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Rapid identification of *mcr*-1-positive *Escherichia coli* from patient urine using a novel lipid-based MALDI-TOF-MS assay

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Abstract

Mobilized colistin resistance (*mcr*) genes confer resistance to colistin, a last-resort antibiotic for multidrug-resistant Gram-negative infections. In this case report, we describe a novel lipid-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) diagnostic used to rapidly identify an *mcr-1*-positive *Escherichia coli* directly from a patient with a urinary tract infection without the need for *ex vivo* growth.

INTRODUCTION

The plasmid-mediated colistin resistance gene (*mcr*) confers resistance to colistin (polymyxin E), a last-resort antibiotic used to treat multidrug-resistant Gram-negative infections [1]. An *mcr* (*mcr-1*)-positive *Escherichia coli* was initially identified in PR China in 2015 during a routine surveillance project on antimicrobial resistance in food animals [2]. Since then, *mcr* genes have been identified from human samples globally (*mcr* 1–10) and in 2016 *mcr-1* was first identified in the USA from an *E. coli* isolate cultured from a urine sample collected from a female patient in Pennsylvania [3].

The *mcr* gene resistance mechanism is via plasmid-mediated expression of the lipid A modification enzyme, phosphatidylethanolamine transferase. This enzyme transfers a phosphoethanolamine (PEtN) to one of the terminal phosphate moieties of lipid A, the membrane anchor of lipopolysaccharide (LPS)-reducing membrane electronegativity and the binding affinity of colistin [4]. Previous studies have demonstrated the ability to identify the addition of a PEtN and other colistin resistance markers using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) [5–8].

Plasmid-mediated *mcr* genes can be transferred horizontally to different bacterial species [9], and because of this, *mcr* gene identification has rapidly increased in prevalence [10]. Therefore, accurate detection and global surveillance is necessary. Currently, the identification of colistin-resistant pathogens from urine requires culture, identification via MALDI-TOF MS, antimicrobial susceptibility testing (AST) and polymerase chain reaction (PCR) for gene identification. This process can take 48 to 72 h [11]. Using a novel lipid-based MALDI-TOF MS that allows rapid detection of colistin resistance direct from patient samples without the need for *ex vivo* expansion is paramount for improving surveillance efforts. In this case report, we identify an *mcr-1*-positive *E. coli* directly from urine using MALDI-TOF MS, which was subsequently confirmed by culture and PCR.

CASE PRESENTATION

An adult female patient attended an outpatient medical centre for a routine follow-up appointment for a diagnosis of *Helicobacter pylori*-associated gastritis. The diagnosis was made at a clinic appointment 3 months prior, where she presented with nausea and was treated with pantoprazole and metronidazole. However, she did not complete the course of antibiotics due to complaints of side-effects, including metallic taste, vaginal itching and dysuria. The dysuria persisted until the follow-up appointment 3 months after the initial appointment. At the follow-up appointment, she also presented with frequency and urgency. The patient's past medical history consists of chronic obstructive pulmonary disorder (COPD) and a

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Abbreviations: AST, antimicrobial susceptibility testing; FLAT, fast lipid analysis technology; LPS, lipopolysaccharide; MALDI-TOF MS, matrix assisted laser desorption ionization-time of flight mass spectrometry; mcr, mobilized colistin resistance gene; MIC, minimum inhibitory concentration; PCR, polymerase chain reaction; PEtN, phosphoethanolamine.
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Table 1. Antimicrobial susceptibility testing results including MIC and MIC interpretations for all antibiotics tested

Antibiotic	MIC (µg ml^{-1})	MIC interpretation
Colistin (polymyxin E)	32	Resistant
Tetracyclines	16	Resistant
Sulfamethoxazole/ trimethoprim	320	Resistant
Ampicillin/sulbactam	16	Intermediate
Amikacin	2	Sensitive
Aztreonam	1	Sensitive
Cefazolin	4	Sensitive
Cefepime	1	Sensitive
Ceftriaxone	1	Sensitive
Ertapenem	0.5	Sensitive
Gentamicin	1	Sensitive
Levofloxacin	0.12	Sensitive
Meropenem	0.25	Sensitive
Nitrofurantoin	16	Sensitive
Piperacillin/tazobactam	4	Sensitive
Tigecycline	0.5	Sensitive

known history of drug abuse, which was being managed with methadone. Six months prior, the patient visited the outpatient clinic and tested positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which developed into viral pneumonia.

Due to the incidental urinary symptoms, the patient was assessed for a urinary tract infection (UTI). With no significant findings on a physical examination, a urine sample was collected from the patient for a point-of-care urine dip test. The dip test was negative for nitrates, blood, or protein, but highlighted a small amount of leukocyte esterase. This implies a small increase in white blood cell count detected in the urine, and thus potentially a UTI. Thereafter, a clinical diagnosis was made of acute cystitis without haematuria and the patient was prescribed a 7-day course of ciprofloxacin to complete, 250 mg twice a day. The urine collection was cultured to determine bacterial load and individual colonies were selected for pathogen identification using Vitek 2 MS (bioMérieux, Inc., Durham, NC, USA). The pathogen was identified as *E. coli* at a concentration of greater than 100000 c.f.u. ml^{-1}, confirming diagnosis of a UTI. Subsequently, AST was conducted and reported 2 days after collection. AST revealed resistance to tetracyclines and sulfamethoxazole/trimethoprim and intermediate resistance to ampicillin. Sensitive minimum inhibitory concentrations (MICs) were shown for amikacin, aztreonam, cefazolin, cefepime, ceftriaxone, ertapenem, gentamicin, levofloxacin, meropenem, nitrofurantoin, piperacillin and tigecycline (Table 1).

Finally, at a subsequent follow-up, the patient informed her providers that she started the ciprofloxacin course 2 days late due to confusion regarding its use, and then subsequently stopped the antibiotics early due to further side-effects of vaginal bleeding and rash. She presented with the feeling of 'hot' urine, but no urinary symptoms of dysuria, frequency, or urgency. A repeat urine dip and culture showed no signs of nitrates, blood or leukocytes, and a borderline finding of 40000 c.f.u. ml^{-1} of haemolytic streptococci. As this does not surpass the diagnostic threshold, and she had no clinical symptoms, she was deemed to not have a secondary UTI.

The patient's urine sample was collected for further analysis using a novel rapid lipid extraction protocol that allows for diagnosis directly from clinical samples, such as blood and urine cultures, without the need for growth by MALDI-TOF MS. This method is termed fast lipid analysis technology (FLAT) [12]. Briefly, 1 µl of urine, without concentrating, was spotted on a MALDI plate and overlaid with 1 µl of buffer, consisting of 0.2 M anhydrous citric acid and 0.1 M trisodium citrate dihydrate. The MALDI plate was incubated at 110 °C for 30 min to extract membrane lipids and rinsed with endotoxin-free water to neutralize the pH and remove cell debris. After drying, 1 µl of 10 mg ml^{-1} norharmane matrix suspended in 2:1 chloroform–methanol was spotted onto the extracted lipid sample on a MALDI plate. Subsequently, the sample was analysed using a Bruker Microflex LRF MALDI-TOF MS (Bruker Daltonics, Billerica, MA, USA) in the negative ion and linear mode. The time to results after the sample was collected, including FLAT and MS analysis, was under 1 h [12].

Spectra of lipid analysis by MS showed ion species at *m/z* 1797 and *m/z* 1920, which is consistent with *E. coli* and addition of a PEtN (Fig. 1) [5]. The addition of PEtN suggested the presence of an *mcr* gene. PCR was conducted for confirmation. Results from PCR showed that the *E. coli* was positive for *mcr-1*, confirming the results from MS analysis. AST was conducted for colistin using the broth dilution method published in the Clinical and Laboratory Standards Institute (CLSI) M07 methods [13]. Via broth microdilution, the MIC for colistin was determined to be 32 µg ml^{-1}, indicative of colistin resistance (resistance >2 µg ml^{-1}).

DISCUSSION

MALDI-TOF MS is a rapid, easy-to-use, readily available technology in most clinical microbiology settings, making it an ideal technology for pathogen identification [14]. Our novel lipid extraction method, FLAT, allows for rapid, direct-from-clinical-sample identification of colistin resistance and *mcr* genes using a MALDI-TOF MS platform in under 1 h. Typical identification of colistin resistance requires culture and AST, which can take 24–72 h after sample collection [15]. Furthermore, several challenges and limitations exist for colistin AST [16]. The rapid and accurate nature of the lipid-based MALDI-TOF MS assay make it a strong tool for initial screening and potential global surveillance of colistin resistance.

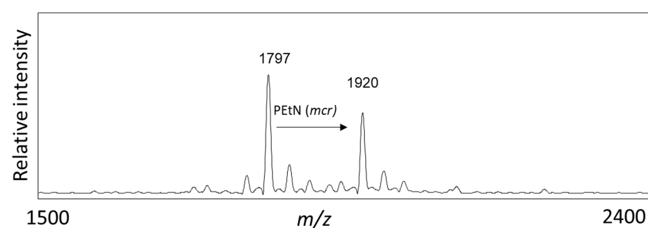

Fig. 1. MALDI mass spectrum of *mcr-1*-positive *E. coli* directly from urine. The *m/z* of 1797 is the known marker for *E. coli*, while the *m/z* of 1920 (Δ*m/z* 123) represents the addition of a phosphoethanalomine (PEtN), signifying the presence of an *mcr* gene.

Globally, *mcr* genes have been universally isolated with the vast majority of *mcr* genes being found in China and Southeast Asia [10]. In the USA *mcr* genes are much rarer [10]. The majority of *mcr* genes have been isolated from the environment and livestock, with human isolates comprising <5% of all isolated *mcr* genes [17]. To date, there have been 10 different homologous *mcr* genes (*mcr-1–10*) discovered, with the most prominent being *mcr-1* [18].

Due to nephrotoxicity and other negative side-effects associated with colistin use, treatment of infection with colistin is reserved for cases where no other antibiotic is clinically viable due to multidrug resistance [19]. The increasing prevalence of extended-spectrum beta-lactamase (ESBL)-producing bacteria and carbapenem-resistant *Enterobacteriaceae* has created a higher demand for colistin use. However, with increasing use of colistin, colistin resistance has increased, and thereby untreatable, pan-resistant bacterial infections have emerged [20], leaving few to no treatment options, leading to untreatable infections in patients. In this case report, the infection was treatable without use of colistin as AST showed susceptibility to several different antibiotics. Despite being treatable, the ability to transfer horizontally makes the presence of an *mcr* gene problematic. Poor antimicrobial stewardship, less than optimal prescribed antimicrobial treatment, and failure to take fully prescribed courses of antibiotics allow for the continuous spread of resistant genes, creating a major public health concern.

The current standard methods for identification of *mcr* genes are sequencing and PCR [21]. Unlike our lipid-based MALDI-TOF MS assay, these molecular methods require culture and thus their time to results is much longer. Other limitations of molecular methods include the need for more resources that may not be readily available, such as primers, increased cost, less reproducibility, and higher likelihood of contamination. The simple, rapid nature of a lipid-based MALDI-TOF MS assay will allow for a much-improved surveillance method for *mcr* genes. A major strength of sequencing over MALDI-TOF MS is the ability to differentiate *mcr* genes or identify novel genes or strains. However, a rapid test, direct from sample, is critical to improve patient treatment and outcomes.

CONCLUSION

The *mcr* genes confer resistance to colistin, an antibiotic used to treat multidrug-resistant Gram-negative bacteria when no other treatment options are viable. With the increasing prevalence of *mcr* genes and antibiotic resistance, the identification and surveillance of colistin resistance is paramount in improving public health. The ability of *mcr* genes to spread via horizontal gene transfer is especially problematic. Patients with sensitive antibiograms can continue to transmit *mcr* genes to other highly resistant organisms. In this case, although the infection did not require colistin treatment, the patient's record of not completing antibiotic treatment allowed for improper treatment of infection and continuing spread of the *mcr* gene to potentially highly resistant organisms. Thus, this case highlights the need for proper surveillance and treatment. In this case report, we demonstrate that our novel lipid-based MALDI-TOF MS assay provides an improved direct-from-sample surveillance method for *mcr* gene identification, potentially improving public health outcomes.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
A waiver of consent was granted by the University of Maryland Medical Center Internal Review Board (HP-00064919). Potential identifiers were removed as per suggestion by the editor.

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