RAPID, LOW-COST CARBAPENEMASE DETECTION USING A SELF-COALESCING STICKER MICROFLUIDIC FOR ENHANCED MANAGEMENT OF CARBAPENEMASE-PRODUCING ORGANISMS IN HEALTHCARE SETTINGS

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ABSTRACT

Infectious diseases caused by carbapenemase-producing organisms (CPOs) present a critical challenge due to high mortality rates and antibiotic resistance. Current detection methods suffer from cost and complexity, hindering timely intervention. We introduce a novel self-coalescing sticker microfluidic device for rapid, low-cost, point-of-care detection of CPOs. Leveraging self-coalescence, our device enables reconstitution of multiple reaction spots, simplifying interpretation. Incorporating machine learning enhances accuracy, with peak performance reaching 0.965 in class identification. Preliminary testing demonstrates feasibility, detecting a class B carbapenemase in 15 minutes with comparable results to standard assays. This rapid, low-cost device addresses the urgent need for CPO class identification.

KEYWORDS

Diagnostic, carbapenemases, rapid, low-cost, microfluidic

INTRODUCTION

Threat of carbapenemase producing organisms

CPOs present a formidable challenge in healthcare due to their ability to rapidly spread antibiotic resistance (Figure 1). These organisms have carbapenemase genes encoded on mobile genetic elements, facilitating transmission between bacteria, leading to the rapid spread of resistance and the potential for large outbreaks in healthcare settings [1,2]. This underscores the urgent need for detection and containment strategies. Unlike other mechanisms of carbapenem resistance, which are often chromosomally encoded and less transmissible, CPOs pose a unique threat due to their ability to disseminate swiftly within and beyond hospital environments [1].

With different carbapenemase classes exhibiting unique antibiotic resistance profiles, rapid and accurate identification of CPOs and their specific enzyme classes is critical for guiding appropriate antibiotic treatments and infection control measures. Patients with CPO infections are at increased risk of receiving ineffective antibiotic treatment, leading to higher mortality rates [3]. With the emergence of antibiotic combinations that are effective against certain carbapenenase classes (such as Klebsiella pneumoniae carbapenemases, class A) while ineffective for others (New Delhi metallo-β-lactamases [NDMs], class B), accurate detection and classification of carbapenemase classes are essential to guide clinical treatment decisions [1,4]. Despite recommendations from the CDC for active screening, most clinical microbiology laboratories do not perform carbapenemase detection due to limitations in available testing methods [2]. Existing molecular assays are too expensive for routine use, lateral flow assays offer a low cost solution but can only detect a single carbapenemase class (rather than all four), and phenotypic tests are hampered by prolonged workflows and subjective interpretation [1]. In this context, there is a critical need for innovative screening tests which aim to combine simplicity, rapidity, and sensitivity to address the diverse diagnostic challenges posed by CPOs.

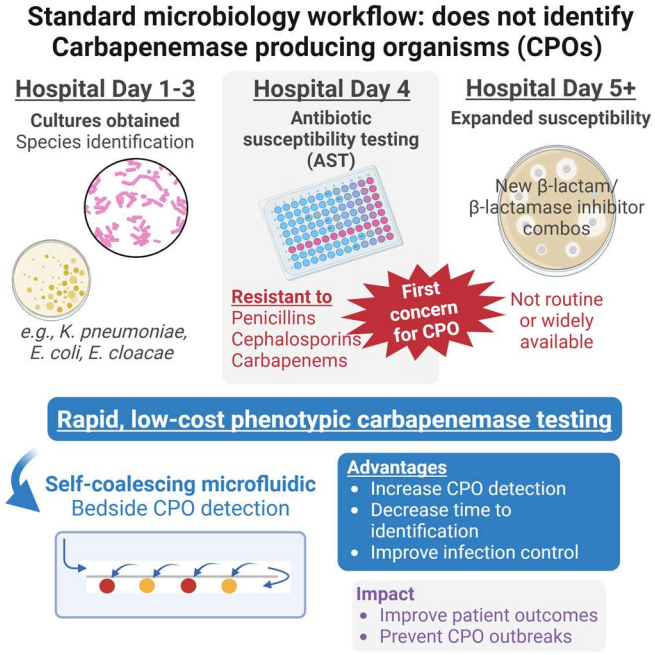

Figure 1: Study overview with standard microbiology workflow and the novelty of our rapid carbapenemase testing approach.

Addressing these challenges, we leverage self-coalescence to create the first low-cost diagnostic of any carbapenemase at the point-of-care that is easy to interpret for any user.

Leveraging self-coalescing microfluidics

Conventional microfluidic devices often require specialized expertise or costly external equipment for operation, posing significant barriers to accessibility. Challenges arise from the precise fluid flow conditions and small sample volumes required, as well as the logistical complexities of performing multiple assays. In contrast, self-coalescing microfluidics streamline these processes by enabling simultaneous testing of multiple samples on a single, user-friendly chip. Self-coalescence is defined as the flow phenomenon seen when a confined liquid with a stretched air–liquid interface is forced to fold onto itself, enabling reconstitution while also mitigating streaky reaction zones [1,5]. With just a single drop of sample, even minimally trained operators can execute multiple tests, complete with appropriate positive and negative controls. This approach leverages the inherent properties of small-scale fluid flows, utilizing geometric features to passively control fluid movement.

Recognizing the potential to adapt existing assays to microfluidics using self-coalescence and dried reagents, we aimed to enhance the applicability and accessibility of this powerful phenomenon in diagnostic platforms. Traditional self-coalescing microfluidics, although powerful, present challenges: they are small,

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challenging to discern by the naked eye, and demand sophisticated, and hence, more costly fabrication methods. We sought to overcome these challenges through the innovative integration of self-coalescence principles into a sticker microfluidic device.

Sticker microfluidic devices offer a cost-effective solution for leveraging microscale channels without the need for a cleanroom, making them accessible and versatile. They include double-sided roll-based adhesives that accompany various materials like silicon, glass, and polymers. Due to the simple assembly and manufacturing needs, these devices can be prototyped and assembled within an hour [6]. Moreover, the use of sticker microfluidics significantly reduces production costs and simplifies manufacturing, addressing longstanding barriers to scalability and affordability. We recognized that when the self-coalescing phenomenon was incorporated into sticker-based microfluidics, it would be possible to create a rapid, low-cost, colorimetric test of carbapenemase activity at the point-of-care.

Machine learning

A key advantage of our detection scheme is that both positive and negative controls can be integrated onto the same device, greatly simplifying the interpretation of the results. However, a fraction of samples can be difficult to analyze as they may only slightly change color, indicating the presence of a carbapenemase, albeit one of a low activity. To that end, we applied a machine learning approach to create an algorithm that can interpret colorimetric readings and improve the overall accuracy of our approach.

Machine learning plays a pivotal role in advancing the capabilities of our self-coalescing microfluidic device for carbapenemase detection. While our device chemistry builds upon the established Carba NP method, which offers phenotypic detection of carbapenemases, machine learning introduces improvements to enhance sensitivity and specificity [1]. One of the key challenges is confidentially identifying carbapenemase classes using a qualitative reading of Carba NP results. Currently, distinguishing between these variations through visual inspection remains elusive. To address this limitation, we leverage machine learning techniques to analyze the subtle yet distinct changes occurring within short time intervals of the reactions. This integration of machine learning enhances the diagnostic capabilities by enabling rapid and accurate classification of carbapenemase classes.

DEVICE FABRICATION/DESIGN

Our device follows a simple layer by layer assembly process, enabled by laser-cut double-sided adhesives. The manufacturing of this device closely follows our previous work on sticker microfluidics [7].

Laser Cutting

The microfluidic sticker layers of the device are assembled using sheets of 0.13 mm silicone roll-based double-sided tape attached to 250 µm PDMS sheets (Rogers HT6240-0.01). These sheets were manually assembled using squeegees to apply pressure and remove air bubbles. AutoCAD was used to design the tape layers of the microfluidic prior to laser cutting. The assembled sheets were then laser cut (Universal Laser Systems VLS 4.60) utilizing these designs under optimized laser cutting parameters to prevent burning the material. Debris accumulated from laser cutting was simply removed using scotch tape.

PDMS

Polydimethylsiloxane (PDMS) was used to provide an inlet/outlet and to seal the device. 10 g of PDMS was prepared using a 10:1 ratio of Sylgard 184 (Dow Inc.) elastomer base to curing agent. Following a 45-minute degassing process, the 10 g PDMS mixture was poured into a 100 x 15 mm Petri dish and cured in a 65°C oven for a minimum of 4 hours. The cured PDMS was tailored to match the rectangular dimensions of the device using an X-acto knife. Inlet and outlet holes were then created in the PDMS roof using a 1 mm biopsy punch.

Device Assembly

Our sticker microfluidic device [7] consists of 5 layers: a cover slip, a layer with PDMS and tape that has a capillary pinning line (CPL), a second layer of PDMS and tape where fluid will flow, a double-sided tape layer with inlet and outlet holes that seal the channel, and a PDMS roof (Figure 2). These layers are assembled from the bottom up by simply removing the backings off of the double-sided tape pieces and adhering them directly to subsequent layers using manual pressure. Before applying the final tape layer to seal the device, 0.5 µL of the assay reagents are directly spotted via pipette and are given 5 minutes to dry before sealing with the final layers.

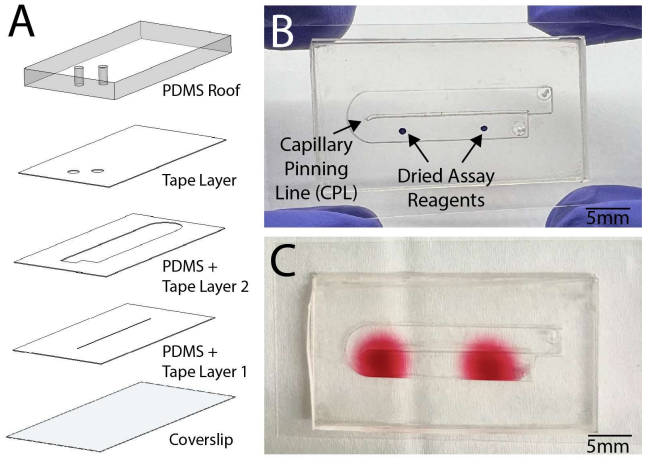

Figure 2: A) Low-cost self-coalescing microfluidic. A) The device consists of 5 layers. B) A capillary pinning line creates a separated flow stream. The sample is loaded into the microfluidic to C) reconstitute the assay reagents.

Self-Coalescence

The self-coalescing phenomenon that occurs over multiple reaction spots is enabled by the CPL. The CPL acts as a trench, forming a Laplace pressure barrier that requires filling before fluid can rapidly flow over it, promoting controlled reconstitution of reagents. We demonstrate this phenomenon by using dried amaranth spots, reconstituted with deionized water containing yellow food dye for contrast (Figure 3). Traditional microfluidics will have reagents streak and smear together when reconstituted (Figure 3A) instead of creating spatially separated and distinct reaction areas (Figure 3B). In our device, when fluid is injected, the CPL in the middle of the microfluidic traps fluid to one side as it fills, then back-fills areas over the reagents. This design detail ensures that as the fluid fills over the lyophilized reagents it is completely static and does not move. If the fluid moved, it would "smear" out the lyophilized reagents and inadvertently mix them together. The successful rehydration of separated reaction spots using a single sample, along with optimized Carba NP chemistry, enabled the use of our self-coalescing microfluidic as a carbapenemase detection device.

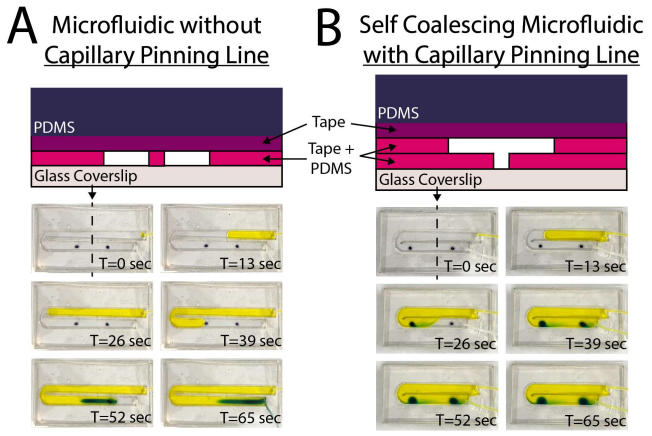

Figure 3. Self-coalescing flow profile showcasing optimal rehydration of reagent spots. A) Without a capillary pinning line (CPL), reaction spots collect at the fluid front. B) The CPL enables multiple reactions that can be easily visualized.

Reagent Chemistry

Our detection chemistry presents an enhanced iteration of the established Carba NP method [1]. The modified Carba NP assay, optimized for laboratory settings, utilizes a reduced reaction volume, resulting in a higher concentration of signal in the final reaction while significantly reducing the overall reagent usage. This adjustment facilitates faster and more distinct color changes, attributed to the concentration-dependent nature of the reactions. Additional modifications to the original Carba NP chemistry have led to a notable reduction in assay performance time to 15 minutes from 2.5 hours, eliminating the need for 37°C incubation, and effectively reducing costs without compromising sensitivity. Moreover, the incorporation of standardized absorbance measurements has minimized variability across results by ensuring consistent interpretation of subtle color changes.

RESULTS & DISCUSSION

Preliminary machine learning testing

Carbapenemases are generally categorized as class A, B, or D enzymes. Our assay protocol encompasses four key reactions to identify these classes: 1) a negative control without imipenem, 2) imipenem with Zn2+ for detecting any carbapenemase activity, 3) imipenem with tazobactam to selectively inhibit class A enzymes, and 4) imipenem with EDTA to inhibit class B enzymes (Figure 4A). The reactions in the assay produce disparate results for the diverse classes, which remain challenging to categorize visually. This variation inspired the use of machine learning to objectively and accurately identifies CPOs and the enzyme class. Preliminary testing of this machine learning approach was conducted using the optimized chemistry in a colorimetric format to determine feasibility.

Using the carbapenemase assay, we collected colorimetric results to form a dataset (n=117) for a multi-class classification algorithm. Specifically, stratified k-fold cross-validation was used to determine the accuracy of various machine learning methods including logistic regression, decision tree, k-nearest neighbors, support vector machine, and Gaussian Naïve Bayes. Our preliminary results show excellent robustness across various models. Using the absorbance readings of the four individual reactions taken at 30 minutes of assay run-time, the classification algorithm was able to correctly identify carbapenemase classes, with a peak accuracy of 0.965 (Figure 4B). These preliminary results showcased feasibility of CPO class identification using the combination of colorimetric results and machine learning approaches.

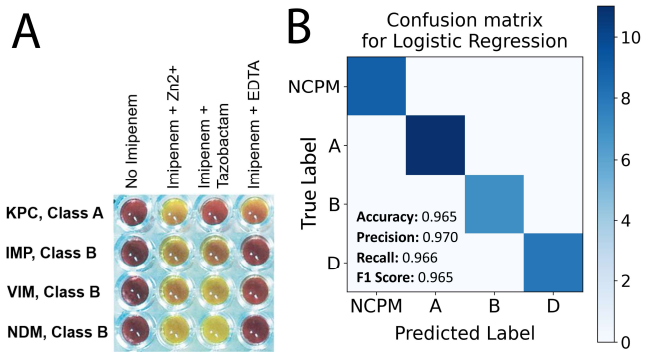

Figure 4. Machine learning for carbapenemase class detection. A) In house carbapenemase assay generates signal patterns that can distinguish carbapenemase class (A/B) and family. B) Preliminary dataset accurately predicts carbapenemase class (35 samples, gradient represents # correctly classified).

CPO classification using the microfluidic

To ensure consistent reagent performance when transitioned to a microfluidic format (Figure 5A), we performed preliminary testing with an isolate that produces a class B carbapenemase (NDM-1), demonstrating how this device can detect a CPO from a positive culture (Figure 5B). Carbapenemase detection was enabled by the addition of the optimized Carba NP chemistry dried directly onto the microfluidic. The negative control is a dried 20uL spot of phenol red/ZnSO4, the second spot contains a dried solution of phenol red/ZnSO4/imipenem, and the third spot contains phenol red/imipenem/tazobactam. This combination represents the presence of a carbapenemase (yellow color, 2nd spot) that is class B (color change despite tazobactam, 3rd spot). This 15-minute test showed comparable results to that of standard enzymatic reaction assays, which shows the feasibility of the self-coalescing sticker microfluidic for CPO class identification.

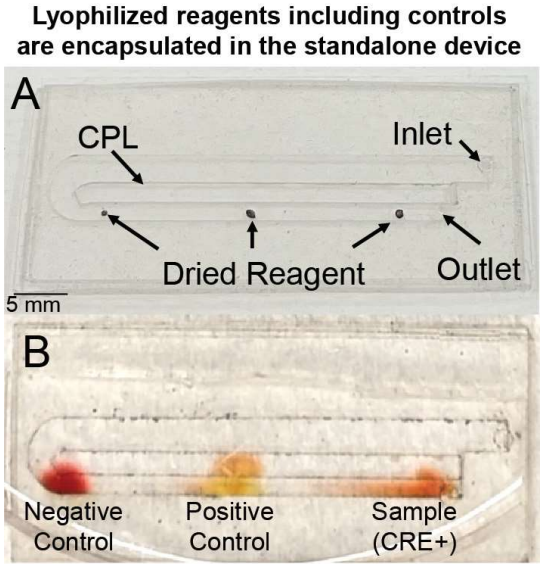

Figure 5. CPO classification using the A) self-coalescing microfluidic. B) The microfluidic demonstrated successful rehydration of the negative control (phenol red/ZnSO4), positive control (phenol red/ZnSO4-/imipenem), and the sample test spot (red/imipenem/tazobactam). Results demonstrate the presence of a class B carbapenemase.

Future work

We aim to enhance the functionality and applicability of the microfluidic device for carbapenemase detection by addressing several limitations of the current device. In future work, we will incorporate a fourth reagent zone in the microfluidic device to better mimic the design of the our modified Carba NP chemistry, thus enabling the comprehensive detection and differentiation of all carbapenemase classes. Additionally, our device has the potential to further classify carbapenemases into families as well as classes. Use of carbapenemase family signatures would allow epidemiologic characterization of enzymes in a population and the rapid identification of a new signature, indicating potential introduction or emergence of a novel carbapenemase. No available methods yield signals for novel carbapenemases, which must be confirmed by sequencing. Therefore, machine learning of quantitative device imaging results could provide low-cost, rapid detection of novel carbapenemases and inform containment strategies to prevent outbreaks.

Additionally, ongoing validation testing of the microfluidic device with 39 well-characterized CPO isolates will ascertain the effectiveness of machine learning classification in real-world clinical settings. More specifically, we aim to generate a substantial dataset of 468 smartphone images for machine learning (3 repeats x 39 isolates x 4 time points), a significant advancement considering previous studies utilized as few as 38 microfluidic images for similar smartphone enabled diagnostics [8]. Utilizing convolutional neural networks (CNNs), we will split the images randomly into a training set (80%) and a testing set (20%), employing transfer learning to supplement a pretrained CNN architecture with our target dataset specific to carbapenemase classification. We will explore popular CNN architectures like Inceptionv3 and AlexNet, alongside data augmentation and preprocessing methods, to optimize model performance, aiming for >95% accuracy in automated carbapenemase detection.

Concurrent optimization endeavors will include fine-tuning channel dimensions, running buffer viscosity tests, and the spotting/drying process of lyophilized reagents. Ultimately, our goal is to establish a robust operational system wherein healthcare professionals can effortlessly administer patient samples into the device, capture images with a smartphone at predefined intervals, and receive prompt and accurate results from a machine learning algorithm delineating the presence, class, and family of carbapenemase. Moreover, we will explore the device's shelf stability potential by encapsulating lyophilized reagents within metallized heat-sealed bags with desiccants, and assessing performance over a 12-month period.

CONCLUSION

Our self-coalescing sticker microfluidic device offers an innovative solution in diagnosing CPO infections by integrating self-coalescing flow principles with sticker microfluidics. The results of our study validate the use of our device as a diagnostic tool for CPO infections. Through modifications to transition our improved Carba NP chemistry to a microfluidic format and the implementation of a machine learning algorithm, we have achieved rapid, accurate, and sensitive screening tests for detecting CPOs and their specific enzyme classes. This development holds immense potential in enhancing patient care by reducing time-to-identification and facilitating timely initiation of effective antibiotic treatments.

The barrier to faster testing has been the lack of easy-to-use tests that can be implemented on demand at or near the point-of-care. Our design combines the advantages of available methods into simple, rapid, and sensitive screening tests for the varied use-cases where CPO detection is needed. With no external equipment required for operation, our standalone device has the potential to integrate seamlessly with standard clinical workflows, without incurring substantial healthcare costs. This rapid and low-cost CPO testing platform addresses a critical gap in current diagnostic methods, promising to enhance CPO detection, reduce time-to-identification, reduce infection case numbers, and facilitate timely initiation of effective antibiotic treatments to improve patient outcomes. This approach holds significant implications for improved patient care and can be readily applied in various healthcare settings.

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REFERENCES

[1] P. D. Tamma, P. J. Simner, Phenotypic Detection of Carbapenemase-Producing Organisms from Clinical Isolates. *J. Clin. Microbiol.* 56 (2018).

[2] Facility guidance for control of Carbapenem-resistant Enterobacteriaceae (CRE) : November 2015 update - CRE toolkit. (2015).

[3] M. J. Satlin, L. Chen, A. Gomez-Simmonds, J. Marino, G. Weston, T. Bhowmick, S. K. Seo, S. J. Sperber, A. C. Kim, B. Eilertson, S. Derti, S. G. Jenkins, M. H. Levi, M. P. Weinstein, Y.-W. Tang, T. Hong, S. Juretschko, K. L. Hoffman, T. J. Walsh, L. F. Westblade, A.-C. Uhlemann, B. N. Kreiswirth, Impact of a Rapid Molecular Test for Klebsiella pneumoniae Carbapenemase and Ceftazidime-Avibactam Use on Outcomes After Bacteremia Caused by Carbapenem-Resistant Enterobacterales. *Clin. Infect. Dis.* 75, 2066–2075 (2022).

[4] D. Yong, M. A. Toleman, C. G. Giske, H. S. Cho, K. Sundman, K. Lee, T. R. Walsh, Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. *Antimicrob. Agents Chemother.* 53, 5046–5054 (2009).

[5] O. Gökçe, S. Castonguay, Y. Temiz, T. Gervais, E. Delamarche, Self-coalescing flows in microfluidics for pulse-shaped delivery of reagents. *Nature* 574, 228–232 (2019).

[6] L. C. Gerber, H. Kim, I. H. Riedel-Kruse, Microfluidic assembly kit based on laser-cut building blocks for education and fast prototyping. *Biomicrofluidics* 9, 064105 (2015).

[7] P. Delgado, C. A. Luna, A. Dissanayaka, O. Oshinowo, J. J. Waggoner, S. Schley, T. Fernandez, D. R. Myers, An economical in-class sticker microfluidic activity develops student expertise in microscale physics and device manufacturing. *Lab Chip* (2024).

[8] M. S. Draz, A. Vasan, A. Muthupandian, M. K. Kanakasabapathy, P. Thirumalaraju, A. Sreeram, S. Krishnakumar, V. Yogesh, W. Lin, X. G. Yu, R. T. Chung, H. Shafiee, Virus detection using nanoparticles and deep neural network–enabled smartphone system. *Science Advances* 6, eabd5354 (2020).

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