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Review Article

Microfluidics: innovative approaches for rapid diagnosis of antibiotic-resistant bacteria

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The emergence of antibiotic-resistant bacteria has become a major global health concern. Rapid and accurate diagnostic strategies to determine the antibiotic susceptibility profile prior to antibiotic prescription and treatment are critical to control drug resistance. The standard diagnostic procedures for the detection of antibiotic-resistant bacteria, which rely mostly on phenotypic characterization, are time consuming, insensitive and often require skilled personnel, making them unsuitable for point-of-care (POC) diagnosis. Various molecular techniques have therefore been implemented to help speed up the process and increase sensitivity. Over the past decade, microfluidic technology has gained great momentum in medical diagnosis as a series of fluid handling steps in a laboratory can be simplified and miniaturized on to a small platform, allowing marked reduction of sample amount, high portability and tremendous possibility for integration with other detection technologies. These advantages render the microfluidic system a great candidate to be developed into an easy-to-use sample-to-answer POC diagnosis suitable for application in remote clinical settings. This review provides an overview of the current development of microfluidic technologies for the nucleic acid based and phenotypic-based detections of antibiotic resistance.

Introduction

The rapid emergence of antibiotic-resistant bacteria has become an increasing threat worldwide, limiting treatment options, causing disease complication, prolonging hospitalization duration and dramatically increasing healthcare expenditure. In the United States, over two million people have suffered from antibiotic-resistant bacteria with ∼23000 deaths every year [1]. While in the EU, ∼25000 multidrug-resistant bacteria-related fatalities were reported [2]. It is predicted that the number of deaths due to antibiotic-resistant bacterial infections will reach ten million by 2050 [3]. There is a clear increase in the use of antibiotics, not only for treating infections, but also for sanitizing surfaces or hands as a preventive measure [4]. In any case, extensive use of antimicrobial agents without proper regulation and/or misdiagnosis have selectively enhanced resistant bacterial populations against various drugs. The evolutionary adaptation against antimicrobial agents can be traced to mutations or the horizontal acquisition of antibiotic resistance genes [5].

Resistant strains can emerge from single point mutations in genes responsible for drug action. For example, single point mutations in the DNA gyrase gene can cause *Helicobacter pylori* to develop quinolone resistance [6]. Previous reports indicated the emergence of multidrug-resistant bacterial strains that are resilient to all antibiotics available on the market [1,7–9]. Unfortunately, the pace of novel antibiotic discovery, even with the exploitation of high-throughput screening technology, has been very slow. To manage infections and minimize the development of

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drug-resistant strains of pathogens, rapid point-of-care (POC) diagnosis and antibiotic susceptibility profiling of pathogens are crucial to ensure an effective outcome. Nonetheless, the conventional methods of bacterial diagnosis and detection of antibiotic resistance are time consuming. Molecular approaches have been exploited as alternatives to speed up the process, however, the operational procedures are often complicated, requiring sophisticated infrastructures, expensive reagents, specialized equipment and highly trained personnel, thus narrowing the implementation in limited resource settings, particularly in remote areas.

Microfluidic systems, integrating several molecular processes into a single lab-on-a-chip platform, have become an attractive tool in the field of diagnosis and have been established for various applications including biomarker detection [10]. The advantages of the technique lie in the potential for technological integration. Different functional modules including mechanical, optical, fluidal and electrical can be compatibly combined and tailored for the necessary applications. The microfluidic device can be made of various materials including silicon [11], glass [12], quartz, polymers such as (poly(dimethylsiloxane) or PDMS and poly(methyl methacrylate) or PMMA [13-15] and paper [16]. The choice of materials often depends on the targeted application, the integrated detection system, the cost, fabrication facility and solute compatibility.

Although most microfluidic devices are constructed using microfabrication techniques, various specific fabrication techniques such as thin-film deposition [17], etching [18], bonding [17,19], injection moulding [20], embossing [21] and soft lithography [15] have been very helpful in expanding the possibility of including other materials in the system. Interestingly, at present, 3D printing technology has started to gain popularity and is believed to soon revolutionize the new generation of microfluidic devices, which could be smaller, more complex, easier to control and navigate and more compatible with other technologies [22–25].

The miniaturization of complex fluid handling steps has many advantages that render this process suitable for POC applications, in particular, reducing the amount of sample used, high portability and the ability to integrate complex processes into an easy-to-use system [26-29]. To date, various microfluidic systems have been designed and developed particularly for the detection of bacterial antibiotic resistance. There are two major groups of microfluidic devices, particularly developed for the detection of antimicrobial resistance, miniaturized nucleic acid-based microfluidic detection and phenotypic-based antibiotic susceptibility testing (AST).

Nucleic acid-based microfluidic system for identification of antibiotic resistance

Antibiotic-resistant phenotypes in bacteria occur through mutations or horizontal acquisition of the genes involved in the function or metabolism of antibiotics. For example, the horizontal acquisition of the *mecA* gene, encoding a penicillin-binding protein 2a (PBP2a), can confer methicillin resistance in methicillin-resistant *Staphylococcus aureus* (MRSA) [30,31]. The acquisition of antibiotic resistance-conferring genes such as the $bla_{CTX\text{-}M}$ gene, encoding cefotaximases (CTX-M) type extended spectrum β-lactamases (ESBLs), is the most common cause of β-lactam antibiotic resistance among Enterobacteriaceae [32]. In *Mycobacterium tuberculosis* (TB), single nucleotide mutations in various genes including *katG*, *inhA*, *rpoB*, *embB*, *rrs* and *gyr* have been shown to induce drug resistance [33]. Thus, the direct detection of mutations or genes that confer antibiotic-resistant phenotypes is a common approach for diagnosis. However, such analysis can be quite complex, requiring multiple steps including cell lysis, isolation of genetic material from the cell, amplification of the target gene regions and the detection of the amplified products. Combining nucleic acid detection with microfluidic technology would be highly advantageous as all the complex protocols can be miniaturized on to a single 'lab-on-a-chip' platform, as presented in Figure 1. The microfluidic system significantly reduces not only the amount of samples and reagents required for the analysis, but also the reaction time. Examples of integrated modules in the device for the detection of antibiotic resistance genes are summarized in Table 1.

Sample preparation/nucleic acid isolation

For on-site analysis, sample pre-processing is often required in order to obtain nucleic acids that are suitable for microfluidic analysis [34–41]. Various methods such as exposure to heat, lytic enzymes and different kinds of chemicals have been utilized to facilitate cell disruption in order to extract the DNA from the specimen [38,40,42,43]. Although, in some cases, the lysates can be directly subjected to PCR, loop isothermal amplification (LAMP), recombinase polymerase amplification (RPA) analyses [40,43], additional steps to increase the purity and concentration of the template nucleic acids are often required to improve the accuracy and sensitivity of the test. After cell lysis, DNA can be captured through the adsorption of nucleic acids on certain materials, such as silicon-coated beads and matrices, silicon-based microstructures, super paramagnetic particles (PMPs) and cationic polymers, such as chitosan and

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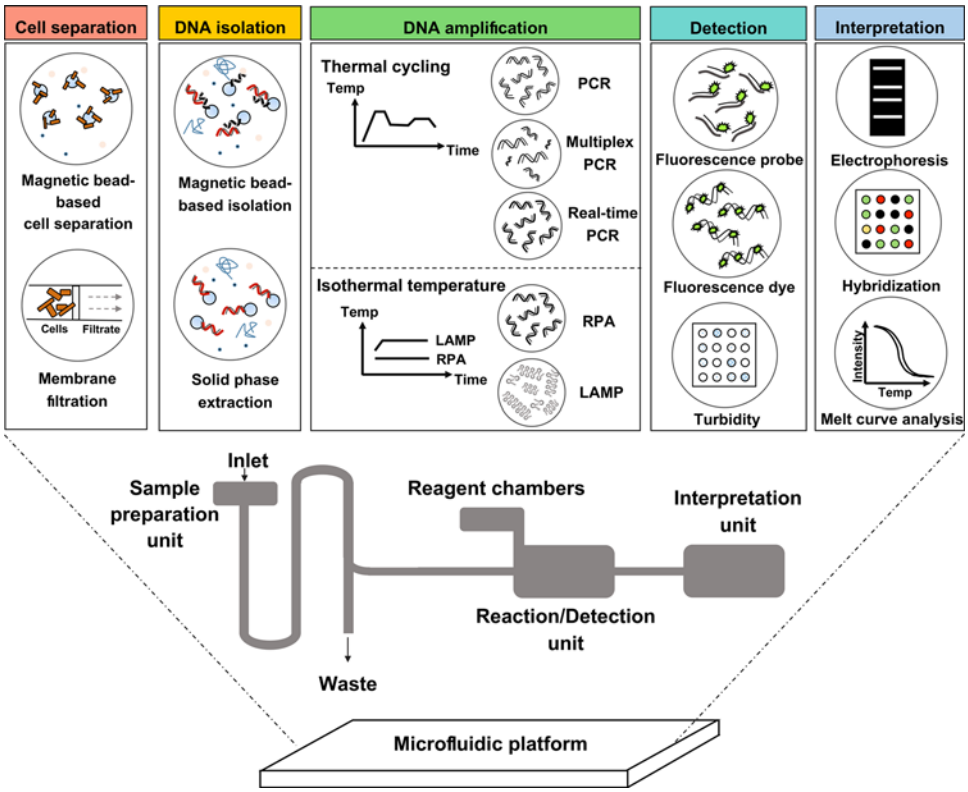

Figure 1. Schematic diagram depicting the workflow of the nucleic acid detection on microfluidic platforms

cellulose [34,35,38,39,44,45]. In addition, to further purify and concentrate the isolated DNA from the specimen, a variety of separation technologies have been implemented in microfluidic applications, including bead capture [43], magnetic capture [43,46–48], isotachophoresis [49–52] and filtration through an immiscible interface [36,37,53,54]. Recently, several platforms have successfully performed single-step nucleic acid purification and concentration, for example, a chitosan-based in-membrane DNA purification system that not only allows the specimen to be substantially concentrated but also stored in the membrane for further use [43]. Immiscible filtration assisted by surface tension (IFAST) allows the DNA of *H. pylori* from stool samples to be isolated and concentrated by 40-fold within 7 min [53]. The recently developed low-cost, stand-alone, integrated microfluidic system, called nucleic acid isotachophoresis LAMP (NAIL), combines isotachophoresis with LAMP detection. Such a system allows rapid detection of *E. coli* O157:H7 from a milk specimen with a sensitivity and specificity comparable to lab-based methods (10^3 cfu/ml) [49].

Amplification and detection methods

Although considerable effort has been focused on developing an effective and rapid method for the extraction and purification of nucleic acids from various kinds of specimens, the amount of genetic material present in samples is under the detection limit of almost all nucleic acid-based detection techniques. The amplification process is often needed to increase the copies of the target nucleic acids to the point where reliable results can be obtained. Two major amplification technologies have been implemented in microfluidic devices i.e. thermal cycling nucleic acid amplification and isothermal amplification processes. For thermal cycling, PCR and related technologies, including multiplex and quantitative real-time PCR, have been widely integrated with microfluidic devices. Although many microfluidic PCR devices have been established [55,56], very few devices have been exploited for the diagnosis of antibiotic-resistant bacteria. In 2013, Chen et al. [57] developed a disposable, integrated, modular-based microfluidic system that can be used to differentiate MRSA from methicillin-sensitive *S. aureus* (MSSA), based on the existence of the *mecA* gene, through fluorescent detection. The detection of single nucleotide mutations in various genes of TB have also been evaluated by an integrated microfluidic TaqMan array card (TAC) with high resolution melt (HRM) analysis [58]. Using this device, PCR reactions can be carried out simultaneously with high confidence. However, the TAC system

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Table 1 Summary of some microfluidic platforms of nucleic acid detection for antibiotic-resistant bacteria

Types of specimen	Pathogenic bacteria/DNA sources	Biomarkers	Microfluidic device substrates	Sample preparation/DNA isolation on device	Amplification methods on the device	Detection methods	LOD	Time of measurement	Reference
Bacterial culture	*H. pylori*	Mutation of DNA gyrase	PDMS	Magnetic beads coated with 16S rRNA probe	Single nucleotide polymorphism polymerase chain reaction (SNP-PCR)	Fluorescence	10^2 cfu/ml	~60 min	[46]
Spiked urine	*K. pneumoniae*	$bla_{CTX\text{-}M\text{-}15}$	PMMA	Anionic exchange DEAE-coated magnetic beads	RPA (off-chip)	Fluorescence (off-chip)	10^3 cfu/ml	45 min	[43]
Genomic DNA	*S. aureus* (MRSA, MSSA)	SG16S, *spa*, *femA*, PVL and *mecA*	PC/PMMA	Thermal and chemical process (off-chip)	multiplex PCR/ligase detection reaction (LDR)	Hybridization/fluorescence	10^2 cfu/ml	40 min	[57]
Bacterial culture	*S. aureus* (MRSA, MSSA)	*mecA*	Double-layered chip fabricated with PDMS	Solid phase extraction (SPE) using silica bead for DNA extraction	LAMP	Calcein dye and observed under UV light	10^3 cfu/ml	120 min	[39]
Genomic DNA	TB	*inhA*, *katG*, *rpoB*, *embB*, *rpsL*, *rrs*, *eis*, *gyrA*, *gyrB* and *pncA*	Not specified	DNA extraction (off-chip)	Real-time PCR (48 different PCR simultaneously)	HRM/TagMan probe with fluorescence	Not specified	90 min	[58]
Genomic DNA	*E. coli*	$bla_{CT\text{-}M\text{-}15}$	AM-EWOD	DNA extraction kit (off-chip)	RPA	Fluorescence	20 pg/μl	10–15 min	[59]
DNA fragment of *mecA* gene (420 bp)	*S. aureus*	*mecA*	Foil substrate casted with PDMS	PCR product of *mecA* gene (off-chip)	RPA	Fluorescence	$<5 \times 10^{-6}$ pg/μl (<10 copies)	20 min	[60]
Genomic DNA	Mixed bacterial genomic DNA	*mecA*, NDM, VIM, IMP, KPC, DHA, OXA23, OXA24, OXA58, CTX-M1, CTX-M2, VanA, VanB	PMMA with parallel of connected reaction sections	DNA extraction kit (off-chip)	PCR on flat PCR apparatus	Fluorescence	1 pg/μl	90 min	[61]
Spiked clinical samples	*S. aureus* (MRSA)	*mecA*	PDMS-based microfluidic system	Specific probe-conjugated magnetic beads targeting DNA of MRSA	LAMP	Optical density of LAMP amplicons using spectrophotometer	0.01 pg/μl	60 min	[48]

requires an expensive real-time PCR platform and HRM analysis software, therefore further development is required for clinical application due to the need for high purity DNA for HRM analysis. Moreover, a multiplex PCR-based microfluidic chip that can be placed in a regular PCR machine has previously been shown to detect up to 13 antibiotic resistance genes from a pool of bacterial DNA [29]. For successful multiplex detection, PCR-based microfluidic devices might not be suitable for field application, especially in remote areas, as the system requires a thermocycler for precise temperature control. Suitable materials and a robust sealing protocol for PCR-based microfluidic fabrication are required as the selected materials have to be resilient to the fluctuations of relatively extreme temperatures experienced during thermal cycling [62].

Isothermal amplification has gained wide interest as an alternative method for integration with the microfluidic platform because expensive instruments, such as a thermocycler or sophisticated protocols are not required, which dramatically reduce the complexity of the systems and device, making them more suitable for POC purposes. Several designs for miniaturized isothermal amplification-based microfluidic systems have been recently reviewed in Troger

et al. [63]. For isothermal nucleic acid detection, the two technologies that are often integrated with the microfluidic system are LAMP and RPA.

LAMP is achieved using *Bst* DNA polymerase, which exhibits strand displacement activity [64]. The high specificity and sensitivity of the LAMP technique typically lie in a specific set of four to six primers that recognize six to eight distinct regions on the target gene. The simplicity of the technique and the isothermal nature of the reaction are highly advantageous as this allows on-site POC application. To date, many kinds of microfluidic LAMP systems have been developed to detect food-borne pathogens [65,66], viruses [67] and multidrug-resistant bacteria [39,48] with high accuracy and high sensitivity. Guo et al. [39] reported the successful amplification of DNA from as low as 1 cfu/μl of bacteria using microfluidic LAMP, enabling rapid detection of the antibiotic-resistant *mecA* gene of MRSA. Notably, an integrated microfluidic LAMP developed by Wang et al. [48] has been able to detect MRSA in clinical samples with extremely high sensitivity. Despite the advantages, the optimal conditions for LAMP require stable operation at a relatively high temperature (60–65°C), which means that an additional thermal module is needed in the system.

RPA is mediated through three core enzymes including recombinase, ssDNA-binding protein (SSB) and strand-displacing DNA polymerase. The orchestrated activities of all these enzymes allow the detection process to be performed not only at a constant temperature of ∼37°C but also with a short reaction time [68]. Moreover, lyophilized RPA reagents can be stored in the cartridge for an extended period of time, which reduces the number of manual handling and tube-based reaction steps. To date, several RPA-based microfluidic devices have been developed for various applications. For example, Lutz et al. [60] proposed a centrifugo-pneumatic-controlled RPA-based microfluidic cartridge that could rapidly detect the *mecA* gene at a high sensitivity with a limit of detection (LOD) of <10 copies [60]. However, detection of the gene from the genomic DNA of *S. aureus* or from the crude DNA of clinical samples has not yet been demonstrated. The recent rapid development of various technologies, including electrowetting and a computerized control system, allows more complex microfluidic designs to be fabricated and a more automated system to be further developed at a rapid pace. Kalsi et al. successfully demonstrated a rapid and sensitive RPA-based quantificative detection of the $bla_{CTX\text{-}M\text{-}15}$ gene using an active matrix electrowetting-on-dielectric (AM-EWOD) digital microfluidic platform [59]. This fully programmable AM-EWOD device allows precise and automated manipulation of reaction droplets, in terms of sizing, positioning and mixing, at the nanolitre scale; therefore, the assay requires a minute amount of samples and reagents. The fluorescent signal monitoring integrated with the system permits a rapid and very sensitive readout with the LOD of a single copy within 10–15 min. Despite fully programmable droplet control using AM-EWOD, it still needs human intervention due to the lack of DNA extraction module incorporated in the system [42]. Moreover, detection of a small volume could cause a marked reduction in sensitivity due to the absorption of nucleic acids on to the surface of the device [60].

Phenotypic-based microfluidic platform for AST

The nucleic acid based approach for the detection of antibiotic resistance demonstrates the ability to detect resistance, however, it does not provide relevant information on susceptibility. It is important that AST confirms susceptibility to antimicrobial agents in individual bacterial isolates for efficient infection treatment. Conventionally, standard AST is determined either by broth or disc diffusion methods. The broth dilution method enables determination of the minimal inhibitory concentration (MIC) of the antibiotic that prevents bacterial growth whereas the disc diffusion method commonly provides qualitative information on whether the bacterial strains are susceptible or resistant to antibiotics [69]. These culture-based assays usually take ∼3–7 days to give complete results [70]. A few days are needed to culture bacteria to the proper density, prior to incubation with antibiotics, in order to reach the limited sensitivity of detection at $\sim 10^7$ cfu/ml [71]. The current AST protocol has several limitations: (i) the growth of bacteria is often measured based on culture turbidity, so false positives can be found in the case of filamentous bacteria, (ii) the test requires large quantities of clinical samples (∼10–30 ml), such as blood or urine [72], (iii) a large amount of antibiotics is required, (iv) interpretation requires complex analytical processes and (v) the techniques only work with culturable bacteria. Due to the aforementioned limitations, numerous microfluidic platforms have been developed for AST. The experimental workflow of microfluidics for AST is depicted in Figure 2. While conventional AST determines the susceptibility of bacteria to the drug based on cell viability and division via the visual inspection of culture turbidity or colony growth, several microfluidic platforms detect biochemical and physiological changes in bacterial metabolic activity [73–75], which improve the sensing capability of the systems. During culture, bacteria utilize glucose and other sugars and produce acid metabolites, causing changes in the pH of the culture medium. Under acidic conditions, a pH-sensitive chitosan hydrogel is swollen, resulting in changes in effective optical thickness (EOT). Integration of this unique pH-sensitive chitosan hydrogel with PDMS microfluidic channels resulted in a microfluidic pH sensor

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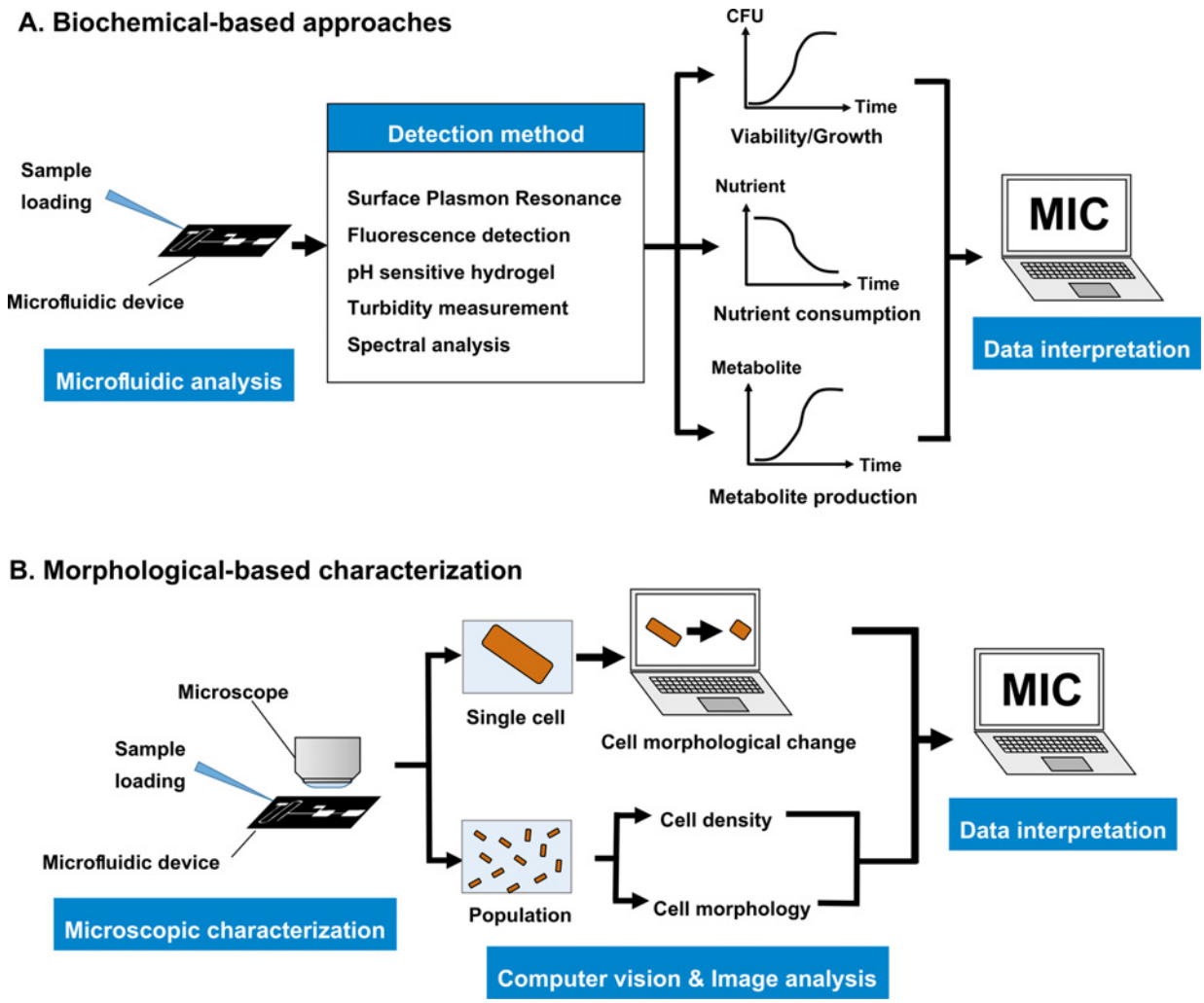

Figure 2. Schematic diagrams depicting the workflow of the phenotypic-based AST microfluidics

Monitoring of bacterial biochemical (**A**) and morphological (**B**) changes in response to antibiotics.

[75]. The chitosan-based pH sensor is very sensitive even to a slight change of pH caused by bacterial growth; thus, antibiotic susceptibility can simply be determined by loading the specimen of bacteria, cultured in a glucose medium spiked with different concentrations of antibiotics, into a microfluidic pH sensor. A change in EOT can be determined by Fourier transform reflectometric interference spectroscopy (FT-RIFS). The MIC values can be derived from the EOT changes. The strategy is particularly interesting as it demonstrates that the confinement of bacterial cells in a high surface-to-volume ratio microchannel allows the rapid accumulation of metabolic products, and therefore, eliminates the need for a long period of pre-incubation and reduces the detection time to <2 h. These techniques are only applicable for a group of bacteria that can produce metabolic acids but can lead to false negative/positive scenarios e.g. a pH change associated with bacteria being killed by antibiotics may arise. Nonetheless, a phenotype microarray (PM) [76] based on this pH sensor with pre-configured antibiotics added into each well will be particularly useful in the clinical setting, as the rapid determination of the MIC of different antibiotics would allow the resistance mechanisms of such pathogens to be readily defined.

Other microfluidic AST strategies exploit the change in bacterial cell phenotypes and properties when exposed to antibiotics. For example, surface-plasmon resonance (SPR) based biosensor platforms have been widely used to distinguish susceptible and resistant strains of bacteria by detecting variations in the refractive index of bacteria when treated with antibiotics in real time [73]. Moreover, a PDMS-based optofluidic device developed by Lu et al. [77], coupled with surface enhanced Raman scattering (SERS) spectroscopy can accurately differentiate MSSA and MRSA

in clinical isolates. The results showed high sensitivity and selectivity compared with that of PCR and multilocus sequence typing (MLST).

Certain microfluidic devices utilize the stress activation of biosynthetic pathways, which are the primary targets of antibiotics [78]. For example, β-lactam antibiotics interfere with the repair of cell wall damage, causing rapid cell death in susceptible strains, but not in resistant strains. Therefore, the design involves a microfluidic system that can cause physical damage to the bacterial cell wall. The device contains channels specifically designed to flow the culture media through the channel surface-immobilized bacteria and monitor the uptake of SYTOX Green dye into the cells. Wild-type bacteria are able to repair the cell wall and will not be eliminated by the flow and hence will not take up the dye. Enzymatic stress with subinhibitory concentrations of lysostaphin, a peptidase enzyme that can be used to damage the cell wall, is used to test the system. Bacterial cell death and rates of killing can be measured in the presence and absence of β-lactam antibiotics. Stress-activated microfluidic platforms have been used to investigate the antibiotic susceptibility of 16 clinically relevant *S. aureus* strains in a blinded study. Full diagnostic results could be obtained within 1 h after introduction of the antibiotic with correct designation of the phenotypes of the tested strains. The results demonstrate the great potential of the stress-based approach microfluidic system for phenotypic AST and for the evaluation of the effects of various stresses on bacteria and their antibiotic susceptibility.

Furthermore, determining single cell growth can also be used to evaluate the antibiotic susceptibility of pathogens [79]. A single cell growth tracking microfluidic device has been designed to immobilize bacteria (either immobilized on to the surface or trapped in agarose medium) to enable visual recording of single cell growth and division under a bright-field microscope in the presence or absence of antimicrobial drugs. Using the image processing algorithms, time-lapse images of a single bacterium in different antibiotic concentrations can be analysed in terms of division, filamentary formation and the swelling of treated cells, etc., which can directly reflect antibiotic susceptibility. It should be noted that the responses of bacteria to antibiotic treatment can be very diverse and specific to the different antibiotic conditions. A more accurate characterization of the responses, based on cellular morphology and division, has been developed. The validity of the results obtained from the single-cell morphological analysis on the microfluidic platform has been verified using four different standard bacteria from the Clinical Laboratory Standard Institute (CLSI) [80]. The results obtained showed excellent agreement with those of the CLSI assay, suggesting that such an approach could be a promising tool for the rapid quantificative diagnosis of antibiotic susceptibility.

Multidrug-resistant *Pseudomonas aeruginosa* (MDRP) has recently become a global concern [81–83]. In 2016, Matsumoto et al. [84], presented a ready-to-use multichannel microfluidic device, preloaded with dried antimicrobials, allowing the simultaneous microscopic observation of the growth of antimicrobial-treated and -untreated *P. aeruginosa* cells [84]. The system only requires 3 h of incubation to evaluate antibiotic susceptibility. The software accompanying the system is almost automated and provides various quantificative results that enable careful interpretation based on cell numbers and the shapes and lengths of treated and control cells. The accuracy of the device has been confirmed with 101 clinical isolates of *P. aeruginosa* and is claimed to be strongly similar to the standard microbroth dilution method [84]. The system nonetheless requires a well-equipped microscope for monitoring bacterial phenotypic changes and a decent computer for image processing, which might preclude the application in a resource-limited setting. The rapid development of an advanced mobile device and a high-resolution camera provides great complementary technology that could aid not only the imaging but also processing of the data. Currently, there is no microscopic attachment or advanced mobile camera that can provide sufficient resolution to unambiguously distinguish changes in bacterial cell morphology. It is clear that significant improvement of this technology is still required before it can be useful for application in resource-limited areas. Moreover, result interpretation mostly relies on image analysis, and the reliability of the algorithm used to classify bacteria of different types into different populations could be a major challenge, especially for a mixed population of bacteria as well as bacteria such as cocci or coccobacilli, whose drug-induced morphological changes might not be easily distinguishable.

The phenotypic-based microfluidic system exhibits a major advantage as some antibiotic-induced physiological changes occur long before growth inhibition can be observed. Standard methods as well as automated instrument methods for AST are mostly based on measuring bacterial growth inhibition, which requires 18–24 h to obtain the results. However, one major challenge for phenotypic-based AST is the requirement of algorithms to translate the obtained results into precise MIC values.

Conclusions

The number of reliable and accurate diagnostic assays for antibiotic-resistant bacteria is increasing, with a view to mitigate antibiotic resistance and provide correct and precise clinical care. The integration and miniaturization of complex biochemical and molecular protocols into a single lab-on-a-chip platform provide tremendous advantages,

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not only in improving the detection limit and reducing the sample amount and consumables, but also in simplifying the complex protocols for various types of medical diagnosis including the rapid detection of multidrug-resistant pathogens. Nucleic acid-based microfluidic platforms require several steps for nucleic acid isolation, amplification and detection, while the phenotypic-based microfluidic platforms require methods and devices to detect changes in the metabolites and physiology of antibiotic-resistant bacteria. Microfluidic platforms have several advantages over the current standard laboratory practice which include: enabling the extensive use of complex multiplexing approaches and formats, the capacity to include sequential sample preconditioning steps of different types, reagent storage, the use of multiple steps involving reagent addition, mixing and washing, the potential for the incorporation of centrifugal steps and the application of a range of detection strategies leading to greater sensitivity and clarity of results. The integration of the microfluidic system with advanced fabrication technologies could soon be revolutionizing the future of diagnosis, especially by moving towards the one-step sample-to-answer POC detection of antibiotic resistance.

Summary

- The field of microfluidics has been exploited for a wide range of applications in the detection of genes or mutations conferring antibiotic resistance as well as physiological or metabolic changes involved in antibiotic-resistant traits.
- Thermocycling and isothermal amplification could be integrated into microfluidic platforms for nucleic acid detection.
- To enable the rapid profiling of antimicrobial susceptibility to antimicrobial agents, various types of phenotypic-based microfluidic AST have been successfully developed.
- The advantages of microfluidic systems for the detection of antibiotic resistance compared with conventional techniques include smaller reagent volumes (1–10 μl), enhanced detection sensitivity (single cell), more rapid (1–4 h) and improved portability. However, the portability aspect needs to be further adjusted due to the requirement of syringe pumps, pneumatic actuators and other ancillary equipment such as a microscope and optical sensing for POC applications.

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Competing interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

AM-EWOD, active matrix electrowetting-on-dielectric; AST, antibiotic susceptibility testing; *bla*, β-lactamase gene; *Bst*, *Bacillus stearothermophilus*; CLSI, Clinical Laboratory Standard Institute; CTX-M, cefotaximases; DHA, Dhahran Hospital; *E. coli* O157:H7, *Escherichia coli* 0157:H7; *eis*, enhanced intracellular survival gene; *emb*B, ethambutol resistant gene; EOT, effective optical thickness; EU, European Union; *fem*A, gene encodes a factor essential for methicillin resistant; *gyr*, DNA gyrase gene; HRM, high resolution melt; IMP, imipenemase gene; *inh*A, isonicotinic acid hydrazide resistant gene; *kat*G, mycobacterial catalase-peroxidase gene; KPC, *Klebsiella pneumoniae* carbapenemase gene; LAMP, loop isothermal amplification; LOD, limit of detection; *mec*A, methicillin resistant gene; MIC, minimal inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; NDM, New Delhi metallo-β-lactamase; OXA, oxacillinase gene; PC, polycarbonate; PDMS, poly(dimethylsiloxane); PMMA, poly(methyl methacrylate); *pnc*A, pyrazinamidase/nicotinamidase gene; POC, point-of-care; PVL, Panton-Valentine leukocidin; RPA, recombinase polymerase amplification; *rpo*B, β-subunit of bacterial RNA polymerase gene; *rps*L, ribosomal S12 protein gene; *rrs*, 16s rRNA gene; SG16S, 16s rRNA gene specific for the genus *Staphyloccus*; *spa*, staphylococcal protein A gene; TAC, TaqMan array card; TB, *Mycobacterium tuberculosis*; *van*, vancomycin resistant gene; VIM, Verona imipenemase metallo-β-lactamase gene.

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