ADVANCED PAPER-BASED ORGANIC ELECTROCHEMICAL TRANSISTORS: A NOVEL APPROACH FOR RAPID POINT-OF-CARE ANTIBIOTIC SUSCEPTIBILITY TESTING

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ABSTRACT

This study introduces a groundbreaking point-of-care (POC) system designed for antibiotic susceptibility testing (AST). At the heart of this innovation is the organic electrochemical transistor, a device that significantly amplifies the electrical signals arising from the redox activities and extracellular electron transfers of pathogens when exposed to antibiotics. This process involves electroactive reactions that either dope or de-dope the transistor's channel, leading to substantial changes in the current flow between the source and drain terminals. Furthermore, our system features an innovative integration with a paper substrate. This design decision significantly simplifies the handling of liquid bacterial cultures, making the process more straightforward and efficient. We have rigorously tested our sensing system using three well-known pathogens: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*, exposing them to leading antibiotics to validate the system's effectiveness.

KEYWORDS

Paper-based organic electrochemical transistors, antibiotic susceptibility testing, point-of-care, disposable, microbial extracellular electron transfer, microbial redox reactions

INTRODUCTION

The development of a rapid, user-friendly, and highly reliable point-of-care (POC) system for antibiotic susceptibility testing (AST) represents a critical advancement in optimizing the use of antibiotics for infection treatment [1, 2]. Such a system is instrumental in combating the escalating challenge of antibiotic resistance by enabling the precise selection and use of antibiotics [3]. Traditional AST methods, both genotypic and phenotypic, despite their advancements, face significant hurdles in terms of technical and operational complexities, particularly within the framework of comprehensive antibiotic stewardship programs [4, 5]. Addressing these challenges, our research team has pioneered an AST methodology leveraging the principles of microbial fuel cells [6-8]. This novel approach facilitates the swift monitoring of bacterial extracellular electron transfer in antibiotic environments, presenting our findings at the 2022 Hilton Head workshop through a compact, efficient model of our methodology [9]. Nonetheless, the generated output signal's weakness was noted, limiting the system's ability to accurately identify pathogens with low or no exoelectrogenic activity. Our current research endeavors are directed at enhancing this technology to surpass these limitations and extend its utility across various clinical settings.

We introduce an innovative sensing system that integrates organic electrochemical transistors on paper substrates, advancing AST capabilities significantly. The adoption of paper substrates simplifies the management of liquid bacterial cultures and promotes safe, eco-friendly disposal, reducing the risk of secondary infections. The breakthrough of our system lies in the organic electrochemical transistor, which offers unparalleled sensitivity and rapid response by detecting subtle changes in conductivity [10, 11]. These changes result from bacterial metabolic activities in response to antibiotics, affecting the transistor channel's doping levels and, consequently, the electronic current flow. Our system has undergone rigorous evaluation using three key pathogens: *P. aeruginosa*, *S. aureus*, and *E. coli*, testing their responses to leading antibiotics (i.e., gentamicin, ciprofloxacin, and cefotaxime). This advancement heralds a significant leap in AST, promising to enhance the strategic deployment of antibiotics in clinical practice, thereby addressing both the challenge of antibiotic resistance and the need for effective infection management.

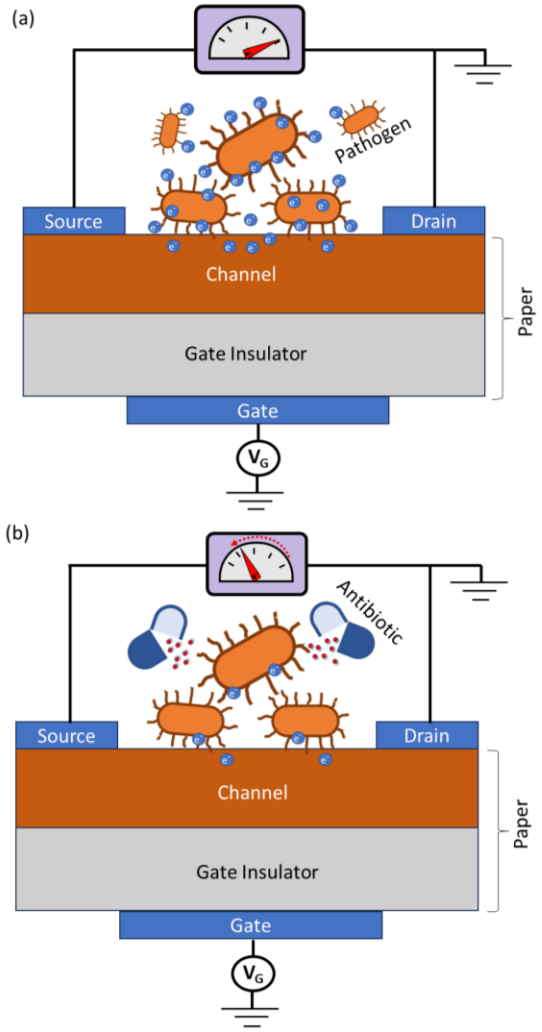

Figure 1: Schematic illustration of our technique using paper-based transistors for AST. Monitoring the electrical output between the source and drain in the presence (a) and absence (b) of antibiotics.

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EXPERIMENTAL PROCEDURE

Device fabrication

The fabrication of the organic p-type transistor, along with all associated circuit components for read-out, was meticulously carried out on Whatman™ 3MM chromatography paper (Figures 2a and 2b). This process used a Xerox ColorQube 8570 wax printer for the precise delineation of hydrophobic zones on the hydrophilic paper substrate [12]. The design and layout for this wax printing were adeptly created using AutoCAD software, ensuring high fidelity to the intended patterns. Following the printing stage, the wax was seamlessly integrated into the paper's matrix through thermal diffusion, achieved by heating the substrate to 140 °C. This step ensured the creation of well-defined, patterned regions for the components. In the next phase, the defined hydrophilic areas on the paper's reverse side were selectively imbued with a poly(vinyl alcohol) (PVA) gel electrolyte, creating an optimal environment for the gate insulator. Conversely, the front side received an infusion of Poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS), an organic semiconducting material, establishing the active channel area of the transistor. Final assembly was completed with the strategic application of electrode materials (i.e., PEDOT:PSS/graphene): the gate electrode was positioned on the underside, while the source and drain electrodes were placed atop, culminating in a sophisticated, fully integrated organic transistor on paper.

The resistors were crafted by permeating the paper with conductive PEDOT:PSS ink, whose resistance can be precisely adjusted [13]. Although the paper fibers naturally insulate electricity, infusing them with this tunable conductive ink transforms the paper into a functional, adjustable resistor for electrical currents.

Preparation of pathogens

Three critical bacterial pathogens—*P. aeruginosa*, *S. aureus*, and *E. coli*—were cultured overnight in Luria Broth (LB) medium, which was maintained at a neutral pH of 7.0. The LB medium was composed of a precise formulation: 10 g/L tryptone for protein source, 5 g/L NaCl for osmotic balance, and 5 g/L yeast extract as a vitamin and growth factor source, all dissolved in 1000 mL of deionized (DI) water to ensure purity and optimal growth conditions. Then, the cultures underwent a centrifugation process at 4000 rpm for 4 minutes, a critical step designed to separate the bacterial cells from the supernatant effectively. The supernatant was carefully removed to isolate the cell pellets, which were then resuspended in fresh LB medium to cleanse the cells and prepare them for further analysis. The resuspension process was meticulously controlled to achieve a target bacterial concentration, measured by optical density at 600 nm (OD_{600}), of 1.0. This specific optical density correlates with a high bacterial concentration of approximately 10^9 colony-forming units per milliliter (CFU/ml), ensuring a consistent and reliable baseline for subsequent experiments and analyses.

Preparation of antibiotics

For our study, we selected a representative antibiotic from each of the three critical families: aminoglycosides, fluoroquinolones, and cephalosporins. These were gentamicin, ciprofloxacin, and ceftazidime, respectively, serving as model compounds to evaluate antibiotic susceptibility. To accurately prepare the final concentrations of gentamicin, ciprofloxacin, and ceftazidime for testing, we used serial dilutions using sterile LB medium. The sterile antibiotic solutions were carefully stored at 4°C and used within a day to ensure their integrity. Specifically, for gentamicin, we prepared concentrations ranging from 2 µg/mL to 32 µg/mL in sterile LB medium. Likewise, for ciprofloxacin and ceftazidime, serial dilutions were similarly executed to obtain concentrations of 0.25 µg/mL to 2.0 µg/mL for ciprofloxacin, and 2 µg/mL to 32 µg/mL for ceftazidime, tailored for the antibiotic susceptibility testing process.

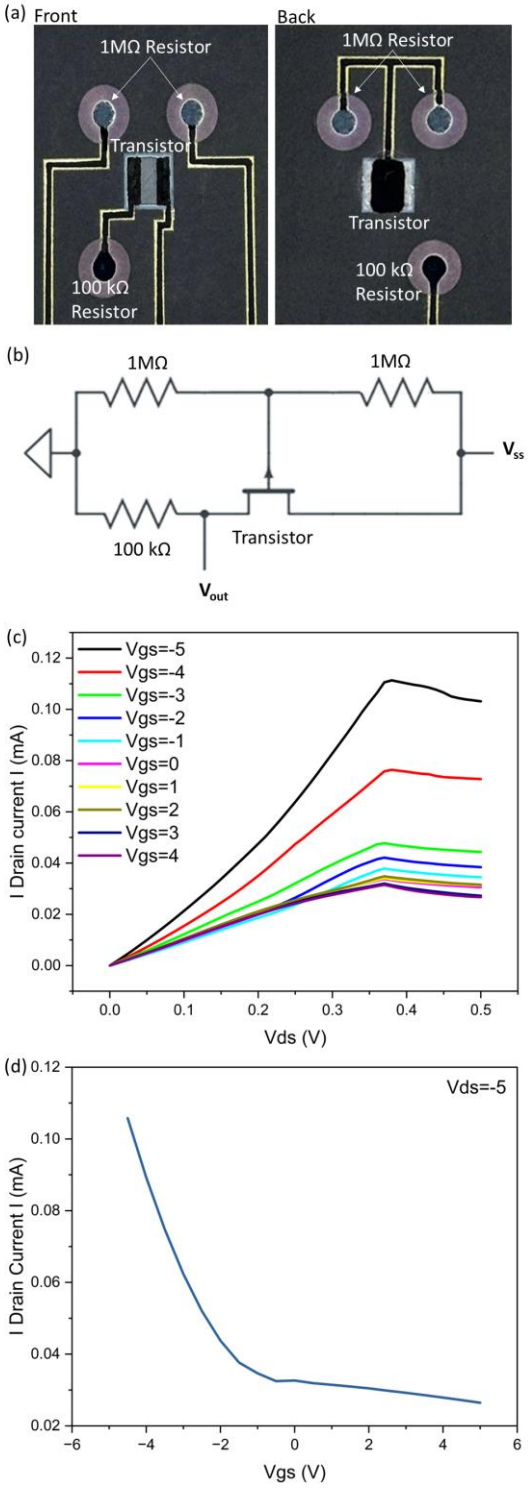


Figure 2: (a) front and back photographs of the transistor-based sensing circuit fabricated on paper, and (b) its circuit diagram illustrating all component details. (c) and (d) delineate the output curves and transfer characteristics.

Antibiotic susceptibility testing (AST)

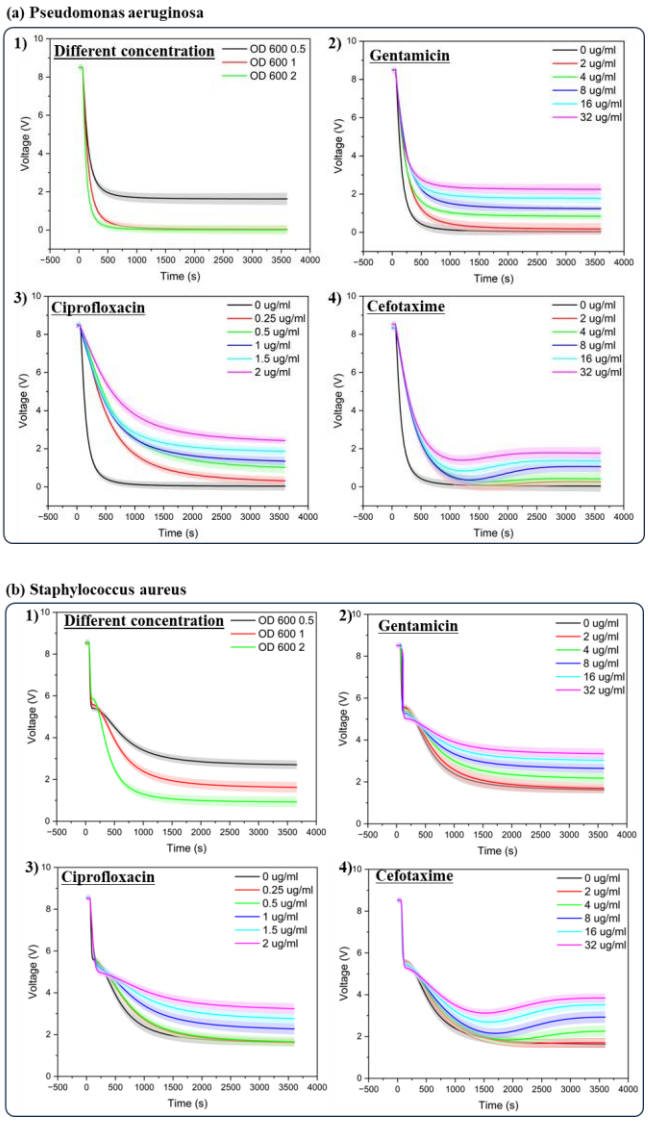
To conduct the AST, 5µL of bacterial suspension, standardized to a concentration of 1.0 OD_{600}, was thoroughly mixed with 5µL of each specific antibiotic concentration. Subsequently, a precise volume of 10µL from this prepared mixture was applied onto the transistor channel.

Electrical characterization

The Keithley 4200-SCS semiconductor characterization system, a sophisticated and highly precise instrument, was employed to assess the transfer and output characteristics of the paper transistor. This system facilitated the meticulous measurement of voltage outputs from the read-out circuit, with all data being accurately captured and recorded through an advanced data acquisition system. This setup ensured comprehensive and reliable analysis of the transistor's performance under various conditions.

Optical analysis

The cross-sectional analysis of the transistor was achieved using a Hitachi SU5000 Field Emission SEM and a V12 Stereo optical microscope, providing intricate structural insights.



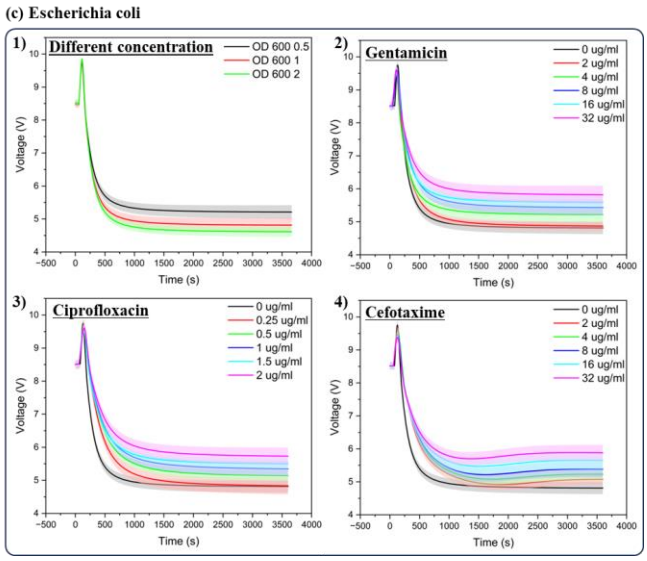


Figure 4: Voltage outputs of our AST system with (a) Pseudomonas aeruginosa, (b) Staphylococcus aureus, and (c) Escherichia coli are presented. (1) These outputs vary with different bacterial concentrations. The AST outputs in the presence of (2) gentamicin, (3) ciprofloxacin, and (4) cefotaxime are also detailed. Upon introducing the sample, continuous and real-time electrical readings are obtained. The electrical signals derived from bacterial metabolic activity are sufficiently sensitive to assess the effectiveness of the antibiotics and to elucidate their mechanisms of action.

RESULTS AND DISCUSSION

Figure 2c illustrates the output curves, while Figure 2d showcases the characteristic transfer of the p-type transistors developed on a paper substrate. Remarkably, these devices exhibit high electron mobility, with a measurement of 4×10^3 $cm^2 \cdot V \cdot s$, and an outstanding I_{on}/I_{off} ratio of approximately 5.5×10^3 at a low operating voltage of −2 V. the transistor displays exceptional electrical characteristics, including a low threshold voltage of −1.3 V, indicative of its superior performance. Bacterial samples mixed with antibiotics are placed onto the transistor channel, which serves as a dynamic sensing node. Here, the modulated current is detected as a voltage output via a read-out circuit interfaced with three resistors (Fig. 2 and Fig. 3). The specificity of resistance values is achieved by meticulously controlling two critical parameters: the conductivity of the PEDOT:PSS ink and its deposition quantity [12, 13]. Moreover, the paper-based channel efficiently absorbed the introduced sample through capillary action, eliminating the need for a fluidic system.

Our transistor-based AST sensor works by leveraging the metabolic processes of bacteria, which produce redox activities and extracellular electron transfers. These processes influence the current flow through the transistor channel, which in turn affects the circuit's output voltage. As the population of active bacterial cells grows, there is a corresponding decrease in output current, particularly because the channel is of the p-type. Notably, the change in output voltage becomes significantly pronounced across all three antibiotics as their concentrations increase. Initially, we expose pathogenic samples to varying concentrations of antibiotics to measure the impact. The output voltage we record varies depending on the concentration of bacteria present. We achieve continuous and real-time voltage monitoring.

Interestingly, *E. coli* strains that are not exoelectrogenic show less sensitivity in their outputs compared to exoelectrogenic strains, such as *P. aeruginosa* and *S. aureus*. [14-16]. Despite this, the electrical signals stemming from the bacteria's metabolic activity are precise enough to determine the antibiotics' effectiveness and understand how they work. For instance, gentamicin, which disrupts cell protein synthesis, causes rapid decreases in voltage, indicating its quick antibacterial action [17]. This is especially true at its minimum inhibitory concentration (MIC) of 4 μg/ml. Ciprofloxacin, which targets bacterial RNA synthesis, triggers slower voltage changes than gentamicin, also with a MIC of 4 μg/ml [18]. Cefotaxime, a beta-lactam antibiotic that interferes with cell wall synthesis, shows the slowest action [19]. Its impact on the voltage curve suggests a delayed effect, with an MIC of 8 μg/ml being optimal for observation.

CONCLUSION

In conclusion, this study pioneers a novel paper-based organic transistor system for fast and reliable point-of-care antibiotic susceptibility testing. Leveraging the transistor's amplification capabilities, our approach enables sensitive detection of pathogenic electrochemical responses to antibiotics. Significantly, the device quantifies antibiotics' minimum inhibitory concentrations. Beyond offering detailed susceptibility profiles, this innovation enhances understanding of antibiotic actions, contributing to more effective treatment strategies. This advancement in microbiological diagnostics represents a crucial step towards optimizing antibiotic usage and combating resistance.

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