Exploiting the Stress Response: Antibiotic Susceptibility Testing of Urinary Tract Infections in Real-Time

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Abstract

Background. Urinary tract infections (UTIs) are among the most common clinically relevant bacterial infections. However, the management of acute, uncomplicated UTIs has become more challenged over the last decade because of increasing antimicrobial resistance due to the empirical prescription of inappropriate therapies. The ability to provide clinicians with antibiotic susceptibility test results rapidly would enable the prescription of targeted therapies and reduce the spread of antibiotic resistance. A new rapid phenotypic approach to antibiotic susceptibility testing is described that relies on monitoring the stress developed by bacteria in the presence of therapeutic compounds using differential impedance sensing methods as a means to determine the effect of the drugs on the cells. Because the development of bacterial stress is immediate, susceptibility results can be obtained rapidly irrespective of the species growth rate.

Methods. The dielectric permittivity of drug-treated and untreated bacterial suspensions was measured directly in urine using differential impedance methods to monitor the corresponding metabolic changes associated with drug induced stress. Measurements were conducted using two identical 100 µL test volume chambers embedded within a credit-card sized cassette and the respective impedances were continuously recorded by a PC-based data acquisition system.

Results. Impedance response profiles were obtained for eight strains of Gram-positive (*S. saprophyticus, S. aureus, S. mitis, and Enterococcus spp*) and Gram-negative (*E. coli, P. mirabilis, S. marcescens, and Klebsiella pneumoniae*) uropathogens treated with six commonly prescribed antibiotics including strains resistant and susceptible to trimethoprim/sulfamethoxazole and ciprofloxacin. Data were also obtained for the yeast *C. albicans*. All profiles for drug-sensitive pathogens were characterized by an immediate and continuous decrease in value with an intensity that is proportional to the drug concentration. This characteristic profile was independent of the mechanism of action of the drug and recognizable in less than 30 minutes. In contrast, the corresponding profiles for drug-resistant strains showed no measurable impedance response when exposed to the same drug. All impedance test results were well correlated with susceptibility data determined by conventional methods.

Conclusions. The data presented here support our hypothesis that the impedance sensing of the cellular stress response is a powerful, fast, and sensitive tool for screening the susceptibility of uropathogens in real time. These results provide a sound technical foundation for the development of a low-cost practical device that can be used for the antibiotic susceptibility testing of uropathogens at the point-of-care.

Introduction

We present a new, non-molecular approach for determining the antibiotic susceptibility of uropathogens without detecting growth or growth-specific metabolites. Our technical approach is based on the premise that therapeutic agents act as chemical stressors which initiate a physiological stress response in the cell. The intensity of this response is also a measure of the effect of the agent on the cell. Because the development of physiological stress is immediate, antibiotic susceptibility results can be obtained in real-time irrespective of the growth rate of the bacterial cell.

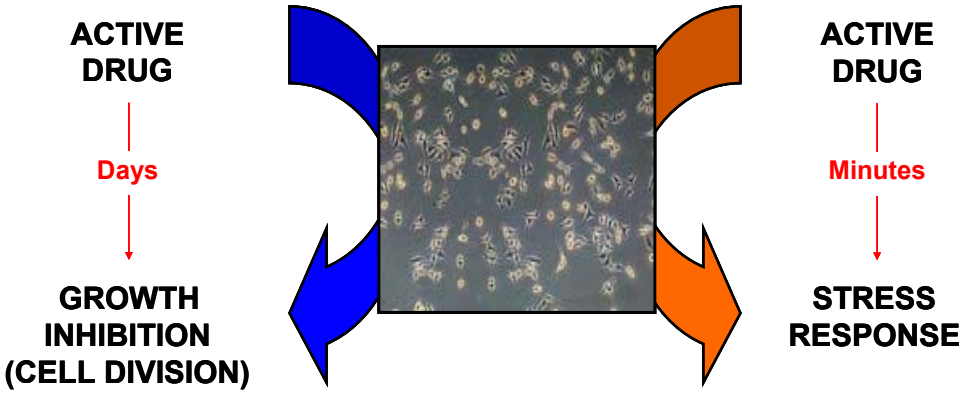


Fig. 1. Two Possible Metabolic Pathways for Measuring the Susceptibility of Uropathogens to Antibiotics.

Detection of the physiological stress response was accomplished by monitoring the dielectric permittivity of a bacterial suspension using differential impedance sensing methods as a means to determine the effects of an antibiotic on a cell.

To demonstrate the advantages of this new method, data are presented for Gram-negative and -positive species treated with three common antibiotics having different mechanisms of action along with one yeast strain treated with both anti-fungal and antibiotic compounds.

Rapid Antibiotic Susceptibility Testing: Stress versus Growth

Assessing the inhibition of bacterial growth in the presence of antibiotics is the conventionally accepted method for determining antibiotic susceptibility. For example, a bacterial strain is judged to be susceptible or resistant to an antibiotic based on the value of the minimum inhibitory concentration (MIC), the amount of drug needed to prevent the replication of the bacterial cells *in vitro*.

An alternative way to characterize the effect of a drug on a bacterial cell is to obtain complementary information that assesses the metabolic status of treated cells directly. For example, an untreated cell maintained under optimal growth conditions will not be stressed. Similarly, the same cell when exposed to an antibiotic to which it is fully resistant will also not be stressed. However when exposed to a lethal concentration of an antibiotic to which it is susceptible, the cell will be highly stressed in its quest to survive.

Methods

The impedance responses from susceptible and resistant clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae,* treated with ciprofloxacin (CIP) and gentamicin (GEN) were measured using the BioSense Z-Sense™ differential impedance sensing platform (Fig. 2). Gram-positive *Staphylococcus saprophyticus* was similarly treated with CIP for comparison. Finally, *Candida albicans* was treated with CIP as well as the anti-fungal fluconazole (FLU) and amphotericin B (AMP) to which it is known to be susceptible.

Fig. 2. Z-Sense™ Differential Impedance Sensing Platform.

All impedance-based susceptibility testing was conducted using following method: One chamber of the Z-Sense™ test cassette was manually filled with a urine-media (1:1 vol/vol) suspension containing either drug-treated or -untreated microorganisms (10^5 CFU/mL; 10^4 CFU/mL for *C. albicans*) while the adjacent reference chamber was filled with untreated cells for direct comparison. The filled cassette was then inserted into the analyzer set at 37ºC and the impedance signals from the two 100 µL test chambers were continuously recorded. The capacitance components of the respective impedance signals were analyzed together to minimize interfering background signals. The resulting Normalized Impedance Response (NIR) profiles continuously register the difference between stressed and unstressed cells.

The bacterial titers were determined at the start and end of each experiment. All experiments were repeated three times (not consecutively). Conventional MICs for all strains were measured using the microbroth method.

Results

The Normalized Impedance Response profiles obtained for all cells susceptible to selected drugs are seen to be qualitatively similar. These profiles are characterized by an immediate and continuous decrease in the NIR values with an intensity that is proportional to the drug concentration used as well as the inherent susceptibility of the cell.

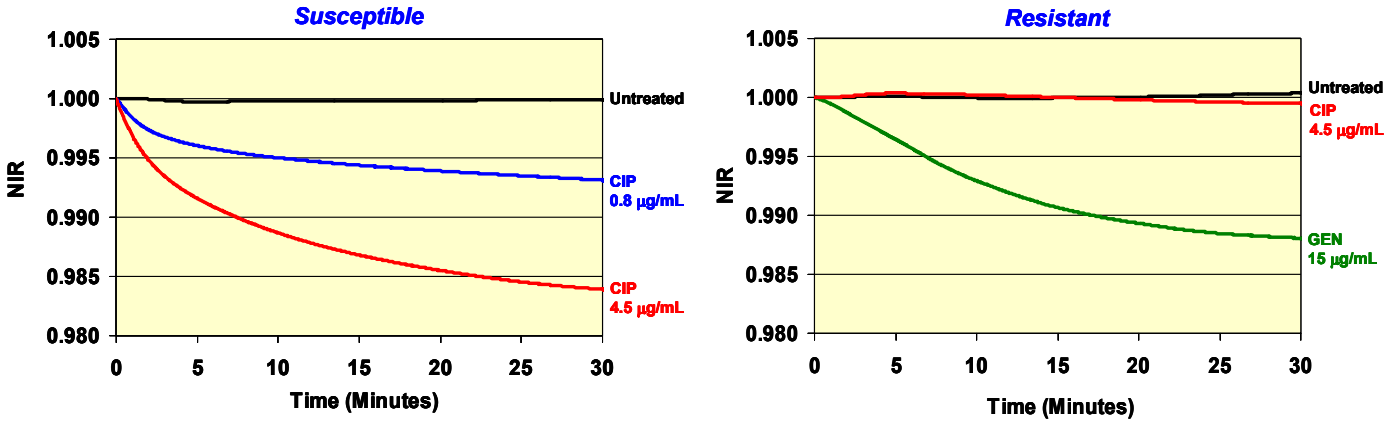
NIR profiles were also obtained for isolates having known resistance to these same drugs. In these measurements, no statistically significant differences between the profiles for the drug-treated and -untreated cells were found, implying the absence of any measurable metabolic response to the drug in resistant cells.

The NIR profile obtained for *C. albicans* in the presence of anti-fungal agents FLU and AMP was consistent with other susceptible profiles while the profile measured for the antibiotic CIP did not deviate from the profile from the untreated cells.

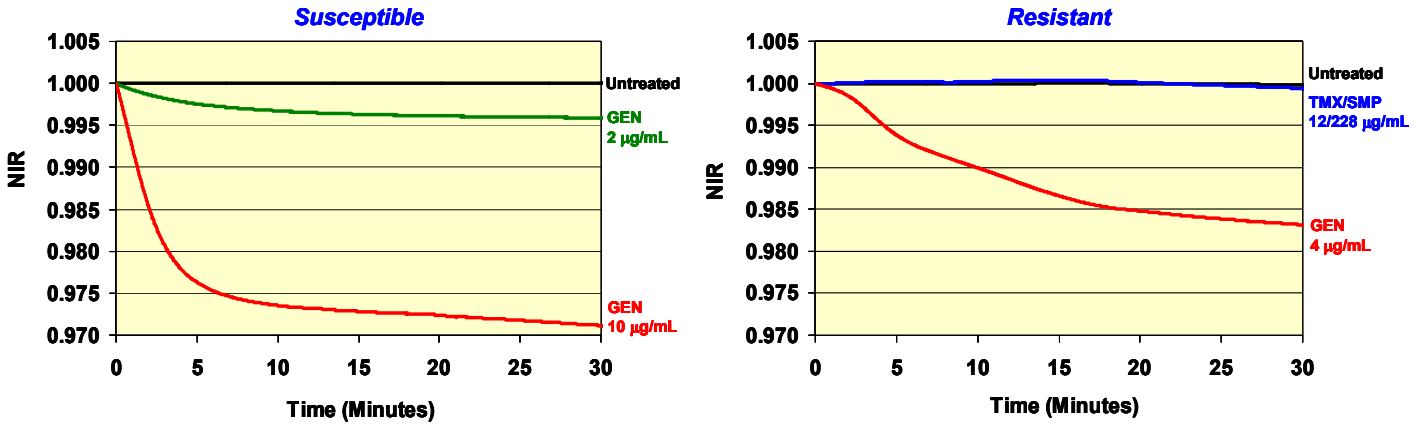
In all cases, significant differences in the NIR profiles for the susceptible cells and drug-resistant cells could be distinguished in 30 minutes irrespective of cell type or the mechanism of action of the drug.

All data obtained with Z-Sense technology were in good agreement with known susceptibilities determined by conventional methods.

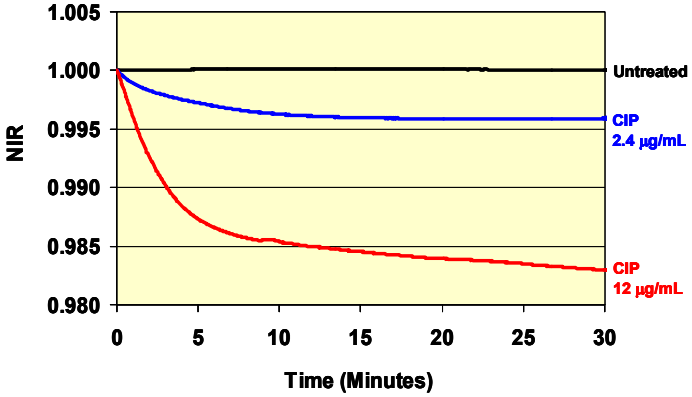
Impedance Response from *E. coli* Treated with CIP



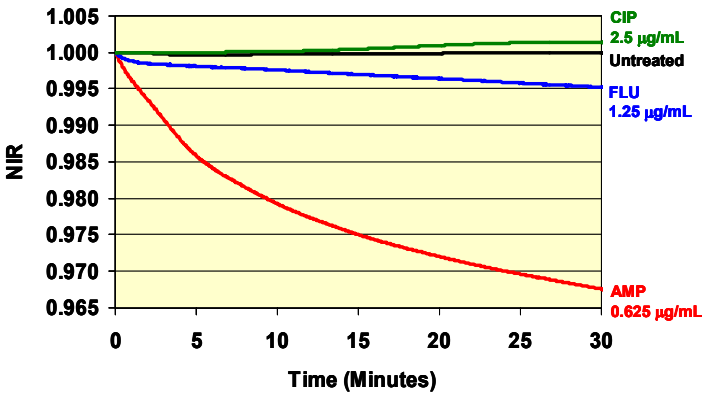
Impedance Response from *K. pneumoniae* Treated with GEN



S. saprophyticus Treated with CIP



C. albicans Treated with FLU and AMP



Conclusions

Drug susceptibility measurements currently require days to obtain results because of the reliance on the growth rate of the cells. This presentation describes an alternative approach that provides commensurate information in real-time by assessing the physiological stress developed by cells in response to a therapeutic agent.

In our studies, we have discovered that a fast, easy, and sensitive way to detect the stress response is to measure changes in the dielectric permittivity of a cellular suspension using differential impedance sensing.

The data presented here show that antimicrobial agents having different mechanisms of action produce a characteristic and concentration-dependent response irrespective of the species. This provides a straightforward means to characterize susceptibility profiles of uropathogens and distinguish between susceptible and resistant cells in real-time.

These results provide a sound technical foundation for the development of a low-cost practical device that can be used for the antibiotic susceptibility testing of uropathogens at the point-of-care.

Acknowledgements

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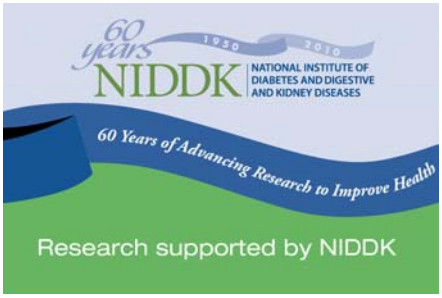

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