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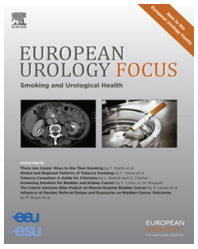

Infections

Integrated Biosensor Assay for Rapid Uropathogen Identification and Phenotypic Antimicrobial Susceptibility Testing

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Abstract

Background: Standard diagnosis of urinary tract infection (UTI) via urine culture for pathogen identification (ID) and antimicrobial susceptibility testing (AST) takes 2–3 d. This delay results in empiric treatment and contributes to the misuse of antibiotics and the rise of resistant pathogens. A rapid diagnostic test for UTI may improve patient care and antibiotic stewardship.

Objective: To develop and validate an integrated biosensor assay for UTI diagnosis, including pathogen ID and AST, with determination of the minimum inhibitory concentration (MIC) for ciprofloxacin.

Design, setting, and participants: Urine samples positive for Enterobacteriaceae ($n = 84$) or culture-negative ($n = 23$) were obtained from the Stanford Clinical Microbiology Laboratory between November 2013 and September 2014. Each sample was diluted and cultured for 5 h with and without ciprofloxacin, followed by quantitative detection of bacterial 16S rRNA using a single electrochemical biosensor array functionalized with a panel of complementary DNA probes. Pathogen ID was determined using universal bacterial, Enterobacteriaceae (EB), and pathogen-specific probes. Phenotypic AST with ciprofloxacin MIC was determined using an EB probe to measure 16S rRNA levels as a function of bacterial growth.

Measurements: Electrochemical signals for pathogen ID at 6 SD over background were considered positive. An MIC signal of 0.4 log units lower than the no-antibiotic control indicated sensitivity. Results were compared to clinical microbiology reports.

Results and limitations: For pathogen ID, the assay had 98.5% sensitivity, 96.6% specificity, 93.0% positive predictive value, and 99.3% negative predictive value. For ciprofloxacin MIC the categorical and essential agreement was 97.6%. Further automation, testing of additional pathogens and antibiotics, and a full prospective study will be necessary for translation to clinical use.

Conclusions: The integrated biosensor platform achieved microbiological results including MIC comparable to standard culture in a significantly shorter assay time. Further assay automation will allow clinical translation for rapid molecular diagnosis of UTI.

Patient summary: We have developed and validated a biosensor test for rapid diagnosis of urinary tract infections. Clinical translation of this device has the potential to significantly expedite and improve treatment of urinary tract infections.

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1. Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections. Enterobacteriaceae species account for >80% of UTIs, with *Escherichia coli* accounting for approximately 75% and 65% of UTIs in ambulatory and hospitalized settings, respectively [1]. Diagnosis via urine culture requires a centralized clinical microbiology laboratory for pathogen identification (ID) and associated antimicrobial susceptibility testing (AST), expressed as the minimum inhibitory concentration (MIC) for an individual antibiotic. AST, based on antibiotic disk diffusion or microdilution, provides an interpretation of MIC results as sensitive, intermediate, or resistant for clinician guidance. The entire process for pathogen ID and AST typically requires 2–3 d. This gap between clinical presentation and the microbiology report leads to empiric prescription of antibiotics.

Evidence-based antibiotic selection for UTI may help to stem increases in antibiotic resistance [2]. Emblematic of poor antibiotic stewardship is trimethoprim/sulfamethoxazole (TMP/SMX). This oral antibiotic was previously the first-line treatment for UTI. However, since TMP/SMX resistance exceeds 20% [3] in North America, ciprofloxacin is recommended in current guidelines for complicated UTI as the resistance rate is lower for this antibiotic [4].

Point-of-care (POC) diagnosis of UTI has potential to improve patient care by rapidly identifying the causative pathogen and the best treatment choice. Electrochemical biosensors are an ideal basis for POC diagnostics as they provide rapid results and high sensitivity, and are small in size and inexpensive. We previously described a 1-h electrochemical biosensor assay for molecular identification of uropathogens [5,6]. Using a biosensor array functionalized with a panel of DNA probes targeting conserved and unique bacterial 16S rRNA sequences, urine samples containing single or multiple bacterial species (polymicrobial infections) can be identified directly [6] in a urine sample. Since the biosensor assay provides quantitative detection, we further adapted the assay for phenotypic AST by detecting differential 16S rRNA levels after brief culture of a sample in the presence and absence of antibiotic [7]. Using the biosensor-based approach, phenotypic AST of common uropathogens against standard bactericidal and bacteriostatic antimicrobials, including ampicillin, TMP/SMX, ciprofloxacin, gentamicin, and ceftriaxone, was demonstrated in prospectively collected urine samples [7].

For the biosensor assay, cells are lysed to release the rRNA that binds to capture and detector oligonucleotide probes at the sensor surface. Probe-rRNA hybridization is facilitated by electrokinetic (EK) hybridization. EK is a microfluidic sample preparation technique that generates electrothermal flow via Joule heating, thereby reducing the assay time and improving the signal-to-noise ratio [8]. Probe-rRNA complex formation is detected using an antibody-horseradish peroxidase HRP conjugate for generation of an electrochemical signal. With EK-facilitated hybridization, a limit of detection of 10^3 colony-forming units (cfu)/ml has been demonstrated [9].

In our previous report, ID and AST were conducted on separate biosensor arrays with different starting samples, urine for ID and cultured urine for AST. Here we report the development and validation of ID and AST assays integrated in a single biosensor assay focusing on detection of Enterobacteriaceae and AST for ciprofloxacin. We also demonstrate that growth quantitation by the integrated biosensor can provide ciprofloxacin MIC results comparable to those achieved by clinical microbiology. The combination of pathogen ID and AST in a single assay provides an effective strategy toward integration into a fully automated POC diagnostic for UTI.

2. Materials and methods

2.1. Clinical samples

Between November 2013 and September 2014, de-identified clinical samples containing $\geq 10^5$ cfu/ml of a single Enterobacteriaceae species ($n = 84$) or without bacteria ($n = 23$) were obtained from the Stanford Clinical Microbiology Laboratory (Table 1). Samples had been preserved with boric acid and stored at 4 °C before testing. CHROMagar Orientation (BD Diagnostic Systems, Hunt Valley, MD, USA) was used for identification of *Escherichia coli* and MALDI Biotyper (Bruker, Billerica, MA, USA) for identification of other species. AST was performed via broth microdilution using VITEK 2 (bioMerieux, Marcy-l'Étoile, France) for a panel of antibiotics.

2.2. Urine culture for biosensor assay

Urine samples were diluted 100× in Muller-Hinton broth and inoculated into wells of ciprofloxacin MIC strips (Merlin Diagnostics, Berlin, Germany). The MIC strips comprised one well without antibiotic and five wells containing lyophilized ciprofloxacin for rehydration with 100 μl of sample to final ciprofloxacin concentrations of 0.25, 0.5, 1, 2, and 4 μg/ml. MIC strips were incubated for 5 h at 37 °C and then immediately tested by biosensor assay or frozen at –80 °C for later assay. Previous studies found no difference in biosensor signal between fresh and frozen cultures [7,10].

2.3. Biosensor functionalization

The biosensor chip (GeneFluidics, Irwindale, CA, USA) is composed of 16 individually addressable sensors. Sensors were functionalized with a

Table 1 – Pathogen species and number of urine samples tested using the biosensor array

Uropathogen	Samples tested	Ciprofloxacin suscepibility		
	(*n*)	S	I	R
Escherichia coli	24	12		12
Klebsiella pneumoniae	24	24		
Proteus mirabilis	11	9	1	1
Enterobacter cloacae	6	5	1	
Citrobacter koseri	4	3		1
Citrobacter freundii	4	4		
Enterobacter aerogenes	4	4		
Klebsiella oxytoca	3	3		
Morganella morganii	2	1	1	
Raoultella ornithinolytica	1	1		
Serratia marcescens	1	1		

S = sensitive; I = intermediate; R = resistant.

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