AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Accelerates Pathogen Identification and May Confer Benefit in the Outcome of Peritoneal Dialysis-Related Peritonitis

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Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) and conventional standard methods were compared for time to pathogen identification and impact on clinical outcomes in peritoneal dialysis-related peritonitis patients. The MALDI-TOF MS method identified the causative microorganisms earlier (average time saved, 64 h for all pathogens), and patients had a shorter hospital stay (mean ± standard deviation, 5.2 ± 4.8 days versus 8.2 ± 4.5 days, $P = 0.001$).

Peritonitis is one of the major complications of peritoneal dialysis (PD) and contributes to technique failure and mortality in PD patients (1, 2). If there is no clinical improvement in PD-related peritonitis (PDRP) by 5 days on appropriate antibiotic therapy, catheter removal is recommended (3). Therefore, early identification of the causative pathogens in PDRP is important for the administration of appropriate antibiotics in a timely manner to improve outcomes. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has been recognized as a fast and reliable method of microorganism identification (4, 5). We conducted a retrospective study to investigate the effects of MALDI-TOF MS on pathogen identification and clinical outcomes in PDRP.

The diagnosis of PDRP was in accordance with the diagnostic criteria recommended by the International Society for Peritoneal Dialysis (3). Two empirical antibiotic regimens were applied: cefazolin with gentamicin for anuric patients (i.e., those with urine output of <100 ml/day) and cefazolin with ceftazidime for nonanuric patients. Patients were hospitalized due to more severe constitutional symptoms (i.e., fever with chills, increasing abdominal pain, refractory vomiting, diarrhea or ileus, or difficult enteral nutrition), delayed response to antimicrobial therapy, ultrafiltration failure, or hemodynamic instability. This study was approved by the institutional review board of the National Cheng Kung University Hospital and adhered to the Declaration of Helsinki.

We investigated the time to pathogen identification (TPI) in PDRP using different methods at the National Cheng Kung University Hospital and Chi-Mei Medical Center. From July 2010 to June 2013, 98 causative microorganisms of monomicrobial PDRP were identified using conventional standard methods and the Vitek or API identification system (bioMérieux, Marcy l'Etoile, France). From January 2013 to September 2014, 57 causative microorganisms of monomicrobial PDRP were identified using the MALDI-TOF MS method. The TPI was defined as the time elapsed between the time samples were received at the laboratory and the time of the positive culture report.

A volume of 10 ml of dialysate effluent (DE) from PD patients with peritonitis was inoculated into Bactec aerobic and anaerobic bottles and then incubated in a Bactec 9240 system or Bactec FX system (Becton Dickinson, Sparks, MD). Culture bottles flagged as positive were removed from the data units and processed for microbiological identification using the conventional standard method or MALDI-TOF MS method. MALDI-TOF MS-based microorganism identification was performed by the MALDI-TOF Biotyper RTC and the Bruker MALDI Biotyper 3.1 software and library (4,613 isolates; Bruker Daltonics).

The main outcome variables of PDRP were evaluated, including time to improvement of peritonitis (DE white blood cells [WBC] count, <100/mm^3 after an overnight dwell), length of hospital stay, catheter removal/transfer to hemodialysis, and in-hospital mortality. Ten patients whose hospital stay was >60 days were excluded, as serious complications rather than peritonitis *per se* may have prolonged the hospital stay and affected the outcome. A P value of <0.05 was considered to be statistically significant. All

Received 29 December 2015 **Returned for modification** 23 January 2016
Accepted 20 February 2016

Accepted manuscript posted online 24 February 2016

Citation Lin W-H, Hwang J-C, Tseng C-C, Chang Y-T, Wu A-B, Yan J-J, Wu J-J, Wang M-C. 2016. Matrix-assisted laser desorption ionization–time of flight mass spectrometry accelerates pathogen identification and may confer benefit in the outcome of peritoneal dialysis-related peritonitis. J Clin Microbiol 54:1381–1383. doi:10.1128/JCM.03378-15.

Editor: C.-A. D. Burnham

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TABLE 1 Causative microorganisms of peritoneal dialysis-related peritonitis identified using the conventional standard method and MALDI-TOF MS ($n = 155$)

Pathogen	No. identified using:		Total no. (%)[a]
	Conventional standard method ($n = 98$)	MALDI-TOF MS method ($n = 57$)	
Gram positive	60	32	92 (59)
Staphylococcus aureus	9	6	15 (10)
Coagulase-negative staphylococci	24	8	32 (21)
Viridans streptococci	14	9	23 (15)
Streptococcus pneumoniae	2	0	2 (1)
Other *Streptococcus* spp.[b]	7	8	15 (10)
Gram-positive bacilli	3	0	3 (2)
Enterococcus spp.	1	1	2 (1)
Gram negative	35	24	59 (38)
Escherichia coli	14	12	26 (17)
Klebsiella pneumoniae	4	3	7 (5)
Other *Klebsiella* sp.[c]	1	0	1 (1)
Proteus sp.	0	1	1 (1)
Pseudomonas aeruginosa	0	1	1 (1)
Other *Pseudomonas* spp.	3	0	3 (2)
Acinetobacter baumannii	4	0	4 (3)
Citrobacter koseri	1	1	2 (1)
Enterobacter cloacae	1	2	3 (2)
Other *Enterobacter* sp.	1	0	1 (1)
Serratia marcescens	4	1	5 (3)
Haemophilus influenzae	1	0	1 (1)
Burkholderia spp.	0	2	2 (1)
Pasteurella multocida	0	1	1 (1)
Fusobacterium sp.	1	0	1 (1)
Other	3	1	4 (3)
Candida spp.	2	1	3 (2)
Mycobacterium tuberculosis	1	0	1 (1)

[a] Percentages are presented as rounded values.
[b] *Streptococcus* spp. other than viridans streptococci or *S. pneumoniae*.
[c] *Klebsiella* spp. other than *K. pneumoniae*.

TABLE 2 Time to pathogen identification in peritoneal dialysis-related peritonitis using the conventional standard method and MALDI-TOF MS

Pathogen	No. of pathogens identified; time to pathogen identification (mean ± SD) (h) using:		*P* value (difference [h])
	Conventional standard method	MALDI-TOF MS method	
All pathogens	98; 135 ± 55	57; 71 ± 37	<0.001 (64)
Gram positive[a]	60; 129 ± 26	32; 77 ± 47	<0.001 (52)
Gram negative[b]	35; 130 ± 32	24; 65 ± 17	<0.001 (65)
Staphylococcus spp.	33; 127 ± 27	14; 90 ± 67	<0.001 (37)
Streptococcus spp.	23; 132 ± 24	17; 64 ± 18	<0.001 (68)
Escherichia coli and *Klebsiella* spp.	19; 130 ± 25	15; 63 ± 15	<0.001 (67)
Gram-negative pathogens other than *E. coli* and *Klebsiella* spp.	16; 131 ± 39	9; 69 ± 21	<0.001 (64)

[a] Includes *Streptococcus* spp. (viridans streptococci, *S. pneumoniae*, and other *Streptococcus* spp.) and *Staphylococcus* spp. (*S. aureus* and coagulase-negative staphylococci).
[b] *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., *Acinetobacter baumannii*, *Citrobacter koseri*, *Enterobacter* spp., *Serratia marcescens*, *Haemophilus influenza*, *Burkholderia* spp., *Pasteurella multocida*, and *Fusobacterium* species.

statistical analyses were performed using the JMP software (SAS Institute, Inc., Cary, NC, USA).

There were 116 patients (48% male) with 155 episodes of PDRP; 98 isolates were identified using a conventional standard method, and 57 isolates were identified to the species level using the MALDI-TOF MS method. There were no significant differences in patient characteristics and comorbidities between the two groups. MALDI-TOF MS allowed the direct pathogen identification from positive culture bottles of DE, accounting for 94.9% of all cases (96.6% of Gram-positive and 91.7% of Gram-negative bacteria, respectively). The causative microorganisms of PDRP are shown in Table 1. Coagulase-negative staphylococci and *Escherichia coli* are the most common pathogens for Gram-positive and Gram-negative bacteria, respectively.

The comparison of TPI between the conventional standard method and the MALDI-TOF MS method is shown in Table 2. MALDI-TOF MS identified the causative microorganisms of PDRP earlier than the conventional standard method. The average time saved was 64 h for all pathogens, 52 h for Gram-positive bacteria, and 65 h for Gram-negative bacteria. There was no significant difference in the time taken for the blood culture bottles to flag positive from laboratory receipt for all pathogens between the two study groups.

The outcomes of PDRP are shown in Table 3. For patients with improvement of peritonitis, the time to WBC count of $<100/mm^3$ was not different between the two groups. For patients needing hospitalization and who had subsequent improvement of peritonitis, the length of hospital stay was longer in the conventional standard method group (overall mean ± standard deviation [SD], 8.2 ± 4.5 days versus 5.2 ± 4.8 days, $P = 0.001$; *Streptococcus* species-related mean ± SD, 8.3 ± 3.6 days versus 3.8 ± 3.2 days, $P = 0.005$).

To the best of our knowledge, this is the first study to demonstrate the clinical effects of integration of MALDI-TOF MS with an automated blood culture system into the process of pathogen identification in PDRP. The results show that the MALDI-TOF MS method can identify the causative microorganisms much earlier than the conventional standard method. The average time saved was 37 to 68 h for different pathogens. Because of the low colony counts (0 to 10^4 CFU/ml) of the infecting microorganisms in the DE of PDRP (6, 7), a large-volume culture (e.g., culturing the sediment after centrifuging 50 to 100 ml of DE) can increase the bacterial number inoculated into a blood culture bottle and further shorten the incubation time, contributing to early pathogen identification.

Early pathogen identification in PDRP using MALDI-TOF MS was associated with shorter hospital stays. However, there was no significant benefit in the time to improvement of peritonitis or catheter removal/transfer to hemodialysis, which may largely depend on the choice of initial empirical antimicrobial regimen. MALDI-TOF MS alone does not reduce the time required for antimicrobial susceptibility testing, and the subsequent antibiotic regimen should be guided by the antibiotic susceptibility profile (3, 8).

In conclusion, we demonstrate here the clinical effect of pathogen identification using MALDI-TOF MS in PDRP. The integra-

TABLE 3 Outcomes of patients with peritoneal dialysis-related peritonitis using the conventional standard method and MALDI-TOF MS

Outcome characteristic	Conventional standard method	MALDI-TOF MS method	*P* value
Overall no. ($n = 145$)	96	49	
No. (%) with improvement	73 (76)	39 (80)	0.63
Time to WBC count <100/mm^3 (mean ± SD) (days)	6.1 ± 3.0	6.7 ± 3.8	0.37
No. (%) with hospitalization management	46 (63)	21 (54)	0.42
Time to WBC count <100/mm^3 (mean ± SD) (days)	6.7 ± 3.4	6.8 ± 4.3	0.64
Length of hospital stay (mean ± SD) (days)	8.2 ± 4.5	5.2 ± 4.8	0.001
No. (%) with no improvement	23 (24)	10 (20)	0.63
Transfer to hemodialysis	23 (100)	9 (90)	0.30
In-hospital mortality	1 (4.4)	1 (10)	0.52
Length of hospital stay (mean ± SD) (days)	26.1 ± 13.5	19.5 ± 17.1	0.17
Those with improvement			
No. with *Staphylococcus* spp. ($n = 42$)	29	13	
Time to WBC count <100/mm^3 (mean ± SD) (days)	5.6 ± 2.3	9.4 ± 8.6	0.03
No. (%) with hospitalization management	12 (41)	7 (54)	0.34
Time to WBC count <100/mm^3 (mean ± SD) (days)	5.9 ± 3.0	7.3 ± 3.1	0.35
Length of hospital stay (mean ± SD) (days)	7.3 ± 3.4	5.4 ± 4.4	0.30
No. with *Streptococcus* spp. ($n = 38$)	22	16	
Time to WBC count <100/mm^3 (mean ± SD) (days)	5.9 ± 2.3	5.9 ± 3.1	0.97
No. (%) with hospitalization management	20 (91)	8 (50)	0.008
Time to WBC count <100/mm^3 (mean ± SD) (days)	6.1 ± 2.4	5.5 ± 2.6	0.59
Length of hospital stay (mean ± SD) (days)	8.3 ± 3.6	3.8 ± 3.2	0.005
No. with *E. coli* and *Klebsiella* spp. ($n = 20$)	10	10	
Time to WBC count <100/mm^3 (mean ± SD) (days)	5.3 ± 1.3	6.6 ± 4.1	0.35
No. (%) with hospitalization management	8 (80)	6 (60)	0.63
Time to WBC count <100/mm^3 (mean ± SD) (days)	5.6 ± 1.2	6.7 ± 5.2	0.58
Length of hospital stay (mean ± SD) (days)	5.8 ± 1.3	4.7 ± 2.7	0.34

tion of MALDI-TOF MS with direct identification from a positive blood culture bottle can promote early pathogen identification and timely pathogen-directed antibiotic therapy and may confer a benefit in patient outcomes.

ACKNOWLEDGMENTS

This work was supported by grants from the Ministry of Science and Technology (102-2314-B-006-015).

We thank Robert M. Jonas (Texas Lutheran University) for helpful comments on the manuscript.

We declare no conflicts of interest.

FUNDING INFORMATION

This work, including the efforts of Ming-Cheng Wang, was funded by Ministry of Science and Technology, Taiwan (MOST) (102-2314-B-006-015).

REFERENCES

1. **Fried LF, Bernardini J, Johnston JR, Piraino B.** 1996. Peritonitis influences mortality in peritoneal dialysis patients. J Am Soc Nephrol **7:**2176–2182.
2. **Boudville N, Kemp A, Clayton P, Lim W, Badve SV, Hawley CM, McDonald SP, Wiggins KJ, Bannister KM, Brown FG, Johnson DW.** 2012. Recent peritonitis associates with mortality among patients treated with peritoneal dialysis. J Am Soc Nephrol **23:**1398–1405. http://dx.doi.org/10.1681/ASN.2011121135.
3. **Li PK, Szeto CC, Piraino B, Bernardini J, Figueiredo AE, Gupta A, Johnson DW, Kuijper EJ, Lye WC, Salzer W, Schaefer F, Struijk DG, International Society for Peritoneal Dialysis.** 2010. Peritoneal dialysis-related infections recommendations: 2010 update. Perit Dial Int **30:**393–423. http://dx.doi.org/10.3747/pdi.2010.00049.
4. **Kothari A, Morgan M, Haake DA.** 2014. Emerging technologies for rapid identification of bloodstream pathogens. Clin Infect Dis **59:**272–278. http://dx.doi.org/10.1093/cid/ciu292.
5. **Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, Raoult D.** 2009. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis **49:**543–551. http://dx.doi.org/10.1086/600885.
6. **Ludlam HA, Price TN, Berry AJ, Phillips I.** 1988. Laboratory diagnosis of peritonitis in patients on continuous ambulatory peritoneal dialysis. J Clin Microbiol **26:**1757–1762.
7. **Fenton P.** 1982. Laboratory diagnosis of peritonitis in patients undergoing continuous ambulatory peritoneal dialysis. J Clin Pathol **35:**1181–1184. http://dx.doi.org/10.1136/jcp.35.11.1181.
8. **Cho Y, Johnson DW.** 2014. Peritoneal dialysis-related peritonitis: towards improving evidence, practices, and outcomes. Am J Kidney Dis **64:**278–289. http://dx.doi.org/10.1053/j.ajkd.2014.02.025.