Microfluidics as an Emerging Platform for Tackling Antimicrobial Resistance (AMR): A Review

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Abstract:
Antimicrobial resistant bacteria have been recognised as a global threat and require a robust and collective response from every stakeholder of the society and public health institutions. Currently available technologies to tackle antimicrobial resistance (AMR) have been found to be time-consuming, expensive, labour intensive and central lab-based which poses increasing challenges, especially in remote areas where resources are limited. In contrast to most of the conventional technologies, microfluidics has become an enabling platform for AMR testing, allowing simple, robust, cost-effective and portable diagnostics. Various microfluidics techniques have been developed and tested for bacterial identification and determination of minimum inhibitory concentration (MIC), demonstrating a broader understanding of the behaviour of antimicrobial resistant strains, and development of antimicrobial susceptibility testing (AST) tools. Miniaturised microfluidic systems have led to the integration of various tools on a single platform such as sample handling, controlled reactions and on-chip detections towards portable, simple, easy to use and POC diagnostics in clinical environments. However, the benefits of microfluidics technology have not been fully exploited in tackling AMR because current microfluidic-based studies provide valuable information about the antibiotic resistance but are far from deployment in clinical settings. Hence, there is a lack of reliable and accurate systems in the market. AMR is an ever-growing threat and microfluidics must act faster to provide commercially viable techniques to combat resistant bacteria and guide clinical decisions. This article reviews the current microfluidic-based technologies for rapid AST testing and future prospects of the microfluidics technology for tackling AMR in clinical settings.

Keywords: Microfluidics, Point-of-care, Antimicrobial resistance (AMR), Lab-on-a-chip, Antibiotic, Capillary flow, Colorimetry, antimicrobial susceptibility testing (AST), minimum inhibitory concentration (MIC)

1. INTRODUCTION
Antimicrobial resistance (AMR) occurs when microbes become resistant to antibiotics causing complications and limited treatment options. AMR is more significant where antibiotics use is excessive or abusive, and the strains of bacteria grow resistant to antibiotic treatments. This is associated with hundreds of thousands of deaths every year (700,000 annually) while more and more bacteria are becoming resistant every day which could even lead regular injuries, infections or surgeries to death [1]. Global healthcare largely relies on the performance of antibiotics to tackle diseases and perform operations including cancer and organ transplantation. Therefore, failure of antibiotics would lead to less effective therapies and more fatalities [2].

Excessive and unnecessary use of antibiotics is regarded as the most significant cause of bacterial resistance as broad-spectrum drugs are administered for common infections. Another practice is that the drugs are not monitored at hospitals and given to patients for home use which is unavoidable because of the excessive costs associated with patient monitoring at hospitals. Additionally, an alarming situation is that in developing countries antibiotics can be purchased over the counter without prescriptions and used for common infections or illnesses that affect body’s immunity against bacterial infections. Among the most quantitatively used antibiotics sectors is the use of drugs in animal farms to promote growth weight and prevention of infections and diseases. This subsequently results in the spread of AMR as bacteria raised in farms end up in the human body and the environment [3]. The supply and demand issue of new antibiotics has also led to an increase in fatalities due to the microbes becoming resistant to the current drugs [1]. Therefore, a social and public awareness and toughened regulations to combat AMR must be increased globally.

Current technologies for bacteria and its resistant strains identification and antimicrobial susceptibility testing (AST) are mostly central-lab based in hospitals, which generally take days to weeks to get results. These tools and procedures are expensive, laborious and skills based. AST provides essential information about susceptibility of the antibiotics for guiding the infection treatments. The standard AST methods commonly used are disc diffusion for detection of bacterial
strains resistedivity towards antibiotics or broth dilution for
determination of minimum inhibitory concentrations (MIC).
These methods usually take 3-7 days for completion of results
and include longer culture and incubation times, large
amounts of samples and antibiotics, the possibility of false
positives and sophisticated arrangements to interpret results
[4, 5]. Several other techniques have been developed to
address these shortcomings such as PCR based resistant gene
detection, mass spectrometry, genome sequencing and
microbeads technology [6-10]. However, these upgrades are
not sufficient to provide the full benefits required for rapid,
cost-effective and reliable ASTs. Henceforth, a collective
global and befitting response is necessary to tackle the ever-
increasing AMR effect on healthcare systems and societies.
This goal can be achieved by limiting prescription of
antibiotics until necessary, developing new antibiotics for
specific diseases at speed much higher than current supply [1],
and developing inexpensive, robust and near-patient portable
systems for faster identification of microbes, AST testing and
MIC determinations.

There is an ever-increasing demand for developing point-of-
care (POC) diagnostics tools for rapid and near patient AMR
testing. Microfluidics, an essential and fundamental technique
to develop POC devices, is referred to as a flow of minute
amount of liquids in microchannels (micrometre dimensions).
Fundamental to the development of POC diagnostics has been
the use of microfluidics, which offers small sample volume
consumption and the combination of multiple sample-
processing steps into a single device. Microfluidics has been
a critical technology for over two decades to study and
manipulate fluids in microstructures. It has the potential to
provide smart microdevices which can change how the
modern biology, chemical synthesis, and point-of-care
diagnostics are performed. Microfluidics offers many
advantages including minute quantities of samples and
reagents, compact ability, low cost, rapid, high resolution and
sensitive analyses [11]. However, microfluidic-based
technologies have not been applied in clinical settings despite
decades of advancements in the microfluidics technology.
These devices provide valuable information about the
bacteria, its resistance and treatment with antibiotics while
tackling AMR.

Nevertheless, these devices are not widely accepted in the
clinical environment and lack backing from key opinion
leaders (KOL) in the field of clinical diagnostics. The chief
reasons for lacking clinical acceptability include fabrication
and operational complexity, the requirement of resources and
sophisticated equipment such as pumps, optics and statistical
analysis, running costs and portability. Ideally, the technology
to be clinically accepted and deployed for POC diagnostics,
especially for AMR testing, must have the following abilities
in a single, standalone and portable device. It must be able to
detect bacteria, perform AST and determine MIC within few
hours, on-site and near the patient. It must be accurate, reliable
and reproducible, simple to fabricate, assemble and operate,
and cheaper to buy with low maintenance costs as compared
to current technologies. It must be scalable and commercially
available in the market, and more importantly, the results must
be able to guide treatments almost in real-time and save lives.
This review mainly focuses on the current developments of
microfluidic-based AMR testing devices that have the potential to
direct the technology towards the benefit of human health in clinical settings.

2. MICROFLUIDIC BASED TECHNOLOGIES FOR ANTIMICROBIOTIC RESISTANCE

Due to the limitations of conventional AMR techniques
discussed above, microfluidic-based platforms have been
developed to enhance understandings of bacterial resistance,
provide smart AST and MIC testing tools, and development of
the new drugs. This review aims to summarise the recent
developments in microfluidic-based AST and MIC testing
tools in different formats of microfluidics technology such as
detection of biochemical and physiological changes in
bacterial culture [12-14]. For example, Tang et al. [14]
developed a microfluidic-based pH sensor device to detect
small changes in pH. These changes were measured by the
effective optical thickness of the pHSensitive hydrogel,
which swells under acidic conditions. As bacteria utilises
glucose during culture that results in the production of acidic
metabolites and hence, changes in pH occur. Some other
techniques involving bacterial detection include but not
limited to surface plasmon resonance-based biosensors by
detecting refractive index changes of bacteria in culture [12],
and optofluidics devices coupled with surface-enhanced
Raman scattering spectroscopy to specify differences in
clinical isolates [15]. Although these types of studies have the
ability to provide sensitive bacterial detections, however,
the requirement of sophisticated instrumentation such as
miniaturised pH measurement and Raman scattering prove
difficult to be integrated into a portable and straightforward
microfluidics device.

Antimicrobial susceptibility testing (AST) is a crucial
technology to attain information regarding bacterial resistivity
towards drugs and, ultimately, controls the spread of AMR.
Several microfluidic-based platforms have proven that AST
can provide faster results while guiding clinical decisions of
antibiotic use and improving patients’ health [16, 17]. For
example, in a microfluidic-based AST and MIC study,
morphological changes of single bacterial cells were tracked.
Moreover, 189 clinical isolates were studied, and MIC was
achieved within 4 hours [18] that was a significant reduction
in operation time. In another study carried out with the same
platform, an AST of Mycobacterium tuberculosis was
achieved in 9 days that is nearly six times reduction in
bacterial growth in conventional technologies [19]. However,
these results while impressive and achieved in a fraction of
time compared with conventional methods but utilised highly
sensitive optical detection systems and complex statistical
calculations. The prospects of these technologies gaining
access to the clinical market depend on the miniaturisation of
ultra-sensitive detections and robust statistical data analysis.
Rapid and multiplex AST systems were also developed to
study bacterial interactions and drugs responses in parallel on
a single device while utilising reduced amounts of reagents
and samples, and performing analysis in shorter times [20-21].
Sun et al. [22-23] developed an agar-based cell-on-hydrogel
platform to study bacterial interactions with different
concentrations of drugs. The authors generated two-
dimensional gradients in a stacking device and obtained AST
results within 2-3 hours. The device in itself was able to
multiplex assays on a single platform and gels can be pre-
coated in the channels that offer the possibility of POC diagnostics in healthcare. Broth-based dilution method is a widely used technique in clinical studies to perform ASTs and hence has a great potential to be integrated into microfluidics systems and provide rapid and sensitive ASTs. Several broth-based microdilution methods have been developed such as a microfluidic broth-based dilution method (Fig. 1a) was developed to perform AST and determine MIC [24]. The

Figure 1: Microfluidic-based devices for rapid AMR detection. a) PDMS/glass microfluidic device consisted of 3 layers namely air and liquid channel layer and glass substrate and eight chambers with built in micro-valves and circular micropumps. The working principle of the device involves opening and closing of the air and vacuum channels and precise control of movement liquid. (Reproduced with permission from [24].) b) A PDMS based device to generate concentration gradients and perform cell culture in eight 30-nanoliter chambers. (Reproduced with permission from [25].) c) Design and photograph of the drug susceptibility microfluidic (DSTM) device for determination of MIC of five drugs, and microscopic image of the growth of *P. aeruginosa* against piperacillin. (Reproduced with permission from [26].) d) Schematic of the bacterial cell tracking of sub-μm motions illustrating working principle steps i.e. sample handling and bacterial cell tethering on the wells on glass slide, measurement of motions under microscope, addition of antibiotic concentrations into the wells, and analysis of change in bacterial cell motion to identify antibiotic dose profile. (Reproduced with permission from [27].) e) Design and operation of the AST device based on cell trapping. Rod-shaped bacterial cells are loaded into the device, and fraction of *E. coli* cell trap was measured against time. A microscopic photograph of call capturing regions illustrates trapping of cells at different locations in the device. (Reproduced with permission from [28].)
authors performed incubation of vancomycin-resistant Enterococcus cells with five different concentrations of vancomycin in the three-layered PDMS-glass device within five minutes and determined MIC in 24 hours. Sample injection, transport, mixing and dispensing were integrated on an automated platform that provided a flexible, reliable and portable system for clinical applications. The schematic of the integrated microfluidic chip and a photograph illustrating the layers, chambers and working principle of the device are shown in Fig.1a. Another AST microfluidics device (Fig. 1b) based on broth dilutions to generate gradient concentrations and perform cell culture in eight 30-nanoliter chambers was developed by Kim et al. [25]. With 20 µL samples of Escherichia Coli (E coli) and, ampicillin or streptomycin were used to determine MIC in three hours. These broth-based studies were able to generate valuable results in shorter times and ideally can proceed for POC diagnostics because of the use of widely accepted cell culture methods. However, these devices require miniaturisation of pumping systems. The currently available pumping modules (the ones used in these studies) cannot perform AMR tests for diagnostics because of the requirement of the expertise in pump handling, and the bulky nature of these systems such as tubing, connections, and manual fluid handlings makes it difficult to deploy for AMR testing. In addition, it increases the costs of disease treatments per patient and hence almost impossible to afford for modern healthcare systems around the world. Therefore, only inexpensive pumping modules and portable, sensitive detections can lead these techniques to the clinical diagnostics for AMR.

Single cell imaging of the bacteria treated with antibiotics can also provide valuable information about the resistivity of the microbes [26-28] such as Yoshimi and co-workers [26] developed drug susceptibility testing microfluidic (DSTM) device to perform AST of Pseudomonas aeruginosa within 3 hours. The device consisted of five sets of microchannels with one inlet and four narrowly fabricated channels for the microscopic view. Bacteria was introduced into the channels precoated with antibiotics and incubated for 3h as shown in Fig. 1c. The system was used to achieve 101 isolated strains of bacteria by evaluating the cell numbers and shapes of bacterial cells in different chambers. Different types of drugs reacted differently with the bacteria such as susceptible cells elongated upon incubation with ciprofloxacin, ceftazidime, meropenem and piperacillin while amikacin affected the cell shape minorly. In another bacterial imaging technique, Syal et al. [27] developed an imaging and tracking technology to identify sub-µm bacterial cell motions (Fig.1d). E coli O157:H7 and uropathogenic E coli (UPEC) were placed on a glass surface, and their sub-µm movements were tracked upon treatment with antibiotics. The device simply worked in four steps: (i) Bacterial cells (UPEC) spiked in urine samples were loaded onto the glass slide with wells; (ii) microscope was used to monitor and record bacterial cell motions; (iii) antibiotics were added to see the effect of drugs on bacterial cell motions; and (iv) different antibiotic concentrations were performed on the device. The experiments revealed that antibiotic concentrations reduced movements (Fig 1d). The platform was able to determine MIC in 2 hours, and the results were compared with conventional AST methods. This type of method can be fast, reliable and inexpensive because of the requirement of simple and inexpensive device fabrication, and device operation without the requirement of sophisticated pumping systems. However, this requires sensitive microscopic imaging and robust analysis of bacterial response is required to track the shape and movements of bacterial cells. Recently, Balteken et al. [28] developed an interesting microfluidics platform for testing urinary tract infection (UTI) within 30 minutes by capturing bacterial cells on a microfluidic chip and monitoring their growth via a microscope (Fig. 1e). The authors were able to detect growth rate changes of UPEC bacterial cells upon treatment with nine antibiotics in minutes. Additionally, 49 isolates were found resistant to ciprofloxacin within 10 minutes. This system has the potential to be used as a point-of-care device for testing urinary tract infection and guiding correct treatments at the clinic only and if miniaturised microscopic detections and automatic statistical analysis can be performed portably.

Another unique capability of microfluidics is to manipulate smaller volumes and compartmentalising biomolecules into nano-litre sized droplets. Droplet-based microfluidics is well known for the reduction or elimination of contamination between different samples, faster mixing in droplets, and controlled and reproducible droplet generation with biochemical reactions inside these tiny confined volumes [29-31]. Bacterial cells can be injected into droplets (either single or multiple cells) which increases the density of cells per droplet volume and hence, promotes the accumulation of released molecules around the cell. This as a result, reduces the incubation time and provides faster detection of bacterial resistance towards drugs. Droplet-based microfluidics has been used to develop robust and reliable AST testing devices as demonstrated by Aveshar et al. [32]. The authors developed a rapid and scalable AST platform to provide urinary tract infection results within one day. A simple device was constructed to combine nano-litre wells equipped with lyophilised antibiotics with resazurin assay while injecting samples with a standard lab-based pipette (Fig 2a). Firstly, lyophilised antibiotics were placed into the restriction channels, and a 10-µl pipette was used to inject bacteria suspension with resazurin and carrier oil. Secondly, the oil plug followed and separated the wells from each other. Finally, a fluorescence signal was monitored in each well. Clinical isolates were directly studied, and the results were compared with standard automated equipment. The authors also demonstrated the ability of the device to multiplex analyses by parallelising nano-litre wells and to equip with lyophilised antibiotics. The device offers promising results and fulfills the needs of simple operating procedures with inexpensive fabrication and multiplexing ability. However, the idea to deposit lyophilised antibiotics in miniaturised wells requires reproducible and reliable deposition in each well which can be difficult and expensive. Furthermore, the use of resazurin requires a fluorescent signal to be detected from these chambers and hence, a miniaturised fluorescence detection system is also required on-board for sensitive detections and analysis.

Droplet-based AMR research studies involve active droplet generation, and encapsulation of bacterial sample and antibiotics into droplet plugs such as Kaushik et al. [33] developed DropFAST, a bacterial growth detector and AST assessor in pico-litre droplets as shown in Fig 2b. Resazurin based fluorescent assay was used to quantify the effect of gentamycin on growing E coli confined in 20 pico-litre droplets. Bacteria and antibiotic plus viability indicator were injected into the microfluidic chip and droplets were
generated. These droplets were incubated in the incubation zone of the microchip and then passed from a narrow 10-µm wide channel for fluorescent detection and analysis. This system allows faster droplet generations, and lower incubation times in droplets, however, this format of the microfluidics technology requires sequential injection of different concentrations of antibiotics and multiplexing it would require multiple pumping lines, increasing the cost of the AMR tests per patient. Moreover, the variability of bacterial encapsulation in multiple droplets also poses a challenge for statistical analysis of final concentrations in the sample. Similarly, in stokes laboratories [34], E. coli bacteria was cultured in microfluidic droplets (Fig. 2c), and continuous monitoring of individual droplet reaction combinations was performed. The authors detected the lysogene broth culture media for bacterial growth continuously while providing results in 1h incubation time. This technology uses droplet on demand technique which generates droplets via a command controlled interface and this is a significant difference between this technology and the DropFAST which uses active droplet generation technique [33]. On the other hand, this study detects the colour change of lysogene broth during incubation while DropFAST detects fluorescence signal. Droplet on demand systems offer more control over the size, speed and flow control of the droplets. Similarly, Churski et al. [35] attempted to automate the MIC determination and E. coli toxicity testing against ampicillin, tetracycline and chloramphenicol via generation of multiple droplets on demand. This system utilised less than 100 µL of sample and reagent volumes and provided user-programmed bacterial and antibiotic concentrations loaded into the droplets. Furthermore, the droplets were stored in a sequence, incubated and injected into a spectrophotometer for cell growth measurements as shown in Fig. 2d. This format of droplet generation provides an ideal system to automate the AMR analysis with miniaturised droplet detections and has the potential to multiplex the assays in parallel. However, it requires expensive pumping systems such as one available in

Figure 2: Droplet-based microfluidics devices for rapid detection of bacterial cells in confined droplets. a) Stationary nanoliter droplet array (SNDA) AST device illustrating device design and working principle. Two rows of 8 nL well prefilled with lyophilized antibiotics are filled with sample followed by carrier oil, breaking into compartmentalized droplets. The architect of device eliminates evaporation, provides oxygen via oil and allows measurement of direct fluorescence from the wells. (Reproduced with permission from [32].) b) DropFAST platform showing bacteria and antibiotic plus viability indicator encapsulation in droplets, incubation and fluorescence detection of resazurin. Histogram fluorescence analysis of droplets with bacteria provides concentration profiles of bacterial presence confined inside droplets. (Reproduced with permission from [33].) c) Schematic of the generation of E. coli and antibiotics droplets, and detection of the signal from incubation chambers. Droplets coalesce in incubation chamber to initiate reaction. (Reproduced with permission from [34].) d) Schematic of the droplet generation from different volume sizes for automated AST. Known sequence of compositions of droplets are generated and incubated off-chip and polythene tubing. Droplets are then injected into a channel to detect bacterial metabolism via optical fiber and spectrophotometer. (Reproduced with permission from [35].)
the market known as Mitos Dropix Droplets on Demand by Dolomite Microfluidics. Currently, these type of systems are only available for research purposes and further optimisations and miniaturisations are required before they can be used for POC diagnostics. Human blood serum has also been tested in droplet-based microfluidics such as Boidicker et al. [36] developed a platform to detect antibiotic susceptibility in nano-litre droplet plugs using methicillin-resistant Staphylococcus aereus (MRSA) bacteria against multiple drugs. The authors also detected MIC in single experiments using cefoxitin against S aereus, and the device was proven to be useful for differentiating between sensitive and resistive bacteria in human blood serum. All the above-mentioned droplet-based studies at their current stages provide valuable results which detect bacteria and help understand the bacterial response towards antibiotics but lack the ability to be used for near-patient diagnostics.

Overall, the benefits of droplet-based systems include confinement of cells in droplets, which increases surface area for reaction, less incubation time or even no pre-incubation required. In addition, droplets configuration allows multiplexing drug resistance to be studied in parallel format where different combinations of multiple drug concentrations against bacterial growth can be tested on a single platform. This, in turn, provides measurements in shorter times and can lead to more informed clinical decisions providing an opportunity for the use of narrow-spectrum antibiotics in disease treatments. But the droplet generation methods used in these studies require multiple pumping modules, sensitive fluorescent detections and droplet analysis of millions of droplets which hinders their use for POC diagnostics in healthcare and makes them a suitable technology for laboratory-based research.

Figure 3: Paper-based and capillary-flow driven microfluidics devices for AMR detection. a) Paper-based analytical device (PAD) to detect Salmonella typhimurium and E. coli. Sandwich immunoassay using B-galactosidase and colorimetric assay with chlorophenol red-β-D-galactopyranoside was performed on paper. The incubation times were optimized on well array devices and chemometers and the bacterial growth was determined measuring the length of the progression on chemometers. (Reproduced with permission from [45].) b) Schematic of the PDMS/paper device for detection of uropathogens on precoated paper substrate inside PDMS channels and wells. The microchip consists of three layers namely top layer with air vents, inlet and outlet, middle layer to connect layers and bottom layer with chambers to insert paper substrates. (Reproduced with permission from [47].) c) Lab-on-a-stick device schematic illustrating FEP test strips, reagent and sample loading into test strips via capillary action. The FEP test strips were precoated with antibiotics and dipped into the sample upon which reagents released into aqueous sample react with bacteria. (Reproduced with permission from [48].)
As an alternative to active microfluidic-based techniques, paper-based microfluidic devices have become useful due to precise on-paper passive flow control, easy operation, less contamination/evaporation issues, portable and disposable nature, and cost-effectiveness [37, 38]. These types of devices have found important applications in POC and medical diagnostics. Especially the devices are well suited for remote areas where expensive sample handling and sophisticated pumping systems are limited [39]. Several paper-based microfluidics devices detecting AMR have been reported [40-47]. Recently, Srisa-Art et al. [45] developed a paper-based analytical device (PAD) to detect Salmonella typhimurium via on-paper sandwich immunoassay using B-galactosidase as enzyme and colourimetric assay bacteria detection with chlorophenol red-β-D-galactopyranoside (Fig. 3a). The authors also tested bird feces for bacterial detection without any interferences from other pathogenic bacteria where the detection was achieved measuring the length of the coloured band on the paper making it suitable for machine free testing. This technology provides a platform to perform cheaper AMR assays, but the sensitivity of the assay was found to be lower due to the matrix effect in the samples. Nevertheless, this format of paper-based technology can be highly regarded as a cheaper alternative to active microfluidics devices and will get through the commercial challenges once fully optimised. In another paper-based AMR device, Katherine et al. [46] developed a PAD for detecting β-lactamase resistance from the environment such as water. In this study, the authors investigated the bacterial species isolated from water in arrays of fabricated wells on paper and detected via smartphone image analysis. This type of testing can prevent spread of resistant bacteria to the environment at a mere cost and save expensive transportation of water samples to central-labs and laborious testing. To implement this study for POC diagnostics, complete miniaturisation of instrumental tools and extensive research is required including optimization of complex samples such as blood, urine or milk. In another study done by Xu et al. [47] one-step bacteria identification and AST testing were achieved on PDMS/Paper-based device. The device consisted of chambers and sample inlet channel made of PDMS and a paper preloaded with antimicrobial agents and chromogenic substrate (Fig. 3b). Multiple uropathogens were detected on the device for UTI disease via detection of colour changes from the chromogenic reaction in chambers. Detection of three different bacteria on a single device and MIC determination was achieved within 15 hours, which is much quicker than standard methods (3-4 days). This device reduced a significant amount of time for UTI tests and potentially can reduce the cost of tests per patient paving the way for deployment in clinical settings. However, it must fully satisfy commercialisation needs such as requirement of portability, detection and assay reproducibility. Generally, paper-based devices are simple, cheaper, easy to fabricate and more importantly are less reliant on sophisticated instruments for operational procedures. Therefore, significant research is required to overcome technical challenges faced by paper-based POC devices, especially in combating AMR in healthcare.

Fluids can also be driven in polymer/glass microfluidics devices by capillary action known as capillary-driven flow microfluidics that eliminates the need for external pumping systems. These types of devices can easily be operated as the reagents/antibiotics are dry stored in the microchannels where samples are driven into the microchannels by directly placing devices into the sample fluids. Many such devices have been developed and reported for diagnostics applications [48-51]. Reis et al. [48] developed fluoropolymer-based (FEP) lab-on-a-stick test strips to study multi-analyte assays in shorter times (Fig. 3c). By dipping reagent stored test strips into sample liquids, it allowed the liquid to travel via capillary action. Following incubation, the individual test strip (with ten channels) was read in a custom-built scanner. In this way, the device was tested for ABO blood typing, identification of three different bacterial cells and MIC determination using multiple drugs. Although these type of capillary-driven flow devices are immature at this stage, further improvements to these systems can provide a new generation of microfluidics diagnostic tools that can be more sensitive, cost-effective and portable for the global fight against AMR spread. Ideally, capillary-driven flow devices are suited for POC diagnostics because this type of microfluidics has the potential to provide rapid, inexpensive and simple clinical assays in shorter times and near the patient. The reagents can be pre-coated on to the surface of the channels and by merely dipping the device into the sample from patients, incubating and getting results within few hours can lead to the development of the simple POC devices accepted by the clinical environment.

Indeed, there is a global requirement for development of handheld devices to identify different types of bacteria and determine MIC for effective treatments. Current microfluidics approaches (some mentioned in this paper) provide solutions for running assays in smaller microfluidics devices yet require bulky optical detection systems to analyse these results. This, as a result, is limiting the portability and wider acceptability of these devices. An alternative to these bulky optical devices, a smartphone can be used for diagnostics purposes where a mobile phone can be converted into a mini microscope or spectrophotometer while data processing programs can be installed on cell-phones to provide automated results. Several studies have been performed using a smartphone as a microscope or optical detector, such as used for ELISA assay detection in microchip [52] and quantitation of prostate-specific antigen (PSA) in FEP test strips [53]. Recently, Barbosa et al. [53] developed a smartphone-based detection system to quantify PSA using enzymatic amplification and colourimetric assay substrate reaction (Fig. 4a). A magnifying lens and light source was attached to the mobile phone camera to detect the colourimetric signal from the FEP test strips. The system was also adapted to detect fluorescent signal of the assay by adding dichroic mirror and a background UV black light. This smartphone-based detection developed in this study has a great potential to be combined with paper-based or capillary-driven flow microfluidics to yield an ever demanded AMR testing system for deployment in clinical settings. Furthermore, Steve et al. [54] 3D printed an attachment consisting of an array of LEDs to illuminate microtiter plates and optical fibre to capture light from the microwells on the titer plate (Fig. 4b). The smartphone was able to measure the turbidity within 1 min. Clinical isolates of Klebsiella pneumoniae in 78 patients were tested against 17 antibiotics with this smartphone-based device, and the AST accuracy was within the FDA defined criteria, i.e. turbidity
measurement (98.21%), MIC (95.12%) and AST (99.23%). These results while encouraging were demonstrated using a 96 well plate and require time-consuming sample handling steps. Therefore, the powerful detection capabilities of smartphone must also be combined with miniaturised AMR testing techniques already developed to gain fruitful outcome for commercialisation of these tools. In contrast, Kadlec et al. [55] developed a smartphone-based spectrophotometric system for detecting the bacterial growth in microwell arrays (Fig. 4c). The authors pre-coated the walls of the gas permeable wells with antibiotics, cultured bacteria with colourimetric viability substrate and read the signal from a smartphone camera. Using this system, AST profiling of cultured bacteria and uropathogens for UTI was performed. The system was fully miniaturised for UTI testing and can lead towards the POC diagnostics in healthcare. However, further improvements to simplify sample handling and multiplexing in parallel should be exploited to gain access to the clinical diagnostics market. Contrary to optical detections via smartphone, an electrochemical detection method [56] and colourimetric scanning [48] have also been attempted for bacterial identification to build standalone and portable AMR testing tools. Nonetheless, these tools are immature at this stage and require the development of cost-effective and reliable methods to overcome the dilemma of gaining trust from key opinion leaders in clinical diagnostics.

CONCLUDING REMARKS

Current conventional AMR detection technologies provide time-consuming, costly, labour-intensive and central lab-based solutions, limiting their applications. Microfluidics has been developed for decades, and the technology has emerged as a powerful tool for point-of-care (POC) diagnostics of AMR in healthcare providing, simple, robust, cost-effective and portable diagnostics. Various microfluidics devices have been developed to achieve this goal. Miniaturisation and integration of different tools have been attempted to produce handheld or standalone devices for rapid AMR testing. The successes have been registered in research articles; however, the potentials of microfluidics technology in tackling AMR have not been fully achieved in clinical settings. This article reviews some of the most popular and important research studies recorded in the literature.

Microfluidics has not gained confidence in the clinical environment and has not made a breakthrough in the commercial industry worldwide. This is mainly due to the nature of delicate microfluidics innovations and requirement of expertise and sophisticated instruments for commercialisation of these devices. The surface properties of materials require expensive treatments, and hence reproducibility issues can arise from this problem. There is a lack of simplicity and ease of use of microfluidic devices along with scalability issues that have made these microfluidics devices less favourable commercially. Although, there have been multiple research studies (some mentioned above) which provide solutions to tackle AMR, yet no microfluidic-based systems are combating AMR in the field. Therefore, simple, affordable and rapid systems are still a requirement to bridge the gap between research and field-deployable POC diagnostics [57]. Following research in AMR tackling, must be able to provide simple, inexpensive and fully portable systems. Current research studies reviewed in this paper provide a clear view that the focus of the research in the last decade has been miniaturising current assay techniques to
provide rapid AMR testing. However, all the characteristics required by clinicians such as simplicity, cost-effectiveness, portability, accuracy and time-efficiency are not found in a single device. This paper provides a platform to compare different techniques and match suitable technologies to develop new devices which include all the characteristics mentioned above. One of the best and emerging scenario is combining paper-based or capillary-driven flow microfluidics with smartphone-based detection which can be cost-effective, simple and portable. Ideally, these devices are cheaper to fabricate and simple to operate such as pre-coated testing strips, or channels can be dipped in the sample, and the colour development or signal can be monitored via smartphone. The smartphone must be able to analyse the signal, and the results must be provided within a few hours while the patient still stays in the clinic so that on-spot and well-informed clinical decisions can be made for the better treatment of the patients.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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Sensitive Detection of Salmonella typhimurium Using a Paper-Based Microfluidic Device

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