An LC-MS screening platform to efficiently detect clinically relevant antibiotics in cholera patient urine and stool

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Introduction

The transmission and pathophysiology of *Vibrio cholerae* is likely impacted by antibiotic consumption. However, low-cost, high-throughput techniques to detect clinically relevant antibiotics in cholera samples are not available. Analytical testing is important because self-reporting of antibiotics is often unreliable. Therefore, the primary goal of this work is to develop and validate a new method to meet this need. Secondary applications include the capacity to control for the confounding effect of antibiotics on bacterial diversity studies, lytic and lysogenic phage, and clinical outcomes research. Herein, we conducted a study to detect clinically relevant antibiotics via LC/MS, in stool and urine samples collected from cholera patients during a 2015 outbreak in Bangladesh.

Method

Instrumentation

Screening for 14 antibiotics and 3 common non-antibiotic medications (Table 1), in urine and stool samples collected from cholera patients, was performed by a unified LC/MS method using an 1100 series HPLC (Agilent Technologies) integrated with an LTQ XL ion trap mass spectrometer (Thermo Fisher Scientific). Mass spectrometry utilized heated electrospray ionization (HESI) in positive mode. MS acquisition used full and data dependent scanning to enhance identification of parent compounds and their known metabolites. Liquid chromatography was performed on a 2.1 x 150 mm Hypersil Gold aQ column, with gradient elution using acetonitrile/water/0.1% formic acid mobile phase; flow rate was 0.25 mL/min; run time was 20 minutes.

Sample Preparation

The simplicity of urine samples allowed for a “dilute and shoot” approach; however stool sample complexity required additional purification. Urine samples were centrifuged after sonication and an aliquot from each sample was diluted with methanol/0.1% formic acid (1:1). Stool samples were filtered, sonicated, and extracted with methanol. Following protein precipitation, samples were centrifuged; supernatant was diluted prior to injection in the same manner as urine.

Sample Analysis

Representative extracted ion chromatograms of a 17-component artificial mixture are presented in Figure 1. A total of 29 urine samples were investigated for possible antibiotic intervention prior to diagnosis in clinic (Figure 2). Due to differences in excretion patterns, the spectra were analyzed for both parent and known metabolites (1-3) . Commonly detected medications were as follows (detection frequency from high to low): metronidazole, ciprofloxacin, Zofran, azithromycin, ceftriaxone, paracetamol, tetracycline, erythromycin and amoxicillin.

Results and Discussion

Figure 1. Parent ion extracted ion chromatograms (EIC) of artificial mixture of 17 clinically relevant medications in cholera patients. Panel A: EIC of components 1 through 9; Panel B: EIC of components 10 through 17.

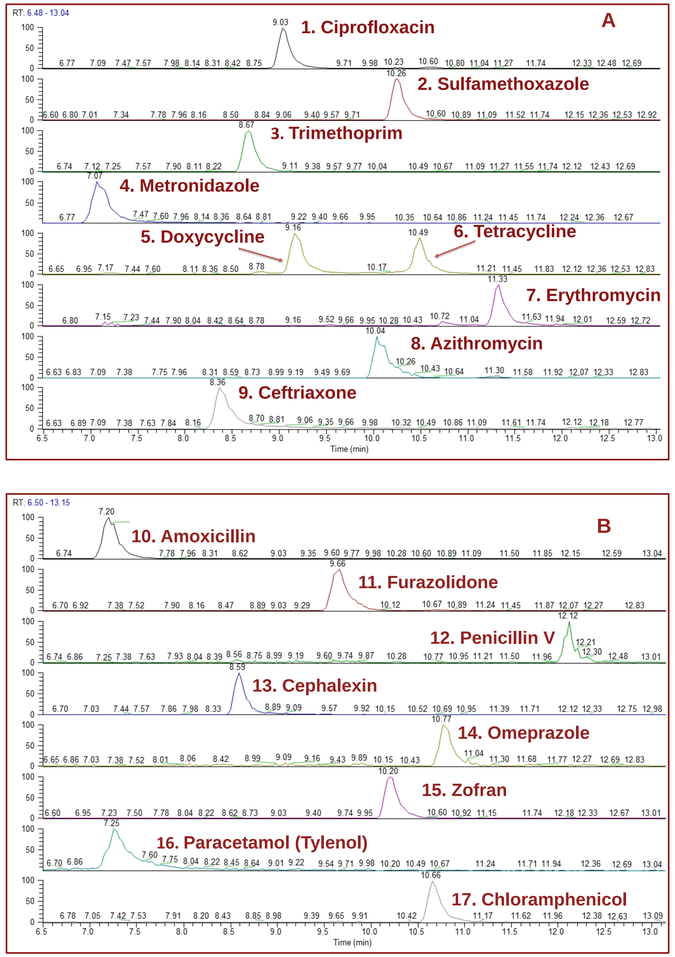
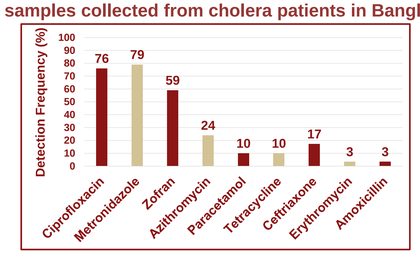


Table 1. Structures and identification numbers for 17 clinically relevant medications in cholera treatment

1. Ciprofloxacin	2. Sulfamethoxazole
3. Trimethoprim	4. Metronidazole
5. Doxycycline	6. Tetracycline
7. Erythromycin	8. Azithromycin
9. Ceftriaxone	
10. Amoxicillin	11. Furazolidone
12. Penicillin V	13. Cephalexin
14. Omeprazole	15. Zofran
16. Paracetamol (Tylenol)	17. Chloramphenicol

Figure 2. Detection frequency (%) of 17 medications in 29 urine samples collected from cholera patients in Bangladesh



Conclusion

- Sample preparation and LC/MS conditions have been established for analysis of 17 medications of diverse structure, in urine and stool samples collected from cholera patients.
- Urine samples provided higher sensitivity for parent compounds and their metabolites, due to lower matrix effect and no need for the extraction procedure.
- Recoveries of parent compounds in stool samples after filtration and extraction were estimated to be within 50%.
- 10 matching urine and stool samples were compared for detection consistency: among commonly detected components, ceftriaxone was detected only in urine samples.
- While stool sample collection is necessary for cholera diagnosis, urine samples are easier to collect and more amenable to LC/MS antibiotic screening.
- Medications were detected in all 29 urine samples collected from cholera patients; within this group, only 3 patients reported taking medications.
- Future studies to investigate the impact of antibiotics on the gut microbiome of cholera patients are under consideration.

References

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