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Paper-based devices as a new tool for rapid and on-site monitoring of 'superbugs'

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Infectious diseases are currently a significant cause of morbidity and mortality, with approximately 700,000 deaths each year worldwide[1]. Viruses, bacteria, and fungi have become increasingly resistant to antimicrobial agents, making antimicrobial resistance (AMR) one of the biggest global health challenges humanity has had to face. Recent reports have highlighted the role pandemics may play in exacerbating AMR through the increased use of disinfectants, alcohol-based hand sanitisers, and antiseptic hand wash[2]. Evidence of antibiotic mis-prescribing in hospitalised COVID-19 patients has also been reported—risking a pandemic-induced spike in AMR[3]. Ultimately, the fate of antimicrobial agents and resulting resistant microorganisms is they are discarded into wastewater, entering the environment as sewage, sludge, and treated wastewater. This results in opportunities for further mutation and horizontal gene transfer (HGT).[2]

One of the critical challenges in combatting AMR is the availability of low cost, rapid diagnostic tools to provide near-real-time, on-site, ultrasensitive detection and determination of the aetiology of infection (e.g., viral, bacterial, fungal) to understand the susceptibility of the microorganism to antimicrobial therapy.

The World Health Organisation established the 'Global Antimicrobial Resistance Surveillance System' (GLASS) in 2015 to provide a standardised approach to AMR surveillance and data collection worldwide. GLASS records phenotypic characteristics, e.g. observation of bacterial growth under antibiotics, while genetic information is absent. The lack of routine collection of genetic sequence data underpinning the resistance phenotype limits the ability to understand and respond to changes in resistance patterns. Both phenotypic and genotypic methods are ideally needed to supplement each other as part of a more comprehensive AMR surveillance programme[1].

There have been various laboratory-based assays developed for both phenotypic and genotypic AMR detection; however, their use for on site measurements remains challenging. In contrast, paper-based devices have shown great promises for point-of-care (POC) diagnostic devices for on-site environmental monitoring of both phenotypic and genotypic AMR. Due to its cellulose nature, paper has several advantages, including lightweight, low cost, biocompatibility, and disposable after use via combustion, as well as capillary action enabling sample process without external power[5]. All the above makes it an ideal substrate for POC devices.

The development of paper-based devices has flourished since 2007 when it was first proposed by Whitesides group[5] for bioassays. They patterned the paper into discrete sections via photolithography, which was then embedded with epoxy-based negative photoresist (SU-8) to create multiple channels. Then the reagents of different bioassays, for example, enzymatic oxidation of iodide to iodine in the presence of the analyte glucose, were spotted and dried in different channels for subsequent diagnostics. The white colour of paper provided a clear contrast for colorimetric-based results, while the capillary action automatically filtered contaminants from the sample, and therefore the result was free from interference. In addition to biosensing, paper-based devices have also been used in sample extraction

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and purification, replacing the conventional tube-based cell lysis, and is more time- and labour-efficient [5].

Table 1 Examples of paper-based devices for both phenotypic and genotypic monitoring AMR

Analytes (ARGs or ARB)	Limit of Detection	Processing time	Storage condition and lifetime	Samples	Detection method
Methicillin-resistant *Staphylococcus aureus* (MRSA)	single copy of mecA gene	36-43 min	35 days under 25◦C, with 85 ± 2.98% efficiency	Blood	Fluorescent LAMP
	single copy of mecA gene	30 min	28 days under 4 ◦C, nitrogen, with 95% efficiency.	Buffer	*In situ* colourimetric LAMP
	100 copies of mecA and 285 copies of ermC genes	30 min	N/A	Buffer	Fluorescent LAMP
Escherichia coli expressing β-lactamase	10^3 CFU/mL	8 h	N/A	Wastewater	Colorimetry
Escherichia coli O157:H7	10^4 CFU/mL	3 h	N/A	Vegetables	Colorimetric ELISA
	10^4 CFU/mL	4 h	30 days stored at 4 °C	Animal	Colourimetry
Cronobacter spp.	10^1 CFU/cm^2	10 h	6 weeks stored at 4 °C	Material surface	Colourimetry

Molecular diagnostics can also be integrated onto paper devices for the detection of antimicrobial resistance genes. This can be achieved via molecular amplification such as loop-mediated isothermal amplification (LAMP) to enhance sensitivity. Such LAMP-integrated paper-based devices have shown ultra-sensitivity and specificity for nucleic acid-based detection for both clinical and environmental samples, which is a significant advancement. Reboud et al.[5] detected malaria species in 98% of infected patients from their finger-prick blood samples by employing paper-based devices. They first extracted malaria DNA from real blood samples via the paper-microfluidic device then carried out the LAMP reaction with reagents introduced into the reaction chamber, followed by a readout with a lateral flow stripe Both the sensitivity and specificity of the devices were higher than the gold-standard real-time polymerase chain reaction (PCR).

As shown in Table 1, paper-based devices have been developed to monitor AMR using phenotypic and genotypic methods and various sample types including good success withenvironmental sample analysis. They offer a rapid and robust detection tool for environmental surveillance even for non-specialists, which is extremely important and useful in low resource settings such as in low- and middle-

income countries. We believe that paper-based devices will provide a rapid sensing platform for near real-time surveillance of AMR and other targets. These results can help to build descriptions of local resistance profiles, which may formulate regional, national or even global monitoring programmes and support the goal of reducing the global One Health burden. The paper-based device can provide complementary genomic understanding to GLASS while transforming environmental monitoring, which has yet to be rolled out in most countries. Furthermore, the advancement of data science, computational approaches such as deep learning, artificial intelligence and the internet of things presents opportunities to have semi- or fully automated systems of environmental monitoring that can not only reflect the current local resistance profiles but also provide insight into emerging risks for timely and effective intervention.

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