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A magneto-DNA nanoparticle system for rapid detection and phenotyping of bacteria

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So far, although various diagnostic approaches for pathogen detection have been proposed, most are too expensive, lengthy or limited in specificity for clinical use. Nanoparticle systems with unique material properties, however, circumvent these problems and offer improved accuracy over current methods. Here, we present novel magneto-DNA probes capable of rapid and specific profiling of pathogens directly in clinical samples. A nanoparticle hybridization assay, involving ubiquitous and specific probes that target bacterial 16S rRNAs, was designed to detect amplified target DNAs using a miniaturized NMR device. Ultimately, the magneto-DNA platform will allow both universal and specific detection of various clinically relevant bacterial species, with sensitivity down to single bacteria. Furthermore, the assay is robust and rapid, simultaneously diagnosing a panel of 13 bacterial species in clinical specimens within 2 h. The generic platform described could be used to rapidly identify and phenotype pathogens for a variety of applications.

The rapid and sensitive detection of pathogenic bacteria is crucial for improving patient care with appropriate antibiotic treatment, preventing the spread of disease, and identifying the source of infection in hospital, home or field settings[1–3]. So far, a variety of diagnostic approaches have been proposed, each varying in sensitivity, specificity, cost and efficacy[4–7]. Strategies based on polymerase chain reaction (PCR) and sequencing have shown particular promise as highly sensitive tools for microbiological identification[8–11]. However, quantitative real-time PCR (qPCR)-based systems are often too expensive for resource-limited environments[12], and current sequencing techniques still lack practical applicability to patient care[5]. Bacterial culture and biochemical staining remain the clinical gold standard, despite their long procedural times (up to several days) and limitations in identifying certain species. There is therefore a need for generic, accurate and point-of-care platforms that allow both pathogen detection and phenotyping. Such systems could have far-reaching benefits in other sectors, including food industries, shipping and export businesses, defence and agriculture.

Here, we report a new diagnostic platform for the rapid detection and phenotyping of common clinical pathogens. The assay makes use of magnetic nanoparticles (MNPs) and oligonucleotide probes to specifically detect target nucleic acids from the pathogen. In particular, we hypothesized that ribosomal RNA (rRNA) sequence information from microorganisms could be used in a robust magneto-DNA assay. Because this magnetic detection strategy allows near background-free sensing, the assay steps are greatly simplified and detection is much faster. For bacterial detection, we selected 16S rRNA (a component of the 30S small subunit of bacterial ribosomes[13]) as the target marker, because a single bacterium contains many 16S rRNA strands (1×10^3 to 1×10^5 strands)[14]. Furthermore, the strands have a high degree of sequence consensus across species (important for general bacterial detection) as well as species-specific variable regions (important for species typing)[15,16]. For bacterial phenotyping (for example, identifying drug resistance), targeting of specific mRNA sequences was carried out in parallel with species detection. In this study, rather than sequencing the whole RNA strand, we established a series of primers and probes for amplification and detection of specific regions of interest within common bacterial types. For signal readout we used a miniaturized micro-NMR (μNMR) system, which requires only small volumes of sample for detection (~2 μl) and is also capable of supporting rapid, high-throughput operations in point-of-care settings[17–19].

Design and validation of the assay

The magneto-DNA assay is based on a sandwich hybridization technique wherein two oligonucleotide probes bind to each end of the target nucleic acid (Fig. 1a). Total RNA is extracted from a specimen, and target regions within the 16S rRNA are amplified by asymmetric reverse transcription-PCR (RT-PCR) to produce large numbers of single-strand DNA with only sense (or antisense) sequences. The resultant DNAs are then captured by polymeric microspheres conjugated with probe oligonucleotides (the bead-capture probe). Subsequently, the overhanging edges of the target DNA are hybridized with MNP-detection probe conjugates (the MNP-detection probe). These magnetically labelled beads shorten the transverse relaxation rate (R_2) of a sample, which is detected by a miniaturized μNMR device. This detection method is both robust and highly sensitive, not only because there are multiple 16S rRNA strands per bacterium (as opposed to a single strand of genomic DNA), but also because there are three steps of signal amplification: (i) PCR amplification of the target nucleic acids; (ii) bead capture and enrichment of target nucleic acids; and (iii) magnetic amplification (because a single MNP can affect billions of surrounding water molecules[17]).

Probes specific to each bacterial target were designed through comparative analyses of 16S rRNA gene sequences from different types of bacterial species (see Supplementary Table S1 for details). By aligning multiple sequences from several different genera, we identified both conserved and variable regions; both types of region were subsequently selected as targets. Primers were designed

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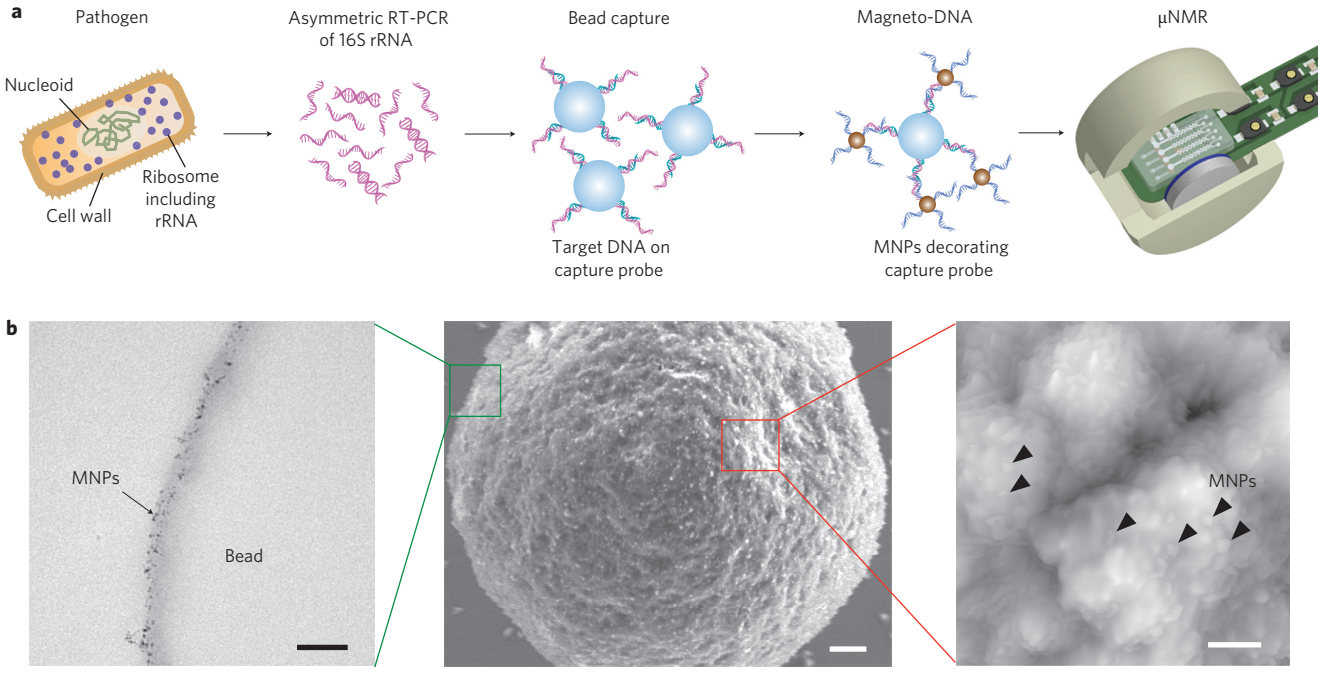

Figure 1 | Magneto-DNA assay for the detection of bacterial 16S rRNA. a, Schematic of the assay procedure. Total RNA is extracted from the specimen, and the 16S rRNA is amplified by asymmetric RT-PCR. Single-strand DNA of the amplified product is then captured by beads conjugated to capture probes, before hybridizing with MNPs to form a magnetic sandwich complex. Samples are subsequently analysed using a μNMR system. **b**, Hybridized probe complexes, as observed by transmission electron microscopy (left; scale bar, 100 nm), scanning electron microscopy (centre; scale bar, 300 nm), and atomic force microscopy (right; scale bar, 100 nm).

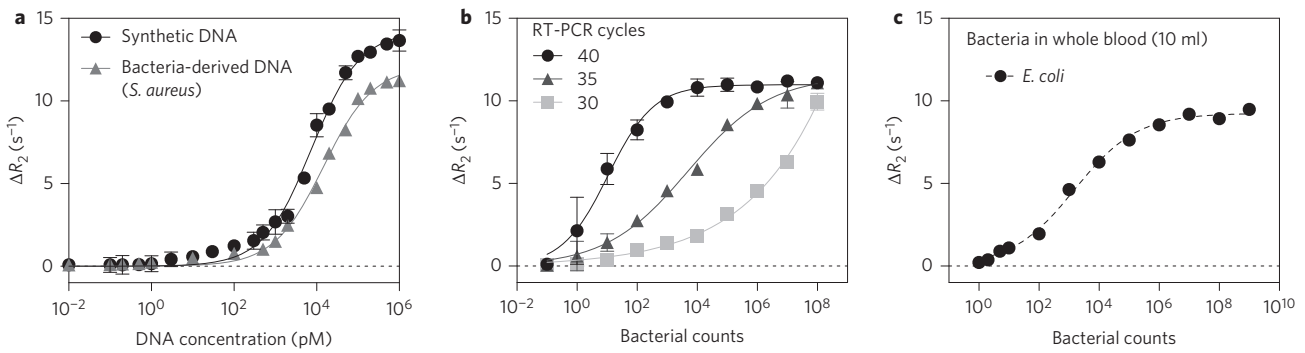

Figure 2 | Detection sensitivity of the magneto-DNA system. a, Serial dilutions of synthetic DNA or bacteria-derived DNA were used as detection targets. Bacteria-derived DNA molecules were obtained via asymmetric RT-PCR of *S. aureus* 16S rRNA (35 cycles). Synthetic DNA had the same sequence as bacteria-derived DNA. The detection limit was ~0.5 pM. **b**, Bacterial detection by magneto-DNA assay. Samples with varying numbers of *S. aureus* were used. Total RNA was extracted and target sequences were amplified by 30, 35 and 40 cycles of RT-PCR. Amplified target DNA were detected using the probe set in **a**. The observed detection limit was a single bacterium, and the dynamic range of detection could be controlled by changing the PCR cycle number. **c**, Bacterial detection in blood. Serial dilutions of *E. coli* were spiked into human blood and processed by first lysing the red blood cells and then extracting the RNA using the same procedure as described above. All experiments were performed in triplicate. All ΔR_2 values were obtained by subtracting the relaxation rate values of the hybridized probe complexes in the presence of target DNA ($R_{2,target}$) by the relaxation rate values of the beads alone ($R_{2,control}$). Data are expressed as mean ± s.d.

to flank the target sequences for hybridization. For each type of bacteria, an optimal primer set was screened to maximize the amount of single-stranded DNA produced by asymmetric PCR. Two oligonucleotide sequences that were complementary to (or near) the 5′ end and 3′ end of the amplicon sequence were selected as probes. One probe type was used as the capture probe and conjugated onto polystyrene beads (diameter, 3 μm), and the other detection probe was conjugated to MNPs (diameter, 20 nm) for magnetic detection by the μNMR system. The numbers of probes per bead and MNP were ~300,000–800,000 and ~16–29, respectively (Supplementary Table S2). After target binding, the beads were densely covered with MNPs, as confirmed by both electron and atomic force microscopy (Fig. 1b, Supplementary Figs S1–S3). The estimated MNP number per targeted bead was $\sim 3 \times 10^5$. Control samples, on the other hand, showed negligible MNPs on the bead surface, which validated the high specificity of the sequence hybridization.

We first determined the detection sensitivity of the magneto-DNA assay, using *Staphylococcus aureus* as the model organism. Both synthetic oligonucleotides and amplified target amplicons from bacteria-derived RNA were prepared. Titration experiments revealed that the limit of detection (LOD) was ~0.5 pM [DNA]

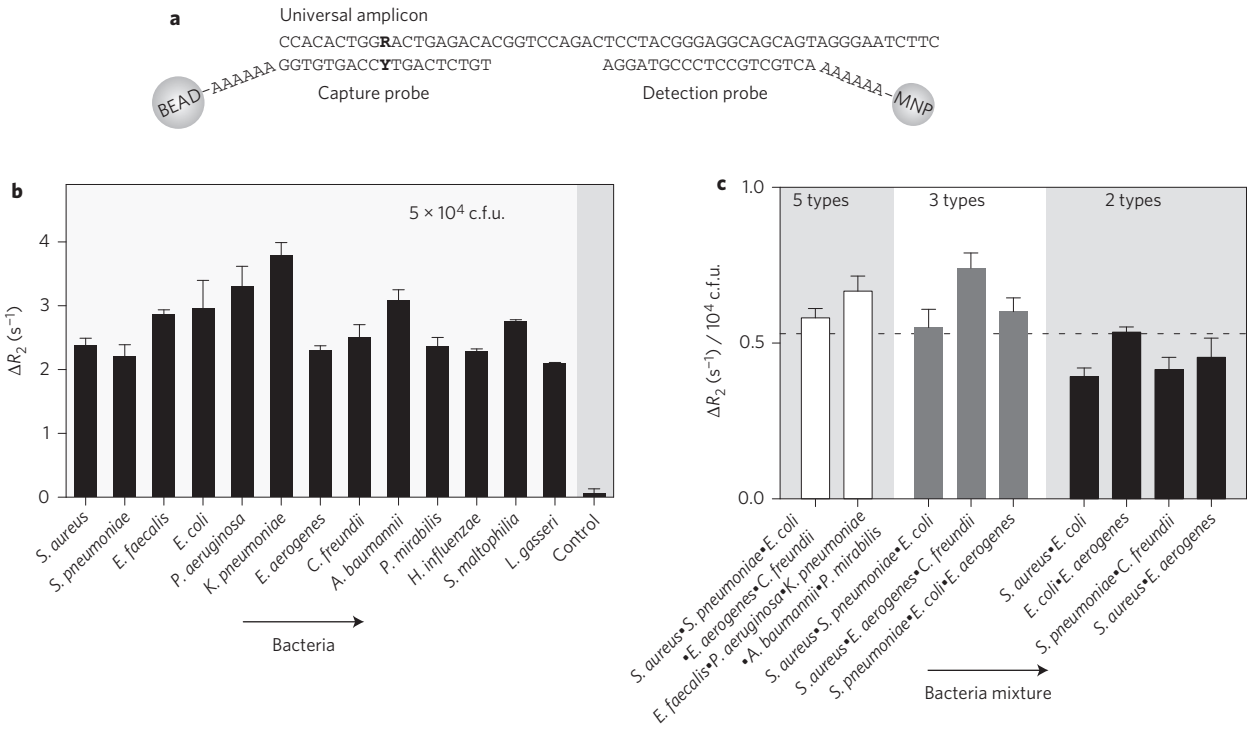

Figure 3 | Universal detection of bacteria using the magneto-DNA system. a, Sequences of universal probes targeting a conserved region of bacterial 16S rRNA. Two probe sequences with a single base difference were blended and used for capture. (**R, Y**) = (A, T) for *Staphylococcus*, *Escherichia*, *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Haemophilus* and *Stenotrophomonas*; (**R**, **Y**) = (G, C) for *Streptococcus*, *Enterococcus*, *Acinetobacter*, *Proteus* and *Lactobacillus*. **b**, Thirteen different bacterial species could each be detected using the universal probes. **c**, Mixtures containing different bacterial types were detected by the universal probes. The observed ΔR_2 values were consistent with the average ΔR_2 value (dotted line) from single species. Data are expressed as mean ± s.d. All samples for the assay were prepared in triplicate.

(Fig. 2a; see Methods for details). Because the sample volume for the current assay was 50 μl, the minimum amount of target DNA needed for detection was 25 amol (or 1.5×10^6 molecules). When samples with varying numbers of *S. aureus* were used, the bacterial detection sensitivity was close to a single bacterium (Fig. 2b). Furthermore, depending on the pathogen density, the PCR cycles could be adjusted to achieve optimal dynamic ranges for detection. Note that errors at lower bacterial concentrations were higher, reflecting sampling errors from serial dilution. Similar results were shown for the detection sensitivity in blood for *Escherichia coli*, one of the major pathogens to cause sepsis. Indeed, it is crucial to achieve early diagnosis of *E. coli* in blood by detecting only a few bacteria. When serial dilutions of *E. coli* were spiked into whole blood and processed for detection, we were able to detect as few as 1–2 bacteria per 10 ml of blood (Fig. 2c). Importantly, we were also able to estimate bacterial load over several log orders. The high sensitivity and robustness of the assay is attributed to the abundance of 16S rRNA (1×10^3 to 1×10^5 per bacterium), which can be easily amplified and detected despite inevitable loss during sample processing. Indeed, because of this high detection sensitivity, an extremely small amount of sample is needed (~0.1% of total volume per sample) not only for detection but also for further characterization by other assays (for example, standard culture, qPCR).

Universal and species-specific detection of pathogens

We next adapted the assay as a generic platform that can comprehensively detect the presence of different pathogens. A set of universal probes were developed by identifying a highly conserved region of 16S rRNA genes from a large pool of different bacterial species (Fig. 3a). Two sequences with a single base difference were selected to serve as the capture probe, and another (single) sequence was selected for detection. When the probe was tested on individual species (Fig. 3b), the observed R_2 values were highly consistent, indicating that different species could be detected using a common probe. Mixtures of different bacterial types could also be detected as a whole (Fig. 3c). The R_2 values normalized by bacterial numbers were within a close range for each mixture, confirming the universal nature of the probes in quantifying the total bacterial load of a given sample.

We further extended the magneto-DNA assay to the identification of different target pathogens. A panel of probes was thus designed to target a hypervariable region within the 16S rRNA gene sequences of different bacterial species (Supplementary Table S1, Fig. S4). To minimize non-specific hybridization, we ensured that the homology of the sequences between genus types was less than 50%. Figure 4a shows an example of bacterial detection. Using specific probes for *Staphylococcus*, the amplified DNA from *S. aureus* 16S rRNA could be detected with negligible background signals from other species. Similarly, all specially designed probes for each bacterial type showed high selectivity with minimal off-target binding (Fig. 4b,c). Notably, the specificity of the magneto-DNA assay was superior to that of qPCR (Fig. 4d, Supplementary Fig. S5), a result that can be attributed to the beneficial features of the sandwich assay. For magneto-DNA sensing, both capture and detection probes must bind to their target to generate a signal. The incubation conditions (for example, stringency) can also be controlled to minimize non-specific binding. Finally, the washing steps following each hybridization cycle in our assay allow removal of any unbound/non-specifically bound targets or probes. The high specificity of the magneto-DNA assay enables reliable and rapid bacterial typing. Even in mixtures of different

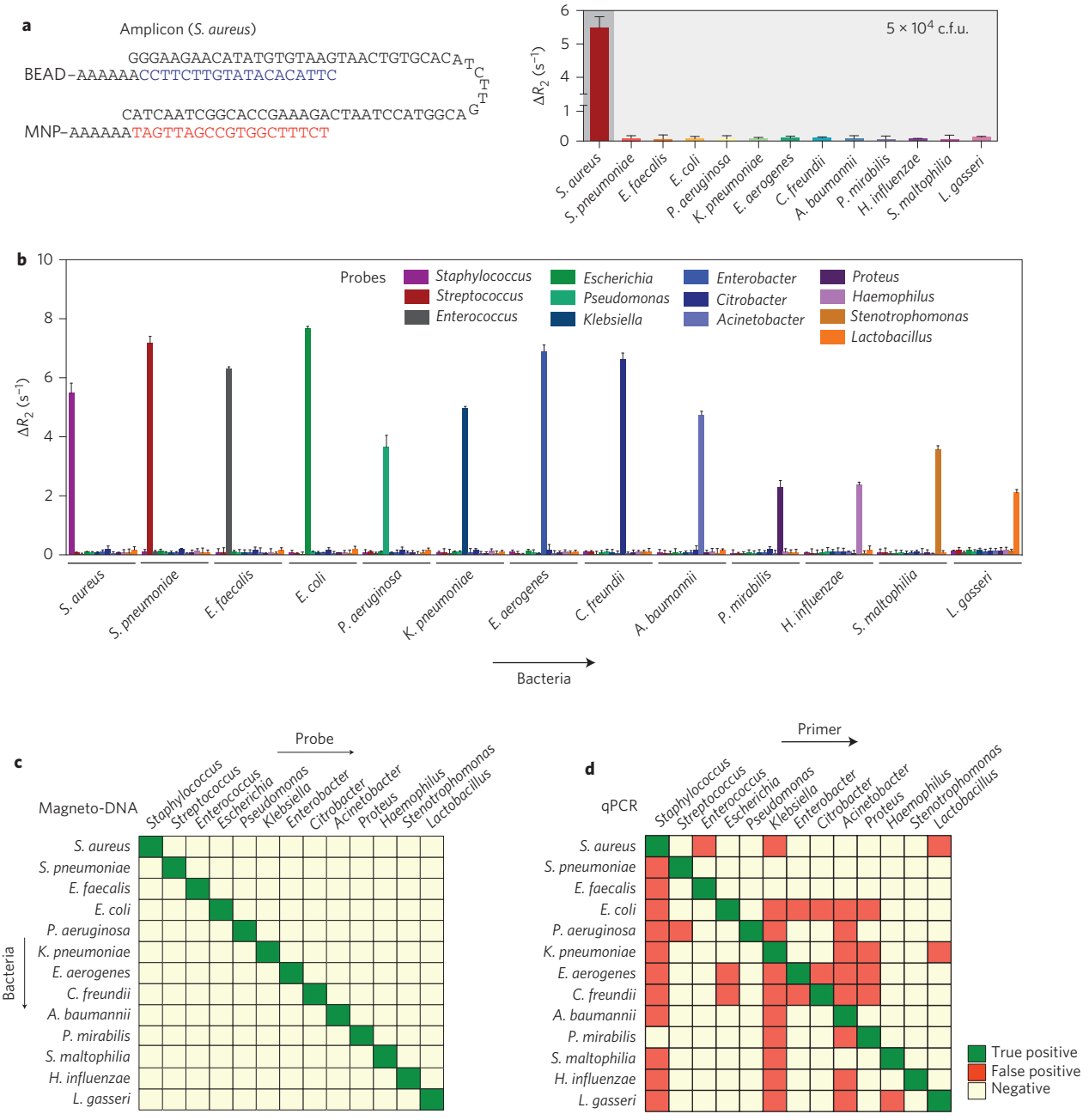

Figure 4 | Differential detection using the magneto-DNA system. Probes targeting hypervariable regions of bacterial 16S rRNA sequences were used to specifically detect various bacterial types. RNA was extracted from bacterial cultures, amplified by asymmetric RT-PCR (35 cycles) using specific primers for each species, and detected using the corresponding probe conjugates. **a**, Probes specific for *Staphylococcus* were used for detecting *S. aureus* (DNA amount equivalent to 50,000 c.f.u.). Target DNA from other bacterial species were added as controls to test off-target binding of the probes. **b**, Relaxation rates for differential detection of various bacterial types. Note the high specific signals and low background noise against other bacteria. Data are expressed as mean ± s.d. All samples for the assay were prepared in triplicate. **c**,**d**, Heat maps comparing the specificity of the magneto-DNA assay with that of qPCR. Specificities in **c** were based on ΔR_2 values from the magneto-DNA assay shown in **b**. Specificities in **d** are relative target amounts obtained from qPCR in Supplementary Fig. S5. Significant signals were marked as positive: positive signals for specific target bacteria were regarded as 'true-positive', and positive signals from non-targeted samples were classed as 'false-positive'.

bacterial species, we could identify and quantify specific bacterial types (Supplementary Fig. S6). Furthermore, by expanding the assay to the detection of mRNA, we could differentiate phenotypes (for example, drug resistance) within closely related species. For example, we were able to detect the mRNA of *mecA* and Panton–Valentine leukocidin (PVL) genes[20,21], which in turn enabled us to identify methicillin-resistant *S. aureus* (MRSA; Supplementary Fig. S7).

Clinical testing

We evaluated the clinical utility of the magneto-DNA assay using patient specimens. Aspirated samples from patients with suspected infections were collected and analysed by conventional culture (procedural time, 3–5 days) as well as the magneto-DNA assay (procedural time, 2 h). Figure 5 shows the results from the magneto-DNA assay. To place these findings in perspective, Table 1 compares our magneto-DNA assay results to those obtained

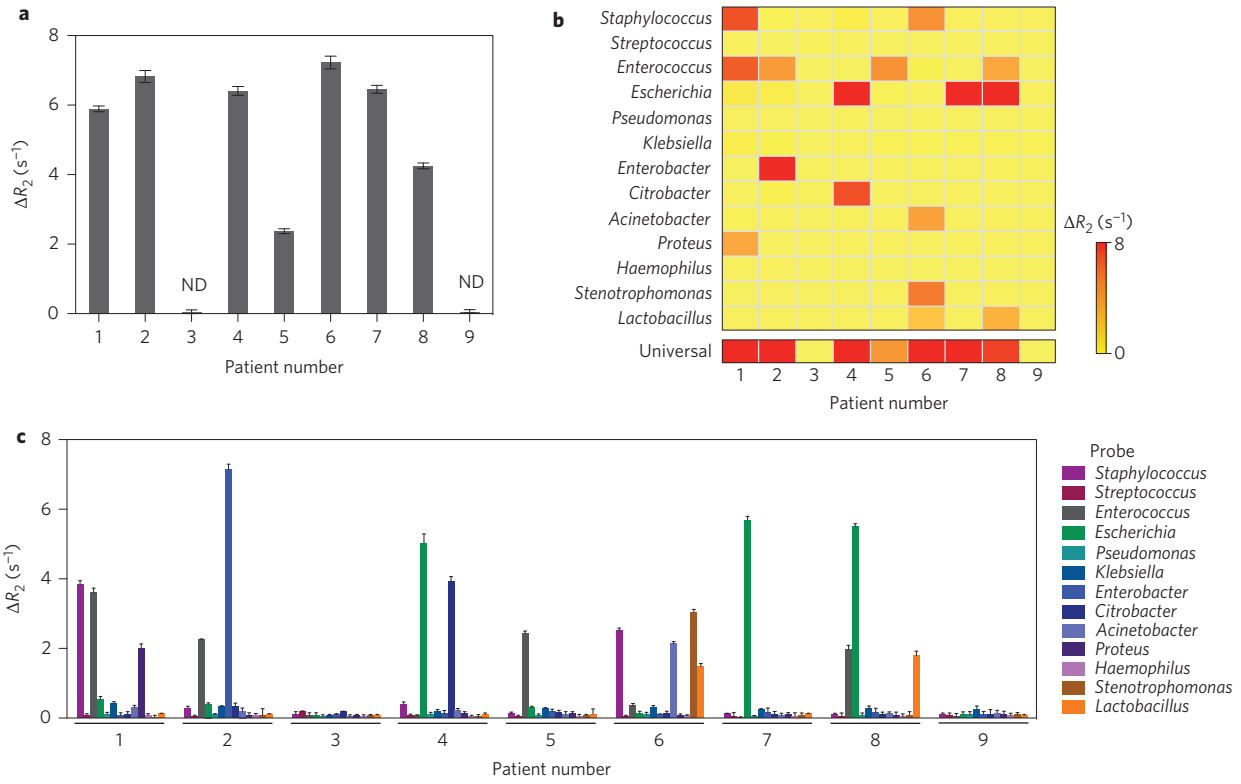

Figure 5 | Diagnosis of clinical samples. a,**c**, Detection of pathogens by the magneto-DNA nanoparticle system using universal (**a**) and specific (**c**) probes for each bacteria type. **b**, Heatmap of obtained ΔR_2 values for universal and specific detection. Clinical specimens (300 μl for each sample) were processed to extract total RNA. This was followed by asymmetric RT-PCR (35 cycles) with universal and specific primers for all bacterial types. The PCR products (equivalent to 0.3 μl volume of sample) were magnetically labelled and detected by μNMR. In **a**, two of the nine clinical samples tested negative, which correlated well with standard culture results. The other seven samples were positive. ND represents samples with no pathogens detected. In **c**, pathogen types are identified within each sample. Note that some samples were infected with more than one pathogen, and the identified bacterial types correlated well with standard culture (Supplementary Table S3). Data are expressed as mean ± s.d. All samples for the assay were prepared in triplicate.

using current gold standard methods. As made clear by this table, the magneto-DNA assay showed excellent accuracy, detecting all bacterial species identified by standard culture. Interestingly, however, the magneto-DNA assay was also able to identify other species (*Citrobacter* in sample 4 and *Acinetobacter* in sample 6) that were left undetected by standard culture. Considering that these organisms could be detected by qPCR (Table 1), it is possible that growth inhibition of species may have occurred during processing. Both *Citrobacter* and *Acinetobacter* belong to a group of Gram-negative bacteria that are poorly targeted by commonly used third-generation cephalosporins because of their capacity to produce inducible beta-lactamase. Thus, the clinical implications of being able to detect such organisms (undetected by standard culture) with the magneto-DNA assay are significant. Furthermore, by having the ability to detect pathogens directly in the specimen, the magneto-DNA assay not only overcomes problems of growth competition, but also minimizes sample contamination. Diagnostic accuracy is thus maximized. Although these results are encouraging, further in-depth studies on these culture-negative organisms are clearly needed. This will require developing the magneto-DNA assay for even higher throughput, and performing larger prospective clinical trials.

Conclusions

In the current study, we have designed a dual probe–nanoparticle system capable of detecting and phenotyping common human pathogens. The method is robust, fast (<2 h), sensitive, accurate and potentially adaptable to a wide variety of other pathogens. It thus has the potential for guiding decisions across various clinical scenarios. We used μNMR as the primary readout for detecting targets labelled with MNPs[17,22]. Although nanoparticle-based magnetic detection methods of whole bacteria have been reported previously, they have largely relied on the use of either small-molecule affinity ligands or antibodies[19,23,24]. These approaches tend to have very limited sensitivity and are not comprehensive enough to be adapted to broad clinical use (that is, they are unable to detect Gram-negative bacteria, resistant strains or specific species). The magneto-genetic assay presented not only overcomes these issues, but also offers a platform technology that can be easily applied to the clinic as well as other point-of-care settings.

Nucleic acid-based techniques allow the specific typing of species with high enough sensitivity to circumvent lengthy culturing processes. 16S rRNA sequence information has been used for bacterial classification and taxonomy, so typing methods based on these databases have emerged as the preferred technique for microbial identification over traditional culture and biochemical assays. Since the advent of genomic sequencing technologies, extensive databases have been established[25], and these have been widely used to develop qPCR assays for the identification of specific targets. Metagenomic studies also represent a novel approach for identifying microbial communities in heterogeneous samples, and could thus be useful for the diagnosis of clinical specimens[26,27]. Despite such advances, however, the development of a robust diagnostic platform for systematically detecting bacteria in point-of-care settings has remained a challenge[28,29]. Various technical and practical problems, such as the propensity for false positives (especially for standard PCR) as well as prohibitive cost issues (for example, Taqman assays, LightCycler probes, DNA/RNA sequencing and so on)

Table 1 | Comparison of the magneto-DNA system and gold standard procedures for bacterial detection in clinical samples.

Patient number	Type of specimen	Magneto-DNA*		Gold standard	
		Universal	Differential	Bacterial culture†	qPCR
1	Urine	Positive (5.9)	*Staphylococcus* (3.8) *Enterococcus* (3.6) *Proteus* (2.0)	*Staphylococcus* (+) *Enterococcus* (++) *Proteus mirabilis* (+++)	Culture results confirmed
2	Pleural fluid	Positive (6.8)	*Enterococcus* (2.3) *Enterobacter* (7.2)	*Enterococcus* (+++) *Enterobacter* (+++)	Culture results confirmed
3	Kidney urine	Negative (<0.1)	None	None	Negative
4	Kidney urine	Positive (6.4)	*Escherichia* (5.0) *Citrobacter* (3.9)	*Escherichia* (+++)	Culture results confirmed and also *Citrobacter*
5	Biliary fluid	Positive (2.4)	*Enterococcus* (2.4)	*Enterococcus* (++)	Culture results confirmed
6	Pelvic abscess	Positive (7.2)	*Stenotrophomonas* (3.0) *Staphylococcus* (2.5) *Lactobacillus* (1.5) *Acinetobacter* (2.1)	*Stenotrophomonas* (+++) *Staphylococcus* (+++) *Lactobacillus* (+)	Culture results confirmed and also *Actinobacter*
7	Kidney urine	Positive (6.5)	*Escherichia* (5.7)	*Escherichia* (+)	*Escherichia*
8	Kidney urine	Positive (4.3)	*Enterococcus* (2.0) *Escherichia* (5.5) *Lactobacillus* (1.8)	*Enterococcus* (++) *Escherichia* (+) *Lactobacillus* (++)	Culture results confirmed
9	Ascitic fluid	Negative (<0.1)	None	None	Negative

*Values for magneto-DNA assay represent ΔR_2 values. Higher ΔR_2 values indicate higher bacterial abundance.
†Detection levels: abundant (>100,000 c.f.u. ml^{-1}) for +++; moderate (10,000 to <100,000 c.f.u. ml^{-1}) for ++; and few (100 to <10,000 c.f.u. ml^{-1}) for +.

have been major obstacles. Here, using validated, readily available bacterial sequence information, we have designed a nucleic acid probe-based magnetic detection assay to identify some relevant human pathogens.

In view of the high sensitivity exhibited by the magneto-DNA nanoparticle system (down to single bacteria), it could potentially be used for the early diagnosis or detection of rare pathogens in dilute samples. In addition, the method has proven to be rapid and robust, with high specificity and low background. Moreover, the magneto-DNA approach has considerable advantages over standard culture and qPCR systems, particularly in terms of assay time and cost, and it can also be easily applied to point-of-care scenarios. Going forward, we envision that this generic approach could have far-reaching applications, possibly in conjunction with newly emerging single-cell magnetic detection methods[30].

Methods

Primer and probe design. To select a target region for amplification and hybridization, we used the 16S rRNA gene sequences of 30 different bacterial genera (from the NCBI nucleotide database). All sequences were aligned using MegAlign software (DNASTAR). A high consensus region was selected for the universal target, and a low consensus (variable) region for the differential target. To detect antibiotic resistance, specificity regions within the mRNA sequences of *mecA* and Panton–Valentine leukocidin (obtained from the NCBI database) were selected as target sequences. To target the 16S rRNA of 13 different genus types (*Staphylococcus*, *Streptococcus*, *Enterococcus*, *Escherichia*, *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Acinetobacter*, *Proteus*, *Haemophilus*, *Stenotrophomonas* and *Lactobacillus*) as well as the mRNAs of the two drug resistance-related genes for MRSA, pairs of specific oligonucleotide probes (length, 18–22 nucleotides) were designed to be complementary to sequences within the target regions, with one hybridizing to a 5′ end of the target and the other to the 3′ end (Supplementary Table S1). Multiple adenine residues (AAAAA or AAAAAA, oligoA) were then added to the external end of the probes, with a thiol group at the terminal end of the oligoA linker. Primers were designed so that all amplicons (~50–70 nucleotides in length) would include the target regions. All oligonucleotides used for the primers and probes were custom-synthesized and provided by Integrated DNA Technologies. Probe specificity was tested by polyacrylamide gel electrophoresis (see Supplementary Methods).

Probe conjugations. For bead-capture probe conjugation, 3 mg of amine-functionalized polystyrene beads (diameter, 3 μm; Polysciences) were first reacted with 292 μg of sulphosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (sulpho-SMCC, Thermo Scientific) in PBS with 10 mM sodium bicarbonate (4 h, room temperature). The activated beads were then washed extensively with PBS. Thiol-modified oligonucleotide probes (capture probes, 50 nmol) were treated with 125 mM dithiothreitol in PBS with 10 mN sodium hydroxide for 2 h, and the mixture was purified by illustra Microspin G-25 columns (GE Healthcare). Sulpho-SMCC-activated beads and the deprotected capture probes were then mixed and reacted for 12 h at 4 °C. Unreacted probes were removed by extensive washing with PBS. To prepare MNP-detection probe conjugates, we used dextran-coated iron oxide nanoparticles with a diameter of 21 nm ($r_2 = 51$ s^{-1} mM^{-1} Fe)[31]. MNPs (0.5 mg), which had 22 free amine groups per particle, were activated with 472 μg of sulpho-SMCC for 4 h, and extensively washed using Amicon Ultra centrifugal filters (Millipore). Thiol-modified detection probes were pretreated for deprotection as described above. The sulpho-SMCC-activated MNPs and detection probes were mixed and reacted for 12 h at 4 °C. The mixture (MNP-detection probe) was purified using Sephadex G-100 columns (Millipore). The amount of oligonucleotides conjugated onto the beads and MNPs was quantified using the Qubit DNA quantification kit (see Supplementary Methods).

Bacteria culture and RNA extraction. All bacteria were purchased from the American Type Culture Collection (ATCC). Bacteria were seeded and cultured in suspension using the following media: *S. aureus* (#25923) in Staphylococcus broth (BD Biosciences); *Streptococcus pneumoniae* (#6318) and *Enterococcus faecalis* (#29212) in trypticase soy broth containing 5% defibrinated sheep blood (Hemostat Laboratories); *E. coli* (#25922) in Luria-Bertani (LB) media (BD Biosciences); *Pseudomonas aeruginosa* (#142), *Klebsiella pneumoniae* (#43816) and methicillin-resistant *S. aureus* (MRSA-mecA+; #BAA-1720 and MRSA-VPL+; #BAA-1707) in trypticase soy broth; *Enterobacter aerogenes* (#13048), *Citrobacter freundii* (#6879), *Acinetobacter baumannii* (#15149) and *Proteus mirabilis* (#7002) in nutrient broth (BD Biosciences); *Haemophilus influenzae* (#49247) in trypticase soy broth containing 5% lysed blood; *Stenotrophomonas maltophilia* (#17671) in trypticase soy broth; and *Lactobacillus gasseri* (#4963) in Lactobacilli MRS broth (BD Biosciences). For RNA extraction, bacteria were first centrifuged (8,000 r.p.m., 10 min), and pellets were treated with Max Bacterial Enhancement Reagent (Life Technologies), then lysed using TRIzol (Life Technologies). After solvent extraction of the RNA followed by precipitation and washing, the final RNA yield was measured using Nanodrop 1000 (Thermo Scientific). To determine detection sensitivity in blood, human blood specimens were collected from healthy donors, and serial dilutions of cultured *E. coli* were added. For RNA extraction, red blood cells were first removed using the ACK (ammonium-chloride-potassium) lysing buffer (MPBio) and then treated with the Max Enhancement reagent. The procedure for RNA extraction then continued as for pure bacterial cultures.

Clinical samples. This proof-of-principle study was approved by the Partners Institutional Review Board. Excess and discarded samples were collected from nine subjects with clinical suspicion for infected bodily fluid or abscess and referred for drainage of such specimens. Specimens were collected using routine image guided approaches by MGH Interventional Radiology physicians and analysed blindly with the magneto-DNA assay, before being compared to conventional culture results. For RNA extraction, 300 μl volumes of specimens were centrifuged to form pellets, and treated as described above. In the case of specimens containing a high content of blood, samples were repeatedly centrifuged and pellets were treated with the ACK lysing buffer to remove the red blood cells, before treating with the Max Enhancement reagent.

RT-PCR. The cDNA from each bacterial RNA or RNA from the clinical samples were synthesized using the reverse transcription system (Promega) with thermal

cycling conditions of 42 °C for 60 min, followed by 70 °C for 5 min (MasterCycler). For asymmetric PCR, cDNAs were amplified using Taq DNA polymerase (Qiagen) and specific primers for each bacteria type (Supplementary Table S1). Either the forward or reverse primers were added in excess (relative to the other primer). The following thermal cycling conditions were used: initiation (94 °C, 5 min); 30–40 cycles of denaturation (94 °C, 30 s), annealing (55 °C, 30 s), extension (94 °C, 30 s); and termination (72 °C, 7 min). The final PCR products were validated by polyacrylamide gel electrophoresis.

Magnetic sandwich assay. For sandwich hybridization, 1–3 μl of the PCR reaction mixture solution was first mixed with the bead-capture probe conjugates and incubated in hybridization buffer (DIG Hyb, Roche Diagnostics) at 40 °C for 15 min. Unbound nucleic acids were removed by washing the beads with hybridization buffer. MNP-detection probe conjugates were then added (50 μg ml^{-1}) and the mixture was incubated in the same hybridization buffer at 40 °C for 15 min. The bead–MNP complexes were washed again with hybridization buffer and finally with PBS. The μNMR measurements were performed using a previously reported miniaturized μNMR device[32]. Transverse relaxation times were measured using Carr–Purcell–Meiboom–Gill pulse sequences with the following parameters: echo time, 3 ms; repetition time, 4 s; number of 180° pulses per scan, 900; number of scans, 7. All measurements were performed in triplicate, and data are expressed as mean ± standard deviation (s.d.). LOD values were determined by calculating 3 × (s.d. of background signal), and limit of quantification (LOQ) values were determined by calculating 10 × (s.d. of background signal)[33].

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Author contributions

H.J.C. designed and performed the research, and co-wrote the manuscript. R.W. and H.L. designed the research and wrote the manuscript. R.W. provided overall guidance. H.L. reviewed the magnetic resonance measurement data. C.M.C. provided guidance and assistance regarding the clinical studies. H.I. performed scanning electron microscopy and atomic force microscopy. All authors discussed the results and commented on the manuscript.

Additional information

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Competing financial interests

Ralph Weissleder is a founding member and consultant to T2 Biosystems.