Screening A Clinical Cohort For Urinary Tract Infections Directly From Specimen via Lipidomics

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1. SUMMARY

Fast and accurate detection of uropathogens is essential for the management of urinary tract infections. Here, we present our effort to detect urinary tract infections in a clinical cohort from Vancouver Island Health Authority hospitals by analyzing urine lipidomic profiles directly without culture by mass spectrometric analysis. Preliminary results shows the fast detection of clinically significant pathogens such as *E. coli*, *K. pneumoniae P. aeruginosa* and polymicrobial samples.

2. BACKGROUND

- Urinary tract infection (UTI) is one of the most common causes of sepsis, accounting for up to 40% of cases. Current methods require 2-3 days for pathogen identification in clinical laboratories.
- Increasingly, protein analysis techniques are becoming standard detection methods, replacing phenotypic characterization and microscopic visualization methods. These techniques are reliable but are often labor-intensive, significantly increasing the cost and burden of diagnostic laboratory support as well as requiring culturing to a pure colony.
- Lipids are essential components of microbial cell membranes, forming the interface between the microbial cell and its environment. These lipids can readily be extracted and visualized by mass spectrometry (MS) with their structural differences resulting in unique mass spectrometric profile (Fig 1).

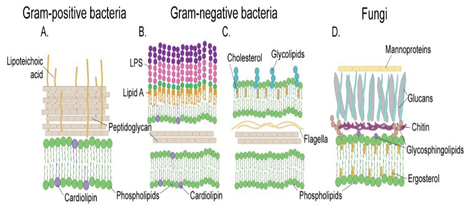
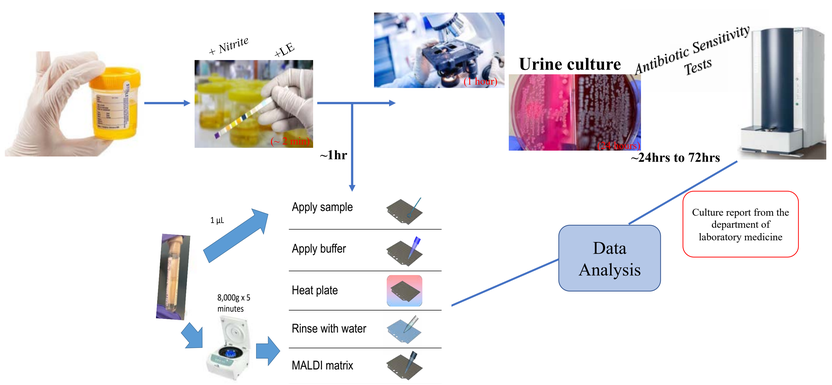


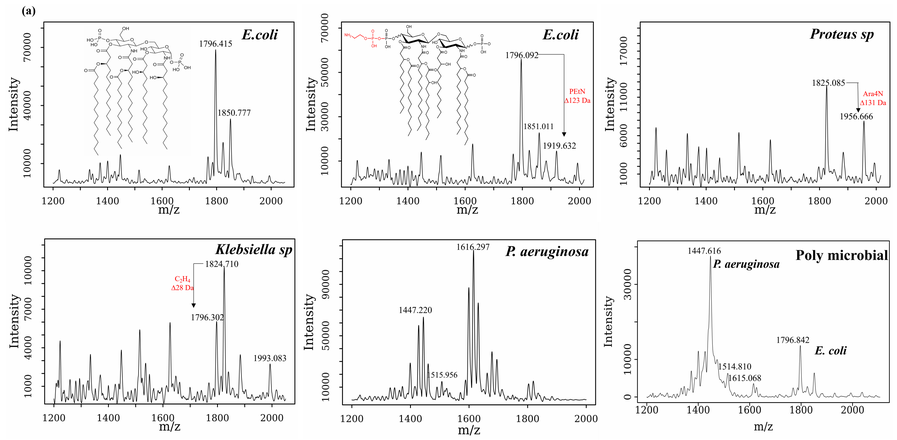
Fig 1: Structure and key features of bacterial cell membrane

3. STUDY DESIGN AND METHODS

- Our lab recently developed the Fast Lipid Analysis Technique (FLAT), a rapid method for direct lipid extraction on a MALDI plate (Sorenson et. al., 2020. *Sci Rep*)
- Briefly, 1 µL of urine was spotted on a standard MFX µFocus MALDI plate. Acidified and incubated in a humidified chamber at 110°C for 30 min. The plate was rinsed and 1 µL of Norharmane matrix (10mg/mL) was added to each spot. Samples were then analyzed using a Bruker Microflex in negative ion mode and automated laser operation
- For Gram-positive optimization, the normal FLAT workflow was used with slight modification where samples were sonicated for 5 min before FLAT.



4. RESULTS



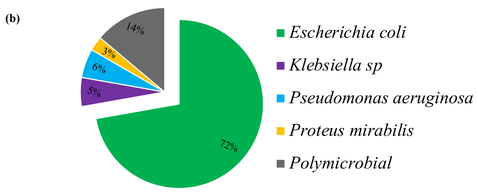


Fig 2: (a) Representative FLAT spectra of detected lipid A from Gram negative uro-pathogens, (b) Pie chart showing detected uro-pathogens.

- An initial cohort of 302 urine samples showed FLAT identified clinically several significant pathogens predominantly *E. coli* and polymicrobial directly from 1 µL of urine (Fig 2a &b).
- Overall, FLAT data produced a sensitivity of 94% and specificity of 99% with positive and negative predictive value of 93 and 99%, respectively for Gram-negatives.
- Two patients were found to harbor a mobile colistin resistance (mcr) gene as noted by chemical modifications to their lipid A by phosphoenthanolamine (Fig 2a).

- Approximately 40% of the samples showed an ion at 1446 *m/z* (Fig 3b), which is reported to be the signature ion for *P. aeruginosa* lipid A (Fig 3a). However, these samples containing this 1446 ion by FLAT failed to produce viable colonies when isolated on a culture plate. Tandem MS results of the 1446 ion confirmed it was a most likely a host cardiolipin (Kim et al. 2011, *Lipid Research*) (Fig 3c).
- Furthermore, FLAT detected an ion at 1230 *m/z* present in all urine samples with blood (Fig 4a). Tandem MS results of this ion compared against a plasma standard analyzed by FLAT confirmed it was a heme dimer (Fig 4c).

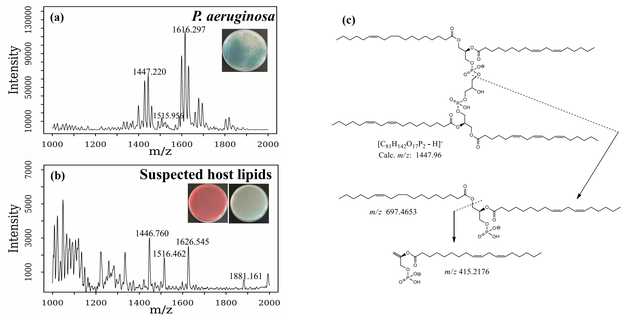
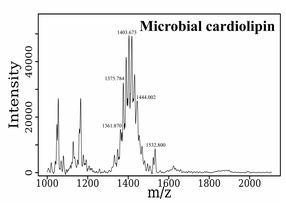


Fig 3: FLAT spectra showing clinical interferences (a) Lipid A spectra of *P. aeruginosa* (b) Suspected host cardiolipin (c) Predicted structure of 1447 ion (heme dimer)



- After adding an extra step of sonication before FLAT to optimize Gram negative cardiolipin, 8 out of 9 urine samples showed cardiolipin signal which is indicative of the presence of Gram-positive pathogen (Fig 5).

Fig 5: Representative FLAT spectra of detected Gram positive cardiolipin after modification (sonication before FLAT)

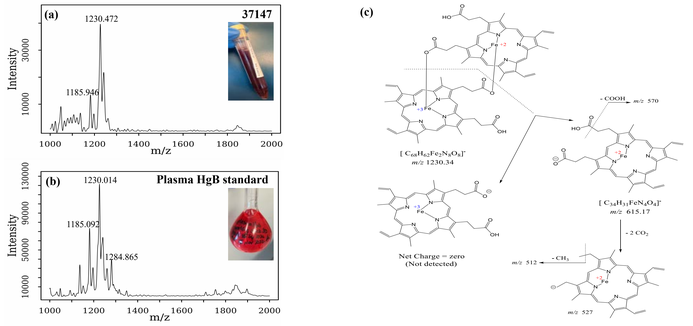


Fig 4: FLAT spectra of clinical interferences (a) Spectrum of patient urine with blood (b) Spectrum of plasma HgB standard (c) Predicted structure of 1230 ion from tandem MS on MALDI-tims-TOF MS via FLATN (Yang et al., 2022. *Anal Chem)*

REFERENCES

- Leung et al., 2017. *Sci. Rep*. 7, 1–10. https://doi.org/10.1038/s41598-017-04793-4
- Zamyatina Alla, 2018. *Beilstein J. Org. Chem.* 2018, *14,* 25–53. https://doi.org/10.3762/bjoc.14.3
- Sorenson et. al., 2020. *Sci Rep* 10, 21536. https://doi.org/10.1038/s41598-020-78401-3
- Yang et al., 2022. *Anal Chem. https://doi.org*/10.1021/acs.analchem.1c05394
- Kim et al., 2011. *Journal of Lipid Research*, 2011. _https://doi.org/ 10.1194/jlr.M010520

5. CONCLUSION

- MS-based phenotypic profiling of membrane lipids can provide accurate identification of Gram-negative and positive bacteria in both mono- and poly-microbial infected UTI samples directly from specimen same day of collection by FLAT without culture..
- FLAT could be used as a rapid lipid-based microbial assay for clinical screening of UTIs.
- This assay does not only identify bacteria rapidly direct from specimen but also detects membrane related antibiotics resistance such as colistin and contamination with blood.
- This lipidomic assay identifies bacteria rapidly (< 1 hr) direct from specimen and as such can be used improve antimicrobial stewardship potentially making this an essential tool in the clinical laboratory.

6. FUTURE STUDY

- To validate FLAT on other biofluids such as bronchoalveolar fluid (BAL), and Cerebrospinal fluid (CSF)
- To increase the sample size for Gram-positive bacterial detection
- To identify fungal pathogens in biological fluids

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