Toward Point-of-care Uropathogen Detection Using SERS Active Filters

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ABSTRACT

150 million people worldwide suffer one or more urinary tract infections (UTIs) annually. UTIs are a significant health burden: societal costs of UTI exceed $3.5 billion in the U.S. alone; 5% of sepsis cases arise from a urinary source; and UTIs are a prominent contributor toward antimicrobial resistance (AMR). Current diagnostic frameworks exacerbate this burden by providing inaccurate and delayed diagnosis. Rapid point-of-care bacterial identification will allow for early precision treatment, fundamentally altering the UTI paradigm.

Raman spectroscopy has a proven ability to provide rapid bacterial identification but is limited by weak bacterial signal and a susceptibility to background fluorescence. These limitations may be overcome using surface enhanced Raman spectroscopy (SERS), provided close and consistent application of bacteria to the SERS-active surface can be achieved. Physical filtration provides a means of capturing uropathogens, separating them from the background solution and acting as SERS-active surface.

This work demonstrates that filters can provide a means of aggregating bacteria, thereby allowing subsequent enhancement of the acquired Raman signal using metallic nanoparticles. 60 bacterial suspensions of common uropathogens were vacuum filtered onto commercial polyvinylidene fluoride membrane filters and Raman signals were enhanced by the addition of silver nanoparticles directly onto the filter surface. SERS spectra were acquired using a commercial Raman spectrometer (Ocean Optics, Inc.). Principal Component – Linear Discriminant Analysis provided discrimination of infected from control samples (accuracy: 88.75%, 95% CI: 79.22-94.59%, *p-value* <0.05). Amongst infected samples uropathogens were classified with 80% accuracy.

This study has demonstrated that combining Raman spectroscopy with membrane filtration and SERS can provide identification of infected samples and rapid bacterial classification.

Keywords: Urinary Tract Infection, Point-of-care Diagnostic, Raman, SERS, Membrane Filtration.

1. INTRODUCTION

Urinary tract infection (UTI) is one of the most common bacterial infections in humans. Each year, over 150 million people worldwide suffer from one or more UTIs, leading to a large burden of disease. In the United States alone, UTIs lead to 10.5 million ambulatory visits, of which 23% are emergency department visits, and are responsible for societal costs in excess of $3.5 billion [1]. Progression of UTI can precipitate severe and life-threatening conditions including pyelonephritis and sepsis. Lower urinary tract infection leads to a 4.4 times increased risk of pyelonephritis. Over 10.3% of severe sepsis cases arise from a urinary source, giving rise to a mortality rate of 8% [2]. As the second most common indication for antimicrobial prescriptions UTIs are a major driver for antimicrobial resistance (AMR) and as a result over 17% of *E. coli* urine isolates in the U.S. are multidrug resistant [3].

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The scale of morbidity and mortality caused by UTIs is exacerbated by a diagnostic paradigm that is characterised by inaccuracy and delay. Screening dipsticks, while rapid and low-cost, are inaccurate. Dipsticks' sensitivity of 80.3% results in missed treatment or delayed recognition of 20% of culture positive patients. While a specificity of 53.7% results in overtreatment of nearly half of culture negative patients [3]. Furthermore, the failure of dipsticks to identify the causative pathogen necessitates treatment with broad-spectrum antimicrobials, leading to drug side-effects and AMR [4]. Microscopy, culture and sensitivity (M,C&S) provides gold standard diagnosis with accurate pathogen classification and phenotypic antimicrobial sensitivity testing. However, the inevitable 48-72 hour delay does not allow for this information to be used in the initial treatment decision and exacerbates workflow pressures as antimicrobial changes are needed as results become available [5]. Accurate uropathogen identification at the point of care will allow for timely narrow-spectrum antimicrobial treatment, providing rapid management of UTIs while avoiding the harms caused by overtreatment with broad-spectrum antimicrobials.

Raman spectroscopy uses the inelastic scattering of light to provide immediate chemical analysis. The technique has a demonstrable ability to provide immediate pathogen identification on cultured samples [6-9]. However, the ability to detect bacteria in urine is limited by low bacterial concentrations, relatively weak bacterial signal and high background signal arising from urine fluorescence. Therefore, processing techniques are needed to enhance signal and reduce noise. Surface enhanced Raman spectroscopy (SERS) utilises surface plasmon resonance of closely applied metal nanostructures to provide Raman signal enhancements on the order of 10^{14} [10, 11]. Multiple techniques have been employed to allow bacterial capture and application of SERS, including pre-culture, centrifugation, evaporation, dielectrophoresis, optical tweezers and immunocapture [12-14]. These techniques, however, are time-consuming or labor intensive. Membrane filtration on the other hand is a simple and well-established technique for microbial aggregation and separation from background media [15]. Membrane filtration paired with SERS has been studied as a means for bacterial detection in food hygiene and microbiology [16, 17]. SERS combined with membrane filtration has the potential to capture uropathogens, remove background urine fluorescence and provide an active surface for SERS. Combining these techniques has not been applied to UTI diagnosis but may potentially allow for rapid and accurate uropathogen identification at the point-of-care.

This work explores the potential role of SERS combined with membrane filtration in uropathogen identification and its application to point-of-care UTI diagnosis.

2. EXPERIMENTAL DETAILS

2.1 Bacterial Suspensions

Samples were created by inoculating cultured and washed bacteria into a background solution at controlled concentrations. A research bank including *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC29212 and *Klebsiella pneumoniae* ATCC13883 was established. Bacteria acquired from North West London Pathology Services were cultured on Columbia blood agar at 37 degrees Celsius for 24 hours. Harvested colonies were stored in a Microbank cryopreservation system at -70 degrees Celsius.

In order to prepare a sample, a single bead from the Microbank cryovial was incubated in 3ml of brain-heart infusion (BHI) for 24 hours at 37 degrees Celsius. The cultured bacteria were centrifuged at 13000 rpm for 2 minutes. The resulting supernatant was discarded, and the bacterial pellet resuspended in 0.85% sodium chloride. This process was repeated a total of 5 times in order to wash off the BHI. The absorption at 600 nm was used to dilute the bacterial suspension to a 0.5 McFarland standard, corresponding to a bacterial concentration of 1.5×10^8 colony forming units per milliliter (CFU/ml). This was subsequently serially diluted in the chosen solution to a final concentration of 10^5 CFU/ml. For this work, bacterial suspensions were created through serial dilution of the bacteria in 0.85% sodium chloride.

2.2 Acquisition of Raman Spectra

A Raman spectrum was acquired from each sample using an IDRaman mini 2.0 (Ocean Optics) handheld spectrophotometer. The IDRaman mini 2.0 uses a 785 nm excitation laser with up to 100 mW excitation power. Raman spectra are collected across a range of 400-2300 cm^{-1}, with a spectral resolution of 12 to 14 cm^{-1}. The point-and-shoot mode was used to acquire Raman spectra. A spectrophotometer mounting system was designed (using Fusion360 3D

design software) and 3D-printed to provide consistent Raman spectral acquisition (Figure 1), and spectra were acquired using Oceanview software (Ocean Optics).

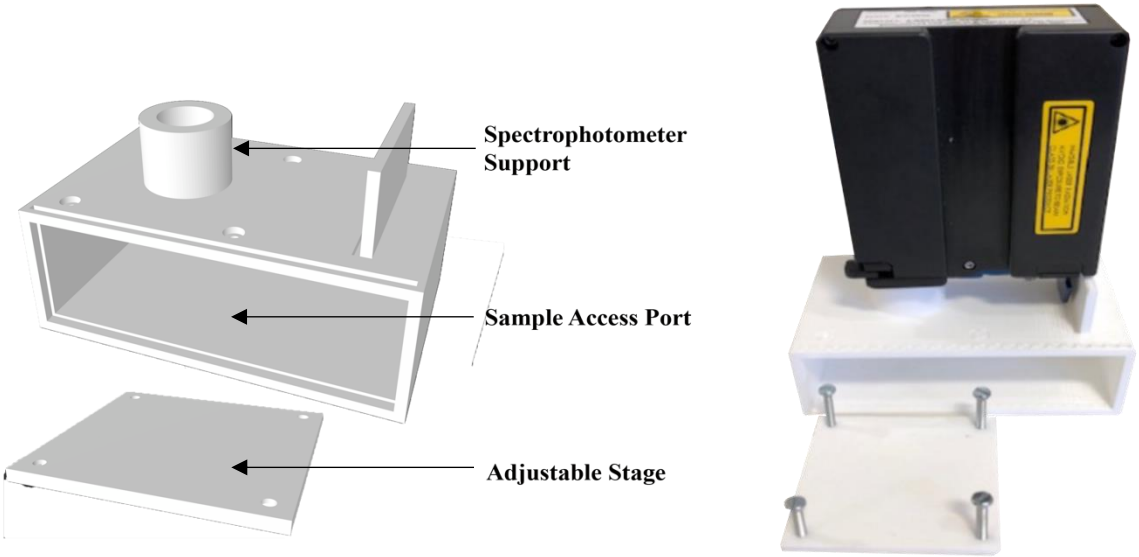

Figure 1. The designed (left) and printed (right) spectrophotometer mounting system. The 3-part system comprised: a holder with specifications made to fit the IDRaman mini without allowing in ambient light (spectrophotometer support); a moveable stage to hold samples at a controlled and consistent focal distance (adjustable stage); and a slot for a sliding door to provide access while avoiding ambient light (sample access port).

2.3 Membrane Filter Selection

Membrane filters were used provide a dual role of bacterial capture in addition to providing a surface for SERS. 0.45 μm pore size membrane filters paired with a vacuum filtration system provide bacterial capture [15]. Commercially available membrane filters of differing materials were assessed to identify the filter with the lowest background signal. Raman spectra were acquired from cellulose ester, polysulphone, nylon and polyvinylidene fluoride (PVDF) membrane filters. Spectra from 5 of each filter type were acquired with a 20 second acquisition time. The mean spectra with confidence intervals were plotted to demonstrate the baseline spectrum and background variability.

2.4 Digital preprocessing

All digital preprocessing and spectral analysis were performed using in-house scripts developed in R software. Background subtraction was performed through iterative restricted least squares subtraction on spectra truncated from 750 to 1750 cm^{-1}. The baseline-corrected spectra were vector normalized to improve comparability by dividing each spectrum by its hypergeometric Euclidean distance.

2.5 Spectral Analysis

The mean spectra were plotted for uninfected (control) samples and for the different uropathogens. Sample classification was performed with Principal Component – Linear Discriminant Analysis (PC-LDA). Principal component analysis (PCA) was used for dimensionality reduction (i.e. feature extraction) and the extracted features were then subjected to supervised classification using Linear Discriminant Analysis (LDA). PC-LDA was performed at two levels: samples were initially differentiated as infected versus uninfected; subsequently, positive samples were subjected to pathogen classification.

2.6 Bacterial Identification with SERS Nanoparticles

Demonstration of proof of principle was approached through bacterial capture using PVDF filters and signal enhancement with silver nanoparticles. A total of 60 bacterial suspensions were assessed, including 20 each of *E. coli*, *E. faecalis* and *K. pneumoniae*, and compared against 20 control samples without inoculated bacteria.

Bacterial suspensions were created according to section 2.1. Bacteria cultured in BHI and washed in 0.85% sodium chloride underwent serial dilution to create 30 ml of 10^5 CFU/ml bacterial suspensions. The samples underwent vacuum filtration on 47 mm PVDF filters for bacterial capture, before pipetting on 200 µl of 40 nm silver nanoparticles (Sigma-Aldrich).

Raman spectra were acquired from the membrane filters as described in section 2.2 (placing the filters within the sample access port of the handheld spectrophotometer mount) using 120 second acquisition times. The spectra were preprocessed before classification using PC-LDA.

3. RESULTS & DISCUSSION

3.1 Selection of Membrane filters

The mean spectra and corresponding confidence intervals were plotted in order to identify the filter type with the lowest background signal and variability (Figure 2). Cellulose Ester, nylon and polysulphone filters exhibited many spectral peaks with high baseline variability in keeping with their respective complex chemical structures. PVDF filters demonstrated a simple baseline with little variability, and as such were selected for further experiments.

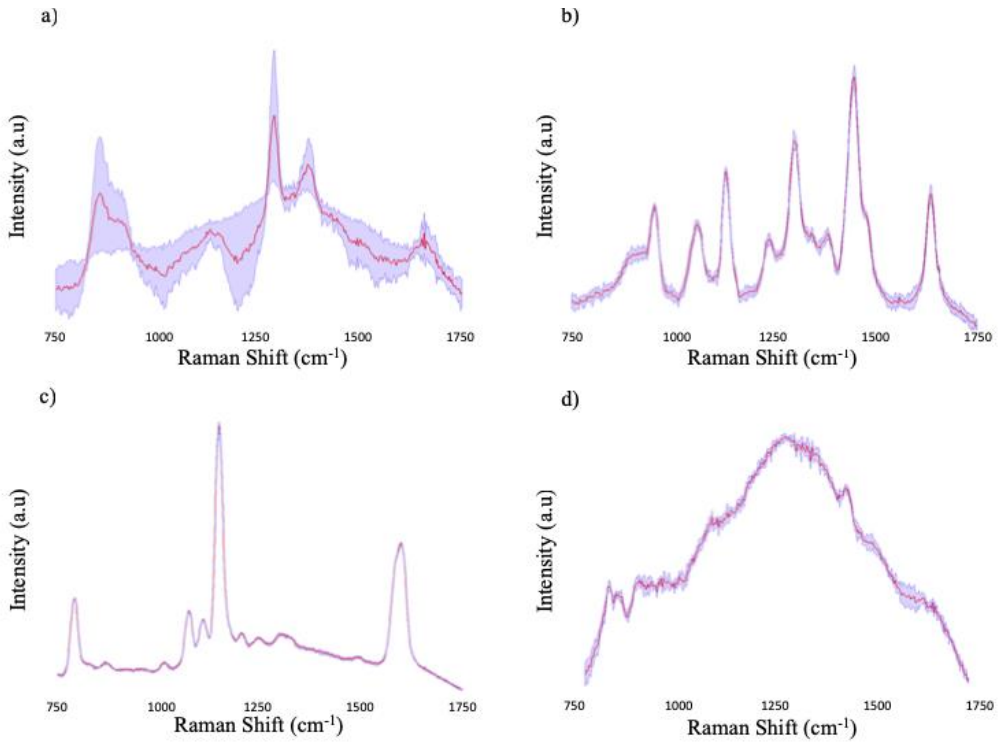

Figure 2. Raman spectra with confidence intervals (shaded area) for a) cellulose ester, b) nylon, c) polysulphone and d) polyvinylidene fluoride membrane filters. Polyvinylidene fluoride filters were selected for further experiments as they exhibit a simple background with little variability.

3.2 SERS Nanoparticle proof or principle

The Raman spectra of uropathogens captured on PVDF filters and enhanced using silver nanoparticles are presented in Figure 3. The spectra exhibited good signal to noise ratios with a range of clearly distinguishable peaks. PC-LDA was applied to the data to investigate the diagnostic potential. While the spectral shapes appear very similar across all samples, PC-LDA was able to differentiate infected samples from uninfected samples with an accuracy of 88.75% (CI:

79.22-94.59%, *p-value* = 0.04) and with a sensitivity and specificity for bacteria detection of 96.67% and 65% respectively (see Table 1).

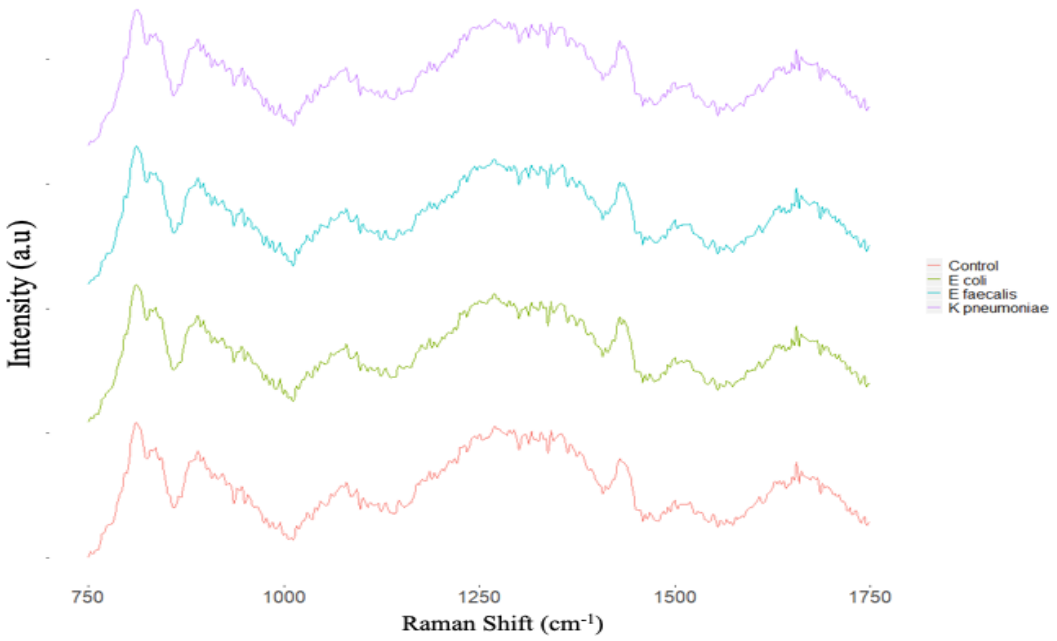

Figure 3. Mean Raman spectra for Controls, *E. coli*, *E. faecalis* and *K. pneumoniae* captured on PVDF filters with surface enhancement provided using silver nanoparticles that were pipetted onto the filter surface.

The spectra for positive samples were submitted for bacterial classification through PC-LDA. While there was clear overlap in the distribution of the 1st and 2nd linear discriminants (Figure 4), it was still possible to classify the uropathogens with an accuracy of 80% (95% CI: 67.17-89.02%, *p-value* <0.05). This corresponded to sensitivity and specificity values both equal to 80%, and the sample classification is shown in Table 1.

Bacterial identification at clinically relevant concentrations demonstrates proof of principle that bacterial capture and Raman signal enhancement was achieved using simple, commercially available materials. Furthermore, with sample processing achieved in less than 3 minutes, bacterial identification is potentially achievable at the point of care. Despite this, the classification accuracy of the positive samples is limited (sensitivity 96.67%, specificity 65%, accuracy 80%) and may be insufficient for clinical decision making. Interestingly, however, the sample classification is likely negatively affected by variable porosity of the membrane filters and variable dispersion of the pipetted silver nanoparticles, which will potentially allow for improvements in bacterial identification in the future.

Furthermore, while demonstrating capture of bacterial Raman signal is potentially possible at the point of care, these methods will require refinement prior to inclusion in point-of-care testing as technical methods such as vacuum filtration and nanoparticle pipetting would be impractical in a clinical setting. Additionally, there are further limitations that will need to be overcome in future studies. The use of reference strain bacteria and controlled bacterial loads simplifies sample classification, while suspension in saline solution reduces background noise and variability therein artificially enhancing signal detection. Thus, current work now aims to progress this technique towards improving bacterial classification accuracy and to provide protocols that better translate to real clinical environments.

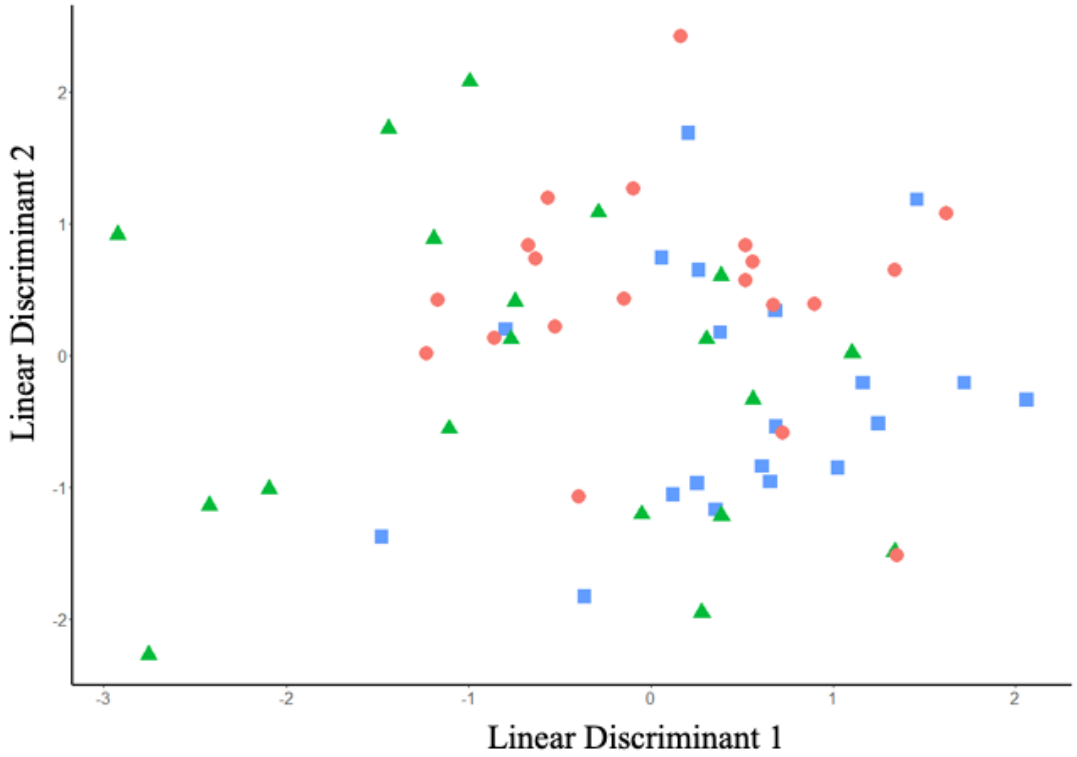

Figure 4. Results of PC-LDA performed on the *E. coli* (●), *E. faecalis* (▲) and *K. pneumoniae* (■) Raman spectra. The data demonstrates that bacterial capture using PVDF filters and signal enhancement based on pipetted nanoparticles provides a degree of bacterial identification but is insufficient for accurate classification.

Table 1. PC-LDA results for classification of infected vs. uninfected samples (top) and for identification of bacteria in positive samples (bottom).

Infected vs. Uninfected		
	Actual	
Predicted	*Uninfected (control)*	*Infected*
Uninfected (control)	13	2
Infected	7	58
Accuracy: 88.75% (CI: 79.22-94.59%, *p-value* = 0.04), sensitivity: 96.67%, specificity: 65%		

Classification of positive samples			
	Actual		
Predicted	*E. coli*	*E. faecalis*	*K. pneumoniae*
E. coli	16	2	2
E. faecalis	2	16	2
K. pneumoniae	2	2	16
Accuracy: 80% (CI: 67.17-89.02%, *p-value* <0.05), sensitivity: 80%, specificity: 80%			

4. CONCLUSION

This work demonstrates that SERS combined with membrane filtration can provide rapid identification and classification of bacteria relevant to UTI. While improvements in diagnostic accuracy and the sensitivity and specificity of bacterial identification are required in order to provide clinical utility, this work demonstrates the potential for future Raman-based diagnostics, as it was achieved using a handheld spectrometer and commercially available reagents and materials. Future work will now focus on further developing this filtration-based method to provide improved diagnostic accuracies and protocols/tools that are suitable for clinical use.

5. ACKNOWLEDGEMENTS

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