bench. Therefore, the LOD was defined with the lowest diluted
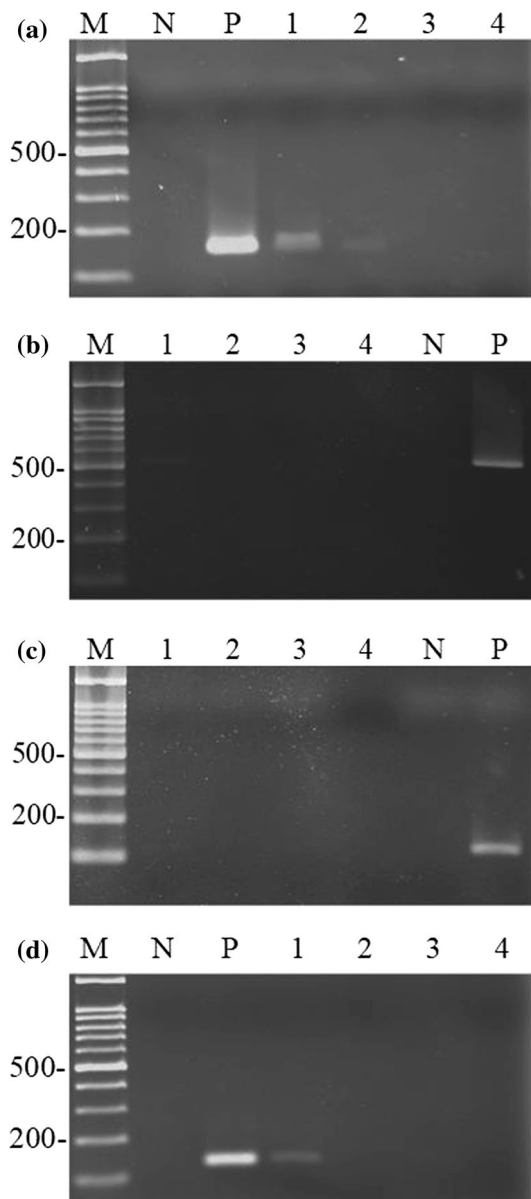
Fig. 7 Limits of detection (LODs) of biofilm-associated genes using mannose-binding lectin (MBL)-coated beads followed by on-chip PCR. **a** LOD for the *icaA* gene from methicillin-resistant *Staphylococcus aureus* (MRSA) captured by eukaryotic MBL-coated magnetic beads. Lanes 1–4: tenfold serial dilutions of MRSA with an initial concentration of 3.2×10^9 CFU/mL (6.4×10^3 CFU/reaction). **b** LOD for the *mecA* gene. Lanes 1–4: tenfold serial dilutions of MRSA with an initial concentration of 1.6×10^9 CFU/mL (3.2×10^3 CFU/reaction). **c** LOD for the *fnbA* gene. Lanes 1–4: tenfold serial dilutions of MRSA with an initial concentration of 6×10^8 CFU/ mL (3.2×10^3 CFU/reaction). **d** LOD of the *fnbB* gene. Lanes 1–4: tenfold serial dilutions of MRSA with an initial concentration of 5.7×10^9 CFU/mL (1.1×10^4 CFU/reaction). In all panels, "M," "P," and "N" correspond to 100-bp ladders, positive controls (genomic DNA, 10 ng/μL), and negative controls (distilled water), respectively

concentration that could be detected by the developed method. The LODs of *icaA*, *mecA*, and *fnbA* are comparable to those reported by Chang et al. (2014), while the relatively high LOD of *fnbB* may be due to poor primer design.

4 Conclusions

This work has demonstrated an integrated microfluidic system that could automate the entire process for detection of BRGs from bacteria known to cause PJIs. The entire process including bacterial isolation, cell lysis, nucleic acid amplification, and optical detection could be performed on a single chip. MBL was first time used to isolate these bacteria from complicate samples, and then molecular diagnosis process was carried out to identify the BRGs. In this work, we tested the pure bacteria in culture medium (LB) to evaluate the performance of this developed system. In the near future, the clinical samples which will be collected by surgeons can be mixed with MBL-coated beads and then analyzed directly in this microfluidic system. The time for bacterial detection can be reduced to 90 min, which is significantly faster when compared with the traditional cultivation method. The LODs of *icaA*, *mecA*, and *fnbA* were comparable to those reported in the literature. It is promising to use the developed microfluidic system for fast diagnosis of bacteria associated with biofilm formation.

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