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Diagnosis of bacterial pathogens in the dialysate of peritoneal dialysis patients with peritonitis using surface-enhanced Raman spectroscopy

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ABSTRACT

Background: Bacterial peritonitis is the most common cause of peritoneal dialysis (PD) therapy drop-out. A quick and accurate diagnosis of the bacterial pathogen can reduce the PD drop-out rate. Surface-enhanced Raman spectroscopy (SERS) can rapidly identify bacteria using chips coated with nano-sized metal particles.
Methods: Known bacteria were loaded in the SERS-chips and illuminated with laser light to establish a reference Raman spectra library. Dialysate from PD peritonitis patients was concentrated by centrifuge and examined with the same SERS, and the resulting Raman spectra were compared with library spectra for bacteria identification. Principal component analysis was used for further confirmation. The same batches of dialysate were sent to routine culture as a reference bacteria identification method. The results of the 2 identification methods were compared.
Results: A total of 43 paired-samples were sent for study. There were 37 samples with bacteria identified but 6 were culture-negative by the reference method. 31 bacteria were identified in paired-samples by SERS, among which, 29 bacteria were exactly the same as those identified by the reference method. Bacteria not included in the reference library spectra cannot be identified.
Conclusions: SERS techniques can rapidly identify bacterial pathogens in the dialysate of PD peritonitis patients.
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1. Introduction

Peritonitis is the most common cause of patient drop-out from peritoneal dialysis (PD) therapy. Infection, especially bacterial peritonitis, is the major pathogenic cause of PD peritonitis [1]. Patients with PD peritonitis are characterized by abdominal pain and turbid dialysate. Severe PD peritonitis may be associated with fever, septic shock or patient mortality rate. Early diagnosis of bacterial pathogens and accurate antibiotic usage benefit microbial eradication and patient cure [2]. In current clinical practice, dialysate from PD peritonitis is sent for bacterial culture, and empirical antibiotics are then administered. Antibiotics chosen will be adjusted according to culture results [2]. It frequently takes 24 h or more for bacterial identification and even longer to report the antibiotic susceptibility test results [3]. A new method mandates quicker bacteria identification.

Raman spectroscopy is a spectroscopic technique that is frequently used to detect the vibrational energy of molecules [4]. To obtain weak Raman scattering, a laser light with wavelength ranging from ultraviolet to near infra-red region is employed. The emitted scatterings from molecules after excited by the laser are collected by a lens and passed through a monochromator. The elastic radiation, or radiation at the corresponding wavelength of laser light, is filtered out by a filter and the remaining of collected light or inelastic radiation is dispersed on a detector and turned into a specific Raman spectrum [5]. The Raman shifts are defined as the wavelength difference between incident and scattered light in the Raman spectrum. Molecules can be identified through their specific fingerprint spectra. However, the signal of spontaneous Raman scattering is very weak and not easily discerned. The Raman signal can be augmented by absorbing molecules on the surface

Abbreviations: MRSAq, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; PD, peritoneal dialysis; SERS, surface-enhanced Raman spectroscopy.

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of Raman substrates, frequently nanosized metal such as gold or silver [6]. Surface enhanced Raman spectroscopy (SERS) can increase Raman scattering intensity by a factor of 10^{10} to 10^{11} [7]. SERS techniques can be applied in bacterial identification [8–10]. It is a culture-free detection method for the quick diagnosis of bacterial infection in body fluids [11]. To detect bacteria in a fluid sample, we frequently need to evaporate water from the aqueous sample to allow the contact of bacteria with the SERS substrate. The presence of water in the sample can cause uncertain spreading of aqueous samples on the SERS substrate and result in problems of reproducibility [12]. Recently, a sensitive cylindrical SERS substrate array was developed. The cylindrical SERS chip allows spontaneous contact of the specimen with SERS substrate and increases the sensitivity and reproducibility of detection [13].

In the current study we used cylindrical SERS substrate array to identify bacteria in the dialysate of PD peritonitis. The cylindrical SERS was fabricated by decorating silver nanoparticles on the tip of 2-mm diameter polymethylmethacrylate (PMMA) rod. The minimum sample volume for one analysis is small and can be <5 μl. Also SERS spectra can be acquired without drying the samples [13].

2. Methods

2.1. Patients

PD patients with abdominal pain and turbid dialysate in China Medical University Hospital from June 2014 to May 2015 were recruited for study. We retrieved 40 ml of dialysate from patients with PD peritonitis. 10 ml of the dialysate was sent to dialysate routine examinations, including white blood cell (WBC) count, red blood cell count, and differential count. These examinations were performed manually by hospital bacterial laboratory technicians. We included only dialysate with WBC over 100/μl and neutrophil count over 50% of the WBC count according to PD peritonitis criteria [14]. Peritonitis patients who used antibiotics before dialysate sampling were excluded. Dialysate retrieval from patients was performed after getting patient informed consent. The study followed the regulation of the institutional review board.

2.2. Reference bacterial culture

Ten milliliters of dialysate was inoculated into aerobic (BD BACTEC Plus Aerobic/F) and another 10 ml into anaerobic (BD BACTEC Plus anaerobic/F) blood culture vials (BD) separately as reference culture [15]. These vials were sent to a hospital laboratory for bacterial identification. These vials were incubated in Phoenix Automated Microbiology System (BD) for microbial identification following the manufacturer`s manual [16]. Reference cultures were used as positive control of the study.

2.3. Establishment of standard Raman bacteria spectra library

Bacteria commonly seen in PD peritonitis were purchased from ATCC (American Type Culture Collection) such as methicillin-sensitive *Staphylococcus aureus* (MSSA, ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), Group A Streptococcus (GAS, ATCC 19615), *Escherichia coli* (*E. coli*; ATCC 25922), *Enterococcus faecalis* (ATCC 29212), *Klebsiella pneumoniae* (ATCC 70063), *Pseudomonas aeruginosa* (ATCC 27853) and *Acinetobacter baumannii* (ATCC 10591). Other bacteria were isolated from hospital patients such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus salivarius*, *Streptococcus oralis*, *Corynebacterium striatum*, and *Enterococcus faecium*. These samples were used as standard bacteria to establish a reference Raman spectra library. The bacteria were grown in culture plates. A sterile culture loop was used to fetch the bacteria and dissolve them in 10 ml distilled water. To avoid contaminating the bacterial wall with culture material, the bacteria solution was centrifuged at 700 rpm for 10 min separate bacteria from the WBCs, immune-cells and cellular debris. The precipitated bacteria was then washed with 10 ml distilled water and centrifuged again. The resulting bacterial precipitate was dissolved in distilled water and adjusted to a concentration of 5 McFarland (MCF) (3×10^9 CFU/ml) [17], which was then further diluted by distilled water to different concentrations. 3 μl of dialysate was loaded into a cylindrical Raman SERS-chip (Labguide Co.) [13]. The SERS-chip was illuminated with laser light and detected by a Raman spectrometer (QE Pro, Ocean). The laser wavelength was 785 nm with a laser power of 20 mW. The integration time was 5 s. The resulting spectra were analyzed and served as the standard reference spectra library.

2.4. Bacteria identification using SERS-chips

Ten milliliters of dialysate from peritonitis patients was centrifuged at 700 rpm for 10 min to separate bacteria from the WBCs, immune-cells, and cellular debris. The supernatant, containing target pathogens, was then mixed with 10 ml distilled water and centrifuged at 13,000 rpm for another 10 min to concentrate the bacterial samples. The precipitate was subsequently diluted with distilled water to an optical density of 5 MacFarland (MCF) [17]. Concentrated dialysate (3 μl) was loaded onto a Raman SERS-chip. The SERS-chip was illuminated with a Raman spectrometer. The resulting spectrum was then compared to spectra from the reference spectra library using RM.View software (Ocean) to identify possible bacteria in the sample.

2.5. Principal component analysis

The Raman spectra (400-2000 cm^{-1}) of reference bacteria and dialysate bacteria after RM.View software analysis were further analyzed by principal component analysis (PCA) using SPSS ver 22 [18]. Dots of PCA from dialysate bacteria that co-localized with dots of reference bacteria were deemed dots from the same type of bacteria.

2.6. Antibiotic susceptibility test

To determine the antibiotic susceptibility of bacteria, we added antibiotics to bacteria to observe any bacteria-specific Raman shift changes. Oxacillin and vancomycin were added to MSSA and MRSA and incubated for 6 to 24 h. Bacteria after antibiotic treatment were loaded onto the SERS chips and the resulting Raman spectra were compared to that of bacteria without antibiotic treatment. Other bacteria were also tested by different kinds of antibiotics. The concentrations of antibiotics used were higher than or equal to the minimal inhibitory concentration (MIC) [19].

2.7. Statistical analysis

Positive and negative predictive values were calculated using the culture results from hospital laboratory as reference.

3. Results

3.1. The reference Raman spectra from known bacteria

The bacteria from ATCC or hospital patients were washed with distilled water and prepared as MCF = 5.0 (1×) or 1.5×10^9 CFU/ml. Solutions were then diluted with distilled water at 10-fold dilution (10×), 100-fold dilution (100×), 1000-fold dilution (1000×) or 10,000-fold dilution (10,000×). A representative Raman shift of *E. coli* without dilution (1×) was shown in Fig. 1A (left). Raman shift spectra from serial dilutions of *E. coli* were merged and shown in Fig. 1A (right). Raman shifts of different *E. coli* concentrations revealed the same fingerprints. The representative spectra of MSSA (1×) and serial dilutions were shown in Fig. 1B. Similarly, Raman spectra of MRSA without and with

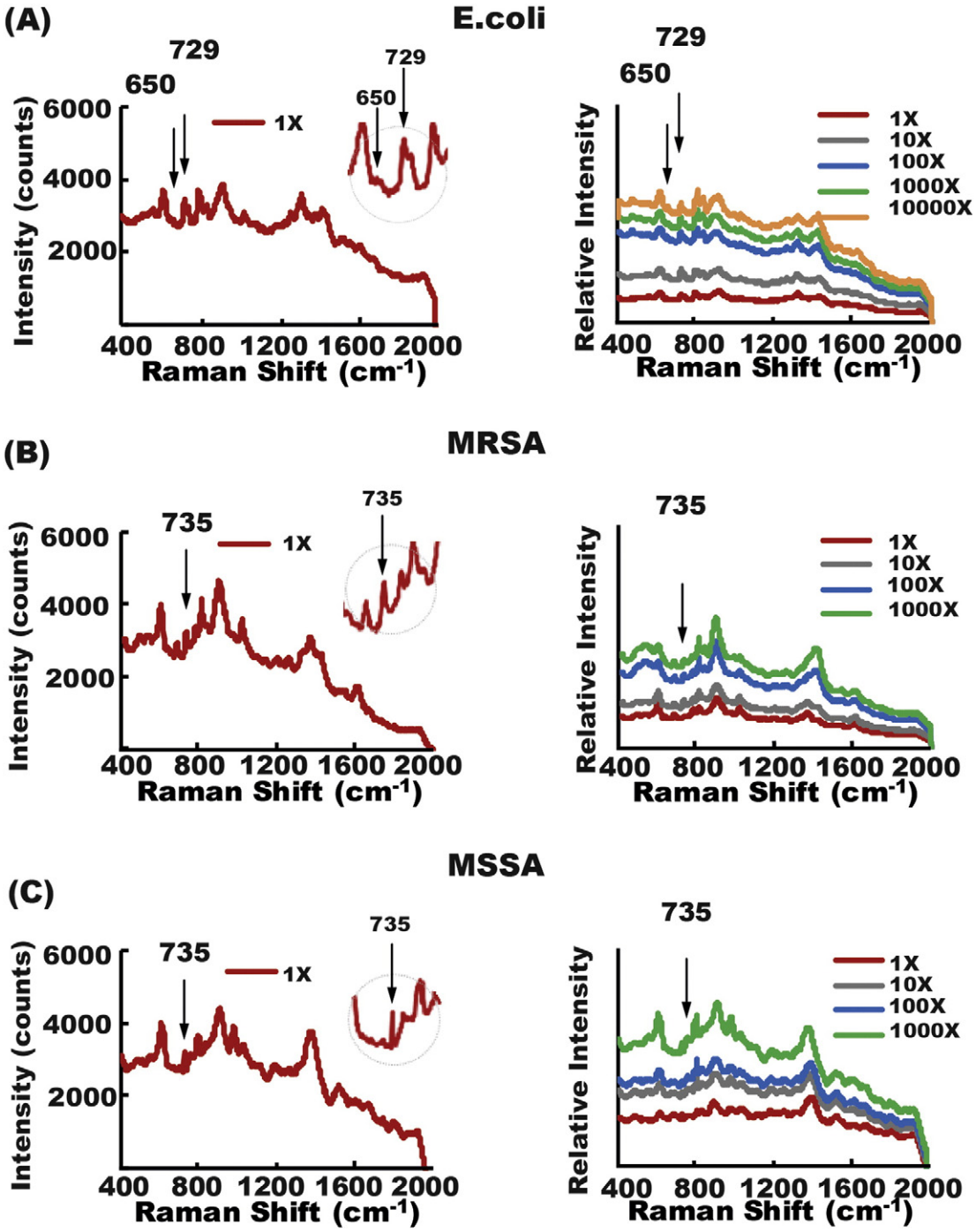

Fig. 1. Raman shifts of *E. coli*, MRSA and MSSA at different bacterial concentrations. Standard bacteria loaded on Raman-SERS chips were illuminated with laser light set at 785 nm with a power of 20 mW and 5-second integration time. The representative Raman shift spectra were shown. The peaks labeled were used as markers for bacteria identification. (A) *E. coli* at a concentration of 5 MacFarland (MCF) (1×) (left) and at serial 10-fold dilution (right), (B) MSSA at 5 MCF (1×) (left) and at serial 10-fold dilutions (right) (C) MRSA at 5 MCF (1×) (left) and at serial 10-fold dilutions (right). *E. coli*, *Escherichia coli*; MSSA, methicillin-sensitive *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*.

dilutions were shown in Fig. 1C respectively. Spectra from other bacteria were shown in Supplemental Fig. 1.

3.2. The identification of bacteria from dialysate of PD peritonitis patients

The Raman shift spectra of bacteria in the infected dialysate from PD peritonitis patients were compared to the standard reference library spectra using RM.View software for bacteria identification. Raman shift spectra of *E. coli* and MRSA in infected dialysate matched well with that in the standard library. The resulting comparisons using this software were shown in Fig. 2. The spectra of the unknown bacteria in dialysate were 97% and 99% similar to *E. coli* and MRSA respectively by software comparison.

3.3. Confirmation of the diagnosis by PCA

The PCA model further confirmed and validated the Raman shifts of bacteria identified by RM.View software. The first two components with the highest cumulative variance were selected to make a score plot. The resulting score plots were set as calibration. The data of the Raman spectra of bacteria from infected dialysate were then processed with data from standard library bacteria. The final diagnosis was made if the

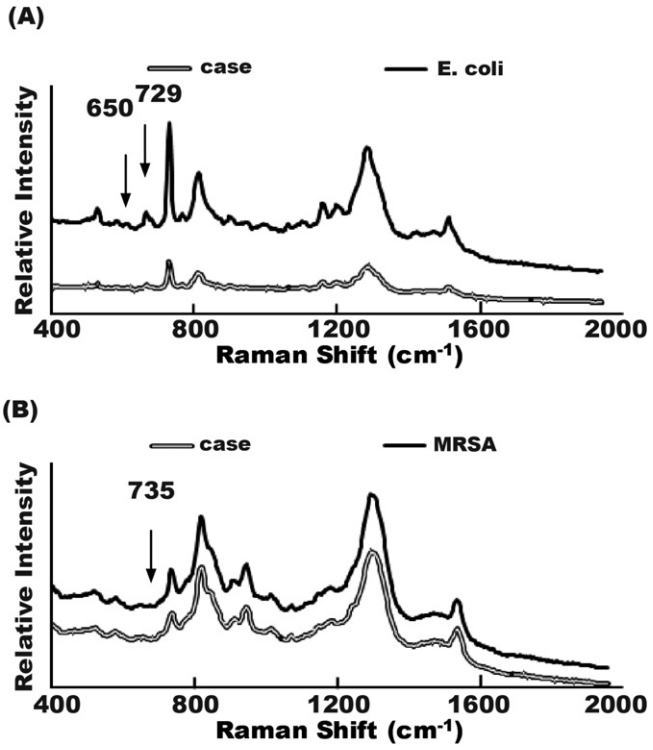

Fig. 2. Comparison of Raman shift spectra of unknown bacteria with the standard library spectra. Examples of comparisons of the Raman spectrum of (A) *E. coli* and (B) MRSA with the spectra of the standard library using RM.View software were shown. The signals of *E. coli* were 97% matched with the signals of standard bacteria and MRSA was 99% matched. The peaks labeled were used as markers for bacteria identification. *E. coli*, *Escherichia coli*; MRSA, methicillin-resistant *Staphylococcus aureus*.

dots of dialysate pathogens fell into a cluster with the dots of bacteria spectra from standard library. The 3 *E. coli* dots from patients formed a cluster with the standard *E. coli* dots (empty diamond) (Fig. 3A), and the 3 MRSA dots from patients also co-localized with dots from the standard MRSA (Fig. 3B).

3.4. Differential between MRSA and MSSA

Staphylococcus aureus is an important pathogen of bacterial peritonitis. The differentiation between MRSA and MSSA is essential for decision for antibiotic use. MRSA can comprise up to 50% of *Staphylococcus aureus* infection, and vancomycin is the drug of choice [20]. When PCA was applied, MRSA dots were located in the upper part of the PCA loading plot but MSSA dots were located in lower part of the PCA plot (Fig. 4). The dots distribution differentiated MRSA from MSSA.

3.5. Antibiotic susceptibility test

Different antibiotics were applied to test the Raman shift changes of bacteria before and after antibiotic treatment. The disappearance of specific signals implicates that the bacterium is susceptible to the antibiotic applied. When cepharidine was applied, the signal intensity at 739 cm^{-1} significantly decreased in the spectrum of *E. coli* 6 h after treatment (Fig. 5A, blue) compared to the signal intensity at the 739 cm^{-1} peak of *E. coli* without treatment (Fig. 5A, purple). The signal intensity of the 739 cm^{-1} peak decreased gradually as the treatment time went on (Fig. 5A, right panel). The signal intensity at the 735 cm^{-1} peak in the Raman spectrum of MRSA without vancomycin treatment (Fig. 5B, purple) also disappeared in the spectrum of MRSA after 6 h of vancomycin treatment (Fig. 5B blue). Similarly, the time-course effect of vancomycin on MSSA can also be seen (Fig. 5B, right panel). The effects of different antibiotics on different bacteria such as gentamicin on *E. coli* also showed similar spectra evolution patterns (data not shown).

3.6. Comparison between the results of dialysate bacteria culture using reference culture and RAMAN SERS

There were 43 paired dialysate samples from 43 PD peritonitis patients including 23 female patients and 20 male patients enrolled for the study. The results were shown in Table 1. Bacteria were isolated in 37 samples by reference culture. There were only 31 samples with bacteria identified by SERS. Among the 31 single bacteria identified by SERS, 29 were the same as their paired-samples by the reference culture. There were 2 mis-matched reports by the two methods. *Klebsiella pneumoniae* was diagnosed by SERS in one paired sample, but *Klebsiella oxytoca* by reference culture. The other was reported as *Staphylococcus epidermidis* by SERS but as *Staphylococcus capitis* by hospital laboratory. The positive predictive value for our SERS tests was 31/31 (100%) with a precision of 29/31 (93.5%) and an accuracy of 37/43 (86.0%). There were 6 culture-negative PD peritonitis samples by the reference culture, and the Raman SERS signals from these 6 paired-samples were also indiscernible. The reference culture results shown as "others" in Table 1 were pathogens not included in our reference bacteria library list, which included 2 kinds of fungus and 4 kinds of bacteria rarely seen, such as *Roseomonas* mucosa in PD peritonitis patients. The Raman SERS signals from these 6 paired-samples were also all unrecognizable because we did not include them in our reference list. The negative predictive value of the study was 6/12 or 50.0%.

4. Discussion

In the current study, we used Raman SERS techniques to rapidly diagnose bacterial pathogens. Raman shifts from known bacteria were used as a diagnostic reference and PCA was used for final diagnosis confirmation. We also used antibiotic solutions to perform antibiotic susceptibility tests. The identification can be made within 1 or 2 h, while the time required for bacterial susceptibility can be reported within 6 h. In the reference method, it frequently takes 24–48 h to report bacterial pathogens and an even longer time for antibiotic susceptibility results.

A rapid and accurate diagnosis of PD peritonitis pathogens is important for treatment [2]. First generation cephalosporin plus gentamicin is the treatment protocol before pathogens can be identified by conventional culture methods in our hospital. We would then change antibiotics after culture results come out. For example, the presence of Pseudomonas implicated the replacement of the first generation cephalosporin by the third generation cephalosporin or anti-Pseudomonas antibiotics [21]. The identification of Gram-positive bacteria such as MSSA, MRSA, or coagulase-negative *Staphylococcus aureus* mandates the cessation of gentamicin or the other aminoglycoside usage [22]. Empirical antibiotic usage varies according to hospital policy or treatment guideline [2,14]. Quicker diagnosis can shorten hospital stay by allowing us to use the right antibiotics and may also help preserve residual renal function by avoiding the use of nephrotoxic gentamicin [23].

Because of the molecular differences in cell wall structure, the Raman shifts are dissimilar between Gram-positive and Gram-negative bacteria. Most of the Gram-positive bacteria express a high intensity signal near 731 cm^{-1} [24], but many Gram-negative bacteria present a high intensity signal around 724 cm^{-1} [24]. Some other signals can also be used to differentiate Gram-positive from Gram-negative bacteria. For example, the signal intensity at or near 655 cm^{-1} was much stronger in Gram-negative than in Gram-positive, which may reflect a signal from proteins of the bacterial outer membrane existing only in Gram-negative bacteria [25]. *E. coli*, for example, has been reported to have a peak at 654 cm^{-1} [24,26]. Our results also showed a higher 650 cm^{-1} signal intensity in *E. coli* than in *Staphylococcus aureus* (Fig. 1).

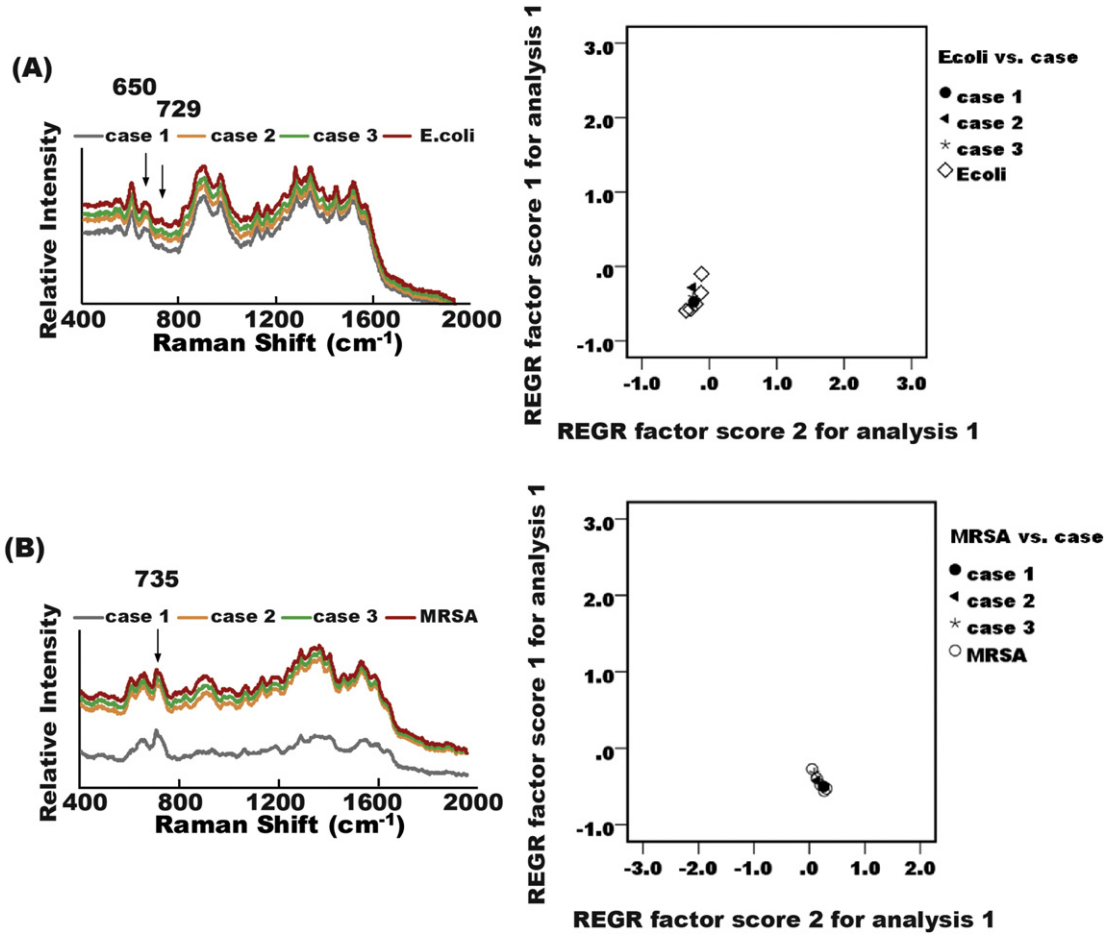

Fig. 3. Detection of *E. coli* and MRSA using Raman–SERS. (A) The merger of Raman shift spectra of standard *E. coli* and *E. coli* from 3 different peritonitis patients revealed a similar fingerprint pattern (left panel). PCA showed cluster of dots of standard *E. coli* with *E. coli* from patients (right panel). (B) Spectra of MRSA from the standard and from 3 different patients showed a similar Raman shift pattern (left panel) and PCA also showed clusters of these MRSA dots (right panel). The peaks labeled were used as markers for bacteria identification. *E. coli*, *Escherichia coli*; MRSA, methicillin-resistant *Staphylococcus aureus*; PCA, principal component analysis.

These signal peaks can be used as markers of bacteria identification and can also be used as a marker of antibiotic susceptibility. The disappearance of a marker signal after antibiotic treatment implicates bacteria susceptibility, and the persistent presence of signal after antibiotic treatment implicates antibiotic-resistance [26].

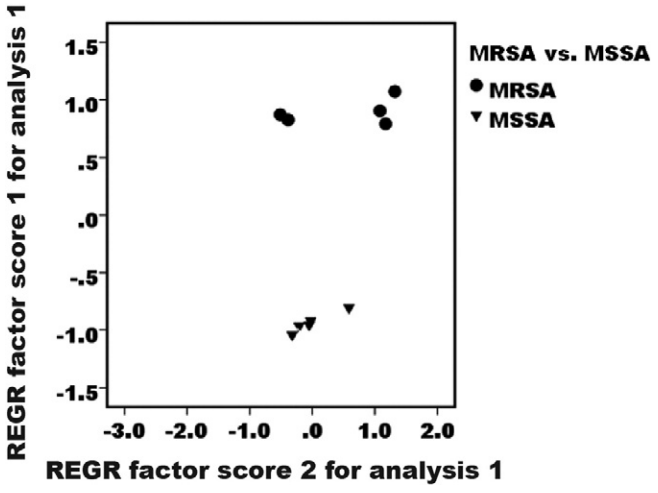

Fig. 4. Differentiation of MSSA from MRSA by PCA analysis. PCA loading plots of Raman spectra from MSSA and MRSA showed clusters of MSSA in the lower part of the plot but clusters of MRSA were shown to be in the upper part of the plot. MSSA, methicillin-sensitive *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; PCA, principal component analysis.

However, the matrix substance used may affect the Raman shift, making specific wavelengths slightly different between chips of different Raman substances [27]. In the study we indicated the high signal intensity frequency in different bacteria for our standard bacteria. The indicated signal intensity can be affected by bacteria concentrations [28]. Therefore, in the preparation of standard spectra library, we used bacteria of different concentrations. The resulting Raman shifts, however, were the same or very similar in their respective bacterial strains. Using bacteria of different concentrations can also test the susceptibility of the chips in detecting its presence. The detection susceptibility was higher for *E. coli* than in *Staphylococcus aureus*. The detection limit of the current device for *E. coli* was 1.5×10^5 CFU/ml. As the loading volume to chip was 3 μl, we could detect *E. coli* in a sample with a bacterial number of 450. Raman shift signals from biological sample are frequently very weak, because biological sample are always inherently very diluted. The target bacteria concentration may be beyond the detection limit of SERS. As the concentration of bacterial pathogen may be lower than detection limit, larger amount of samples and repeated centrifuge can increase bacterial concentration and is frequently necessary for biological sample used in biochip [29]. Centrifugation, membrane filtering, magnetic beads capturing are the conventional methods for sample concentrating. Some novel technique such as hybrid electrokinetics on-chip or electrophoresis methods can also be used for target pathogen concentrating [29,30].

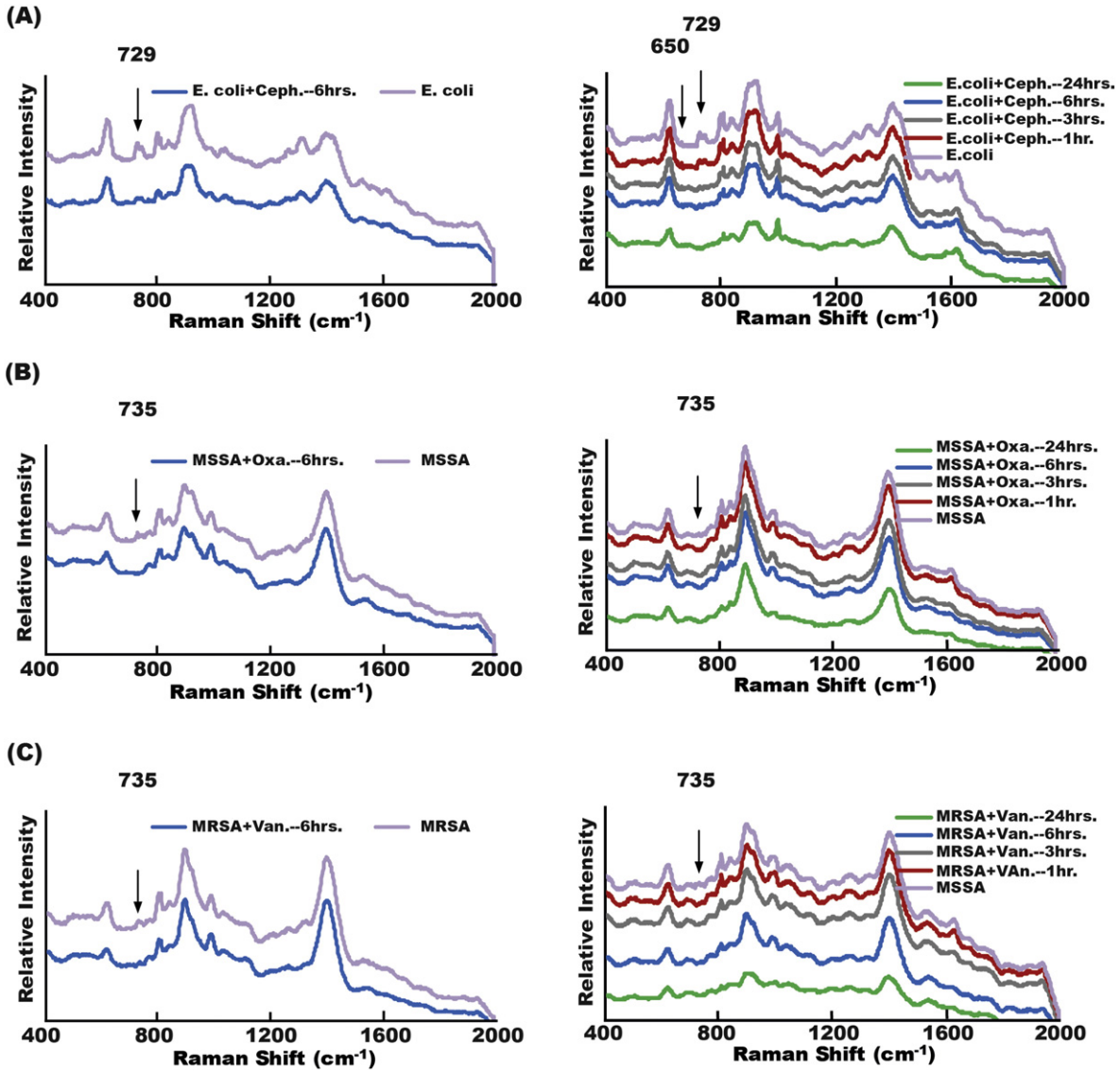

Fig. 5. Detection of antibiotic susceptibility by Raman-SERS. (A) *E. coli* was incubated with cepharidine (128 mg/l), and the Raman shift spectra 6 h after treatment revealed that the signal intensity of the 739 nm peak (left panel, blue) was lower than that of *E. coli* before antibiotic treatment (left panel, purple). The signal intensities of 739 nm peaks decreased as incubation time further increased to 24 h (right panel). (B) MRSA was treated with vancomycin (0.06 mg/l), and the signal intensity of the 735 nm peak decreased 6 h after treatment (left panel). Similarly, the time-course changes of signal intensities at the 735 nm peak can also be seen (right panel). The peaks labeled were used as markers for bacteria identification.

Table 1
Comparison of culture results by reference culture or Raman SERS.

	Reference culture (43)	Raman SERS (43)
Escherichia coli	3 (6.9%)	3 (6.9%)
Enterobacter faecalis	1 (2.3%)	1 (2.3%)
Pseudomonas aeruginosa	2 (4.6%)	2 (4.6%)
Klebsiella pneumoniae	4 (9.3%)	5 (11.6%)
Klebsiella oxytoca	1 (2.3%)	0 (0.0%)[a]
MSSA	3 (6.9%)	3 (6.9%)
MRSA	3 (6.9%)	3 (6.9%)
Staphylococcus epidermidis	6 (13.9%)	7 (16.2%)
Staphylococcus capitis	1 (2.3%)	0 (0.0%)[b]
Streptococcus salivarius	5 (11.6%)	5 (11.6%)
Streptococcus oralis	2 (4.6%)	2 (4.6%)
No growth	6 (13.9%)	Unrecognized signal 6 (13.9%)
Others	6 (13.9%)[c]	Unrecognized signal 6 (13.9%)

MSSA, methicillin-sensitive *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus*.

[a] Was isolated as *Klebsiella pneumoniae*.

[b] Was isolated as *Staphylococcus capitis*.

[c] Included *Candida albicans* (n = 1); *Aspergillus* (n = 1); *Roseomonas* (n = 1), *Pantosea* (n = 1), *Campylobacter* (n = 1), and unrecognized Gram positive *Bacillus* (n = 1).

We concentrate our samples by centrifuge, which has been proved to be able to increase bacterial concentration and successfully increase the culture rate of conventional bacterial cultures [2]. As the dialysate volume could be as large as several hundred milliliters, we had enough samples to collect target pathogen using centrifuge method. Repeated centrifugation can further concentrate the samples when the target pathogen concentrations are too low.

In the process of bacterial concentration, we also washed our bacteria samples with distilled water. The sample washing can avoid the contamination of the bacteria wall by inflammatory cells or proteins, which might affect the resulting Raman shift.

We used cylindrical Raman chips, allowing good contact of bacteria with Raman. With the cylindrical chips, we could detect most of the bacterial pathogens included in our standard bacteria lists. This cylindrical chip also has the advantage of allowing the use of simple Raman spectrometers such as portable or handheld Raman spectrometers without the need of sophisticated devices such as confocal microscopy [11].

Using these sample processing methods, we could detect the presence of bacteria in most of our samples with the aide of computer-

assisted identification of possible bacteria and further manual confirmation. We then finally made our diagnosis by PCA analysis. Using our Raman chips with the sample concentration and computer support, we could make a high diagnosis precision rate.

For those pathogens detected only by reference methods but not by our chips, the reason may be due to our limited standard bacteria pool, which makes detection of these pathogens difficult. For those reported as culture negative by the reference method, we did not detect the pathogens by Raman chips either. Culture negativity may actually be due to non-infectious or infectious diseases [31]. Culture-negative infectious peritonitis may due to antibiotic use before sampling, poor sampling, suboptimal culture method, or unusual organisms such as viruses or bacteria rarely seen [2,32]. We did not detect any bacterial signals in these culture-negative samples, therefore it is likely there were no bacteria in these samples.

The current study was limited primarily by its small patient size. The peritonitis rate is less than 2/100 patients per year in our hospital, it is not easy to collect many peritonitis samples in a short time. The second limitation is that we had only limited numbers of standard bacteria as a reference. Further studies are needed to allow the accumulation of more Raman spectra as references to facilitate the comparison between fingerprints of reference bacteria and spectra of unknown bacteria. Thirdly, the detection limit may need a bacteria concentration of higher than 10^5 CFU/ml. This limitation can be overcome by either increasing the sample amount or further concentrating the samples by repeated centrifugation [14]. Another method to overcome the sample amount problem could be using a Raman spectrometer with higher resolution power. In the current study, a case of *Klebsiella oxytoca* by hospital laboratory was diagnosed as *Klebsiella pneumoniae* by SERS. Both strains belong to Klebsiella genus and are structurally similar [33]. The other discordance pairs were *Staphylococcus epidermidis* and *Staphylococcus capitis*. Both are actually members of coagulase-negative Staphylococci (CONS) [34], and maybe difficult to be differentiated by SERS. A Raman spectrometer with a higher resolution power may be able to differentiate the small Raman shift differences between bacteria of the different species of the same genus. The other way to overcome the problem of species differentiation is to add these species into the reference library. Finally, we did not have experiences of mixed bacterial flora infection. As Raman shifts of many bacteria are very similar, mixed flora infection may make bacteria detection difficult. Further studies may be needed to overcome the problems of multiple bacterial species infection.

In conclusion, we successfully used Raman SERS technique to detect the presence of bacteria in the dialysate of PD peritonitis patients. Raman SERS may be able to shorten the time needed for bacteria identification and antibiotic susceptibility testing.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cca.2016.07.026.

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