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CLINICAL RESEARCH

Association of Local Unit Sampling and Microbiology Laboratory Culture Practices With the Ability to Identify Causative Pathogens in Peritoneal Dialysis-Associated Peritonitis in Thailand

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Introduction: This describes variations in facility peritoneal dialysis (PD) effluent (PDE) culture techniques and local microbiology laboratory practices, competencies, and quality assurance associated with peritonitis, with a specific emphasis on factors associated with culture-negative peritonitis (CNP).

Methods: Peritonitis data were prospectively collected from 22 Thai PD centers between May 2016 and October 2017 as part of the Peritoneal Dialysis Outcomes and Practice Patterns Study. The first cloudy PD bags from PD participants with suspected peritonitis were sent to local and central laboratories for comparison of pathogen identification. The associations between these characteristics and CNP were evaluated.

Results: CNP was significantly more frequent in local laboratories (38%) compared with paired PDE samples sent to the central laboratory (12%, $P < 0.05$). Marked variations were observed in PD center practices, particularly with respect to specimen collection and processing, which often deviated from International Society for Peritoneal Dialysis Guideline recommendations, and laboratory capacities, capabilities, and certification. Lower rates of CNP were associated with PD nurse specimen collection, centrifugation of PDE, immediate transfer of samples to the laboratory, larger hospital size, larger PD unit size, availability of an on-site nephrologist, higher laboratory capacity, and laboratory ability to perform aerobic cultures, undertake standard operating procedures in antimicrobial susceptibilities, and obtain local accreditation.

Conclusion: There were large variations in PD center and laboratory capacities, capabilities, and practices, which in turn were associated with the likelihood of culturing and correctly identifying organisms responsible for causing PD-associated peritonitis. Deviations in practice from International Society for Peritoneal Dialysis guideline recommendations were associated with higher CNP rates.

Kidney Int Rep (2021) **6**, 1118–1129; https://doi.org/10.1016/j.ekir.2021.01.010
KEYWORDS: culture-negative peritonitis; ISPD guideline; lab practice; microbiology; peritonitis
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Received 29 August 2020; revised 9 January 2021; accepted 11 January 2021; published online 2 February 2021

Peritonitis is a major cause of morbidity, technique failure, mortality, and excess health care costs in patients treated with peritoneal dialysis (PD).[1] Identification of the causative microorganism is critical for guiding antimicrobial treatment, optimizing clinical outcomes, promoting antimicrobial stewardship, and minimizing antimicrobial resistance.[2] Culturing the PD effluent (PDE) is key to microbiologic diagnosis, but the yield is highly dependent on laboratory practices. As such, the International Society for Peritoneal Dialysis (ISPD) has recommended that PD centers should set the threshold target of culture-negative peritonitis (CNP) at a level of not more than 15% of peritonitis episodes[1] and provided guidance regarding laboratory practices associated with optimizing yields from cultures.

CNP is defined as clinical features of peritonitis (abdominal pain or cloudy dialysate), dialysate leukocytosis (leukocyte count > 100/μl with > 50% neutrophils), and negative dialysate culture result for any organisms.[1] Rates of CNP previously reported in the literature have been highly variable, ranging from 5% to 41%.[2–5] To date, microbiology laboratory practices with respect to PDE culture yields have not been comprehensively evaluated. The aim of this study was to describe adherence to the guidelines and variations in facility PDE culture techniques and local microbiology laboratory practices, competency, and quality assurance associated with PD-related infections, with a specific emphasis on factors associated with CNP.

METHODS

Study Design and Population

The rationale, design, and methods for international and Thailand Peritoneal Dialysis Outcomes and Practice Patterns Study (PDOPPS) have been previously published.[6,7] This prospective cohort study was conducted in 22 PDOPPS centers in Thailand from May 2016 to October 2017. The study was approved by the Chulalongkorn University Institutional Review Board and local ethics committees. To be eligible for inclusion, PD centers had to provide treatment to at least 20 PD patients at the time of selection. Thailand study centers were randomly selected from the complete list of 140 eligible PD facilities in Thailand using stratified random sampling by facility characteristics according to geographic regions, health care regions, hospital categories, and numbers of PD patients and PD nurses.

Center-Level Characteristics

Facility-level data using uniform and standardized data collection tools, procedures, and processes were completed by the study coordinator at each participating facility. Facility practices regarding culture technique and microbiology laboratory practices were collected yearly via surveys of PD case managers and microbiology laboratory directors in each facility. Any unclear or inconsistent responses were resolved by direct telephone contact with the study coordinators and laboratory managers. The microbiology laboratory survey consisted of 36 questions covering 6 areas of ongoing competency assessment, including sample collection technique, media and condition use, equipment and instruments, identification techniques and methods, method validation and laboratory control, and training of the laboratory staff (Supplementary Questionnaire).

Microbiology Analysis

All cloudy PD bags before antibiotic commencement in consented PD participants were sent to local and central laboratories when peritonitis was suspected. The specimen collections were performed separately and independently from the global PDOPPS. According to individual practices of each facility, 3 to 100 ml of PDE was aliquoted from the bag at the injection port with an aseptic technique for microbiologic culture in local laboratories by the nurse coordinator. The bag with the remaining solution was then placed in an ice-shield box and shipped directly to the central laboratory as soon as possible. Any bags with delayed shipping (>24 hours from the onset of bag collection) were discarded. Peripheral blood cultures may also have been collected at the discretion of the attending physician if they deemed it clinically appropriate (e.g., if the patient was clinically septic). However, the collected blood sample was not required for the central laboratory and not counted in the analysis.

At the central laboratory, 3 bottles of 50 mL of PDE obtained from the submitted PD bags were centrifuged at 3500*g* for 15 minutes, and the supernatants were discarded. The remaining solution (∼5 ml) was mixed with the pellet and injected into BACTEC Plus Aerobic/F and BACTEC Plus Anaerobic/F vials (Becton, Dickinson and Company, Dún Laoghaire, Ireland) as well as spread onto several agar plates, including blood agar, MacConkey agar (Oxoid, Basing-stoke, UK), chocolate agar (Oxoid), and specific agar plates (as needed) for 5 to 7 days at 37 °C for bacterial culture. For fungal culture, the pellet from another 50 ml of centrifuged PDE was streaked on Sabouraud dextrose agar (SDA) and specific agar plates (as needed), then incubated at 25 °C and 37 °C for 15 to 30 days. For mycobacterial culture, the pellet from an additional 50 ml of PDE was inoculated in Ogawa medium slants for 2 months and BACTEC MGIT 960 media for 42 days.

Bacterial pathogens were identified by Gram stain and the VITEK MS system (bioMérieux USA, Hazelwood, MO). Yeast-form fungi were identified by the

API20c AUX kit (bioMérieux, Marcy l'Etoile, France), based on biochemical reactions, and mold-form fungi were classified based on the morphology of their sex spores and conidia. Positive MGIT cultures were examined microscopically to confirm the presence of mycobacteria by using smears stained with Kinyoun stain for detecting acid-fast bacilli. The species from these positive MGIT cultures were then determined by the GenoType Mycobacterium CM and AS assays (Hain Lifescience, Nehren Germany), according to the manufacturer's instructions.

Any discrepancies in organism identification between 2 laboratories were verified from both ends. The local laboratory was asked to send discordant colonies to the central laboratory to reconfirm the pathogen using the culture technique mentioned earlier and the polymerase chain reaction with 6 different primers. A universal bacterial primer (UFUL/URUL primer[8] of the 16S ribosomal RNA [16SrRNA] gene), 2 universal fungal primers (ITS1/ITS4 primer[9] of the internal transcribed spacer [ITS] and 5.8SR/LR7 primer[10] of the 28S rRNA gene), and 3 universal mycobacterial primers (MYCOGEN-F/MYCOGEN-R[11] of 16SrRNA gene, INS1/INS2 primer[12], and IS6110-F/IS6110-R[13] of the insertion sequence 6110 element) were used.

The reaction mixture with fungal DNA was used as a positive control, and reaction mixture without a template was used as a negative control. The experiments were repeated twice. The purified polymerase chain reaction products were then outsourced for Sanger sequencing service (First BASE Laboratories, Singapore Science Park II, Singapore). The sequencing results were subjected to Basic Local Alignment Search Tool (BLAST; National Center for Biotechnology Information; https://blast.ncbi.nlm.nih.gov/Blast.cgi) search against the GenBank database for homology identities.

Statistical Analysis

Data are presented as frequencies and percentages for categorical variables, mean (SD) for normally distributed continuous variables, and median (interquartile range) for nonnormally distributed continuous variables. Peritonitis-related death was defined as the event that occurred during the 50 days after a peritonitis episode. Hemodialysis (HD) transfer was defined as transfer to HD within 50 days after peritonitis onset, with failure to return to PD within 84 days of modality switch date. Categorical data were compared by the Pearson χ^2 test and the Fisher exact test, as appropriate. Continuous variables were tested for normality using the Shapiro-Wilk test and histograms. Differences in continuous data were evaluated by unpaired t test for normally distributed data and the Wilcoxon rank sum test for nonnormally distributed data. No data were transformed.

Microbiologic patterns of PDE culture between local laboratories and the central laboratory were compared, and then the discordant organisms between the 2 groups were explored. The event of interest was the CNP rate (episodes/yr), which was compared between facility/laboratory categories with a test for incidence-rate difference with mid-P values adjustment and incidence rate ratios with 95% confidence intervals (CI). All data were analyzed using Stata 16.1 software (StataCorp, College Station, TX). A 2-tailed P value <0.05 was considered statistically significant.

RESULTS

The Disparity of Culture Yield and Microorganism Identification

During the cohort period, there were 360 peritonitis episodes in 241 participants from 22 participating facilities. Of these, 83 episodes from 79 patients were included in the study (Figure 1). All peritonitis episodes occurred after patients had completed PD training and commenced PD. The demographic data, blood chemistries, and 3-month outcomes comparing participants with positive and negative local culture results are summarized in Table 1. The crude peritonitis rate was 0.40 episodes/yr, and the peritonitis rate in the median facility was 0.37 episodes/yr (interquartile range, 0.30, 0.49 episodes/yr). The rates of culture negativity of each facility are illustrated in Figure 2, with the ISPD reference threshold of 15%.

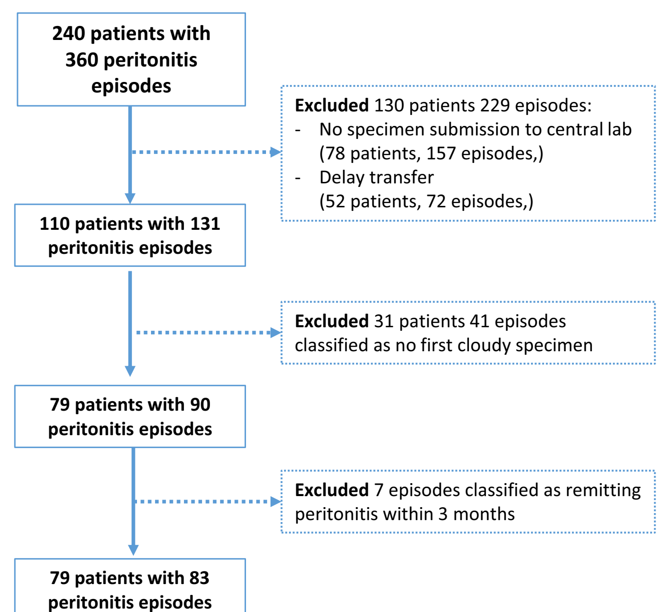

Figure 1. Patient flow diagram and summary of peritonitis episodes. Remitting peritonitis was defined as a combination of relapsing peritonitis within 3 months from the same or different organisms.

Table 1. Characteristics, biochemistries, and outcomes of participants

Variables	Overall	Patients with Local negative culture	Patients with Local positive culture	*P* value
Participant characteristics[a]				
Patients, No	79	31 (39%)	48 (61%)	
Age, yr	57.9 (11.8)	56.7 (12.6)	58.7 (11.2)	0.47[b]
Female sex	48 (61)	22 (71)	26 (54)	0.14[c]
PD vintage years	1.59 (0.48–2.59)	1.70 (0.61–3.56)	1.15 (0.43–2.28)	0.21[d]
PD modality, CAPD	78 (99)	31 (100)	47 (98)	>0.99[e]
Diabetes mellitus				0.77[c]
Yes	42 (55)	17 (57)	25 (53)	
No	35 (45)	13 (43)	22 (47)	
Caregiver				0.40[e]
Family	36 (46)	17 (55)	19 (40)	
Paid	5 (6)	2 (6)	3 (6)	
None	38 (48)	12 (39)	26 (54)	
Education				0.81[e]
Primary school or lower	51 (70)	21 (70)	30 (70)	
High school	14 (19)	5 (17)	9 (21)	
Bachelor or higher	8 (11)	4 (13)	4 (9)	
Marriage status[f]				>0.99[e]
Single	6 (8)	2 (6)	4 (8)	
Married	56 (71)	22 (71)	34 (71)	
Separated/divorced	17 (22)	7 (23)	10 (21)	
Peritonitis episode characteristics				
No. of peritonitis episode	83	32 (39)	51 (61)	
Serum albumin, mean (SD), g/dL	3.0 (0.6)	3.0 (0.6)	3.0 (0.6)	0.92[b]
Serum potassium, mmol/L	3.3 (3.0–3.8)	3.2 (3.0–4.0)	3.3 (3.0–3.7)	0.80[d]
Serum calcium, mean (SD), mg/dL	8.6 (1.1)	8.7 (1.0)	8.6 (1.2)	0.60[b]
PDE leukocyte count, /µl	1950 (400–5700)	1500 (300–4014)	2200 (628–6917)	0.15[d]
PDE neutrophil count, /µl	1805 (252-5096)	878 (196–3531)	1848 (400–5838)	0.23[d]
Hemoglobin, mean (SD), g/dL	10.1 (2.2)	10.1 (1.9)	10.1 (2.4)	0.97[b]
Outcomes[f]				0.02[c]
Alive, continue PD	51 (62)	14 (44)	37 (74)	
Alive, transfer to hemodialysis	9 (11)	5 (16)	4 (8)	
Death	22 (27)	13 (41)	9 (18)	

CAPD, continuous ambulatory peritoneal dialysis; PD, peritoneal dialysis; PDE, peritoneal dialysis effluents; PMN, polymorphonuclear leukocytes; SD, standard deviation.
Categorical values are represented as number (%), and continuous values are represented by the median (interquartile range) or as indicated otherwise.
[a]Characteristics at last peritonitis episode was used for participants with more than one peritonitis episodes.
[b]Unpaired *t* test.
[c]Pearson's χ^2 test.
[d]Wilcoxon rank-sum test.
[e]Fisher's exact test.
[f]Total percentage is not equal to 100% due to rounding.

CNP was significantly more frequent in local laboratories (38%) compared with paired PDE samples sent to the central laboratory (12%, $P < 0.05$).

The organisms detected by the central laboratory but not by the local laboratories included fungi (9%), gram-positive bacteria (8%), polymicrobial infection (4%), gram-negative bacteria (3%), and mycobacteria (2%; Table 2). Concordant and discordant culture results between the central laboratory and local laboratories are provided in Tables 2 and 3 and Figure 3. The characteristics and outcomes of patients with local negative culture results and concordant or discordant central laboratory culture results are summarized in Supplementary Table S1.

CNP Rate and Local Laboratory Practice

CNP was significantly less likely with a number of facility practices, including performing sedimentation, early transfer of inoculated bottles to microbiology laboratories, and restriction of specimen collection to PD nurses (Table 4). Facilities belonging to small hospitals (<120 beds), having lower numbers of patients

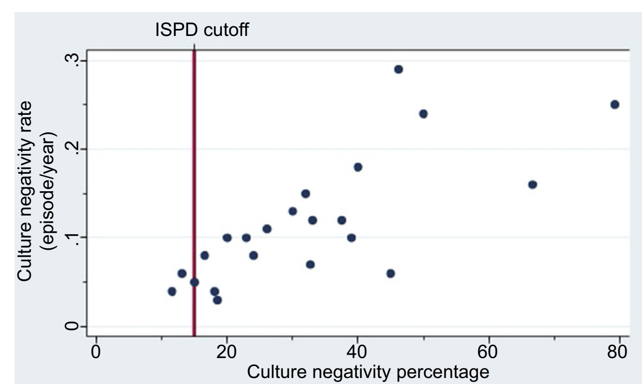

Figure 2. Culture negativity rate for each participating peritoneal dialysis (PD) centers by culture negativity percentage with the International Society for Peritoneal Dialysis (ISPD) cutoff.

Table 2. Peritoneal dialysis effluents culture yields and concordant and discordant microbial culture results between local and central laboratories

Variable	Local labs ($n = 83$)	Central lab ($n = 83$)	Central yes, local yes[a]	Central yes, local no[b]	Central no, local yes[c]
Culture negative	32 (38)	10 (12)	9 (28)	1 (10)	23 (72)
Gram-positive bacteria	29 (35)	36 (43)	27 (93)	9 (25)	2 (7)
Coagulase-negative *Staphylococcus*	10 (12)	10 (12)	9 (90)	1 (10)	1 (10)
Staphylococcus aureus	6 (7)	6 (7)	4 (67)	2 (33)	2 (33)
Streptococcus spp	5 (6)	13 (16)	5 (100)	8 (62)	0 (0)
Enterococcus spp	5 (6)	4 (5)	4 (80)	0 (0)	1 (20)
Others	3 (4)	3 (4)	1 (33)	2 (67)	2 (67)
Gram-negative bacteria	18 (22)	20 (25)	15 (83)	5 (25)	3 (17)
Escherichia Coli	4 (5)	5 (6)	4 (100)	1 (20)	0 (0)
Enterobacter, Pantoea spp	4 (5)	3 (4)	3 (75)	0 (0)	1 (25)
Klebsiella pneumoniae	1 (1)	2 (2)	0 (0)	2 (100)	1 (100)
Acinetobacter spp	4 (5)	3 (4)	2 (50)	1 (33)	2 (50)
Pseudomonas, Brevundimonas, Burkholderia spp	3 (4)	4 (5)	3 (100)	1 (25)	0 (0)
Others	2 (2)	3 (4)	2 (100)	1 (33)	0 (0)
Fungi	1 (1)	8 (10)	1 (100)	7 (88)	0 (0)
Yeast	1 (1)	3 (4)	1 (100)	2 (67)	0 (0)
Mold-filamentous	0 (0)	5 (6)	0 (0)	5 (100)	0 (0)
Polymicrobial	3 (4)	7 (8)	3 (100)	4 (57)	0 (0)
Mycobacterium	0 (0)	2 (2)	0 (0)	2 (100)	0 (0)

[a]The total number of cases where both laboratories had the same result for that row.
[b]The number of cases where the central laboratory disclosed the result listed in the row, but the local laboratories did not.
[c]The number of cases where the local laboratories disclosed the result listed in the row, but the central laboratory did not.
Data are presented as number (%).

treated with PD (<100 cases), having no nephrologist, having smaller laboratory capacity, having limited capability in detection of aerobic organisms, lacking a standard operating procedure in microbial susceptibilities, and lacking local accreditation (LA) certificates were associated with higher CNP rates (Table 5).

Sample Preparation and Collection Practice

Most facilities (91%) collected PDE specimens after a dwell time of at least 2 hours. However, 2 facilities (9%) accepted bags with 1-hour dwell times for microbial cultivation if it was the first cloudy bag. Six facilities (27%) did not allow the patients to collect their first cloudy bag at home but instead insisted that they be collected by PD nurses at the centers. For the 16 centers that allowed patients to collect their first cloudy bag at their homes, 4 facilities (25%) taught them to keep the bag in a 4 °C refrigerator until they could bring the sample to their PD centers. Seven facilities (32%) hung the bag for 15 minutes before collecting the sample, and the other 15 facilities (68%) inverted the bag 2 or 3 times before sampling. In 3 facilities (14%), only PD nurses were allowed to collect the PD samples, whereas the other facilities allowed ward nurses or emergency department nurses to collect PD samples if they first met the patient (Table 3).

Table 3. Discordant microbial culture results between local and central laboratories

Local laboratories	Central laboratory
E faecalis	*S gallolyticus*
Streptococcus group D, not enterococcus	*S sanguis*
Streptococcus group D, not enterococcus	*S sanguis*
Alpha-hemolytic streptococcus	*S mitis/oralis*
Unclassified gram-positive cocci	*S gallolyticus*
Enterobacter cloacae	*Pantoea* spp.
Enterobacter spp.	*Morganella morganii*
No growth	• Gram positive : streptococci (*S gallolyticus* [2], *S equinus* [1], *S sanguinis* [1], *alpha-streptococcus* [1]); *Sta aureus* (1), *A. viridans* (1), *Micrococcus* (1), *Bacillus* (1) • Gram negative : *K pneumoniae* (2), *E coli* (1), *A baumanii* (1), *B diminuta* (1) • Mold : *A flavus* (2), *A niger* (1), unclassified *Aspergillus* (1), *Simplicillium obclavatum* (1) • Yeast: *C albicans* (1), *C parapsilosis* (1), *Rhodotorula diminuta* (1). • Mycobacteria : *M tuberculosis* (2)
Micrococcus spp	No growth

Only 2 facilities (9%) centrifuged the PDE (50–100 ml) and cultivated the pellet (large-volume culture). The other 20 facilities (91%) used bedside inoculation (13 facilities used 1 bottle, 7 used 2 bottles) with total PDE volumes of 3 to 5 ml (2 facilities), 6 to 10 ml (12 facilities), or 12 to 20 ml (6 facilities). Automated blood culture bottle(s) (10 BACTEC, 6 BacT/Alert, or 3 others) were used in 19 facilities (86%), and 1 facility used in-house prepared blood culture bottles. However, none routinely used anaerobic broth as recommended by the ISPD.

Fifteen facilities (68%) immediately transferred the specimens/inoculated bottles to laboratories. Specimens were left in the ward at room temperature in 6 facilities and at 4 °C in 1 facility until laboratories opened for the

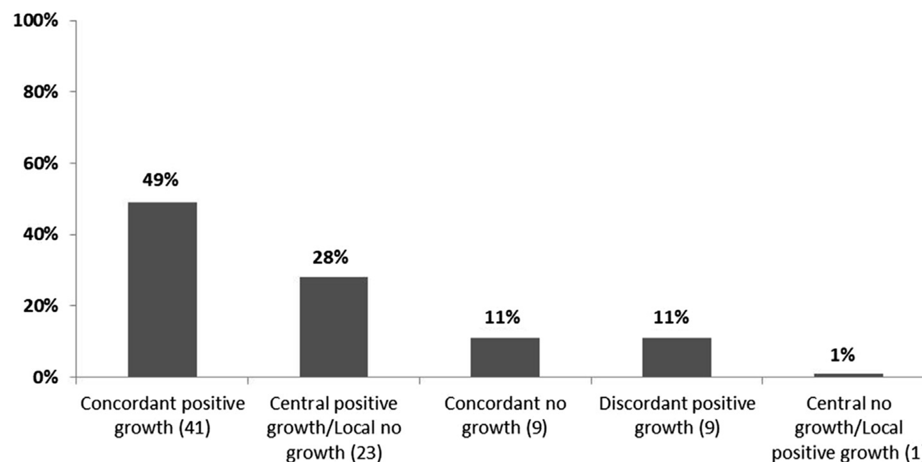

Figure 3. Concordant and discordant organism identifications between central laboratory and local laboratories.

day. Only 5 laboratories (23%) reported that they provided microbiologic services 24 hours a day. Thus, only one-quarter of the facilities followed the ISPD guideline concerning specimen transfer (Tables 4 and 5).

Laboratory Capacity, Capability, and Certification

Two laboratories (9%) received <25 culture specimens/d, and 7 (32%) received >200 culture specimens/d.

Only 2 (9%), 1 (5%), and 6 laboratories (27%) had complete equipment, reagents, and media to culture obligated anaerobes, molds, and mycobacteria, respectively. For example, 9 facilities (41%) used specific fungal media (6 SDA and 3 Mycosel [Becton, Dickinson and Company]), and 21 facilities (95%) used only 35 °C to 37 °C incubation temperatures for cultivating fungi. Twelve laboratories (55%) had outsourced specimen processing for mycobacterium and filamentous mold cultures. However, none of the laboratories with competency in cultivating these fastidious organisms autonomously inoculate the specimen in special culture conditions for capturing the organisms unless the clinician specifically requested it, usually in cases of refractory or repeated CNP or if the pathogen was accidentally grown under routine conditions.

Twenty laboratories (91%) performed antimicrobial susceptibility tests for bacteria, and only 2 laboratories (9%) did so with fungi. Fifteen laboratories (68%) performed minimal inhibitory concentration testing (E test and/or automated susceptibility; e.g., VITEK-2, MicroScan [Beckman Coulter, Brea, CA]). Of note, 21 (95%), 17 (77%), and 20 laboratories (91%) used standard operating procedures and manuals in specimen processing, microbial identification, and antimicrobial susceptibility testing, respectively.

Only 6 laboratories (27%) currently held a standard accreditation certification (International Organization For Standardization [ISO] 15189 or College of American Pathologists [CAP]) and LA, 18 laboratories (82%) had LA, and 2 laboratories (10%) lacked any accreditation (Table 5).

DISCUSSION

The present study found CNP occurred in 38% of peritonitis episodes, which was considerably higher than the maximum 15% recommended by ISPD guidelines[1] and significantly more frequent in local laboratories (38%) compared with paired PDE samples sent to the central laboratory (12%, $P < 0.05$). Lower rates of CNP were associated with PD nurse specimen collection, centrifugation of PDE, immediate transfer of samples to the laboratory, larger hospital size, larger PD unit size, availability of an on-site nephrologist, higher laboratory capacity, and laboratory ability to perform aerobic cultures, having a standard operating procedure in antimicrobial susceptibilities, and obtaining LA. Discordant culture results between the central laboratory and local laboratories were also observed in 8 cases of culture-positive peritonitis. There was marked variation in the capacities, capabilities, and practices of local laboratories, with some laboratories lacking accreditation, as well as in reported PDE acquisition and processing techniques.

Although the outcome of CNP is debatable compared with culture-positive peritonitis, the capacity to identify the pathogen in every episode of peritonitis is important for rationalizing antimicrobial treatment and identifying potential sources of infection, thereby improving treatment outcomes and mitigating the risks of relapsing, recurrent, and repeat peritonitis episodes. Fahim *et al.*[14] reported that CNP had a more favorable outcome than culture-positive peritonitis, whereas the Hong Kong study[2] reported converse outcomes.

Table 4. Associations of peritoneal dialysis effluents culture technique with culture-negative peritonitis in Thailand Peritoneal Dialysis Outcomes and Practice Patterns Study participating sites

		Culture-negative peritonitis		
Variable	All facilities	Incidence rate, median (IQR), episodes/yr	*P* value for incidence rate difference	Incidence rate ratio (95% CI)
Facilities, No.	22			
PDE acquisition				
Collection of first cloudy bag				
Yes	16 (73)	0.09 (0.06–0.12)	0.09	0.72 (0.49–1.07)
No	6 (27)	0.14 (0.12–0.16)		1.00 [Reference]
Dwell time before sampling				
<2 hours	2 (9)	0.13 (0.12–0.13)	0.68	1.13 (0.55–2.10)
≥2 hours	20 (91)	0.10 (0.03–0.16)		1.00 [Reference]
Bag treatment				
Inverted 2–3 times	15 (68)	0.11 (0.07–0.15)	0.2	1.30 (0.86–2.01)
Hanging for 10–15 min	7 (32)	0.08 (0.04–0.18)		1.00 [Reference]
Sampling volume				
≤5 ml	2 (9)	0.14 (0.11–0.16)	0.35	1.28 (0.72–2.16)
6–20 ml	18 (82)	0.10 (0.06–0.15)	0.09	1.00 [Reference]
50–100 ml	2 (9)	0.07 (0.03–0.10)		0.34 (0.04–1.26)
Specimen collector				
Restrict to PD nurse	3 (14)	0.07 (0.04–0.29)	<0.001	0.10 (0.05–0.17)
No restriction	19 (86)	0.10 (0.06–0.15)		1.00 [Reference]
PDE processing				
Sedimentation				
Yes	2 (9)	0.07 (0.03–0.10)	0.03	0.33 (0.07–0.98)
No	20 (91)	0.11 (0.07–0.16)		1.00 [Reference]
Use of automated bottle(s)				
Yes	19 (86)	0.10 (0.06–0.16)	0.19	1.59 (0.78–3.78)
No	3 (14)	0.10 (0.03–0.13)		1.00 [Reference]
Use anaerobic bottle				
Yes	0 (0)	NA	NA	NA
No	22 (100)	0.10 (0.06–0.15)		
Specimen or inoculated bottle transfer				
Specimen storage during off-hours				
No storage	15 (68)	0.08 (0.05–0.13)	0.02	1.00 [Reference]
Storage in room temperature	6 (27)	0.14 (0.10–0.25)	0.65	1.56 (1.05–2.30)
Storage in 4 °C refrigerator	1 (5)	0.11 (0.11–0.11)		1.21 (0.38–2.98)

CI, confidence interval; IQR, interquartile range; NA, nonavailable; PD, peritoneal dialysis, PDE, peritoneal dialysis effluents.
Values are represented as number (%), unless otherwise specified.

Recently, Htay *et al.*[15] used the Australia and New Zealand Dialysis and Transplant Registry (ANZDATA) registry and found that CNP had similar or poorer outcomes than peritonitis due to nonstaphylococcal gram-positive peritonitis but had better outcomes than peritonitis caused by all other organisms.

The median CNP of local facilities was similar to previous reports from Thailand (24%–43%)[5,16–18] and at the upper end of the range reported globally (5%–41%).[2–5] However, such registry and center-specific reports have provided scant details regarding the culture techniques used. Szeto *et al.*[2] demonstrated that higher CNP rates were associated with sampling by a nonspecialist nurse and previous antibiotic exposure. In contrast, a UK survey[19] found no correlation between center-reported CNP rates and PDE processing technique, sampling volume, or center characteristics. In our study, CNP was associated with facilities that deviated from ISPD guideline recommendations concerning PDE preparation, collection, and handling, had limited facility resources (smaller hospital with no nephrologist on site), and had low facility experience (<100 PD cases). CNP was also associated with limited laboratory capacity and capability in identification of aerobic bacteria, lack of a standard operating procedure in antibiotic susceptibility, and lack of a LA.

Although few studies have explored an association between center-level characteristics and the CNP rate, the association between facility size and technique failure has been well established and confirmed by many large registry-based studies.[20–23] Variations in center adherence to the ISPD peritonitis guidelines have also been well demonstrated in Australian[24] and PDOPPS studies[25]; however, their impact on culture-negative rates is obscure.

Table 5. Associations of local facility and laboratory characteristics with culture-negative peritonitis in Thailand Peritoneal Dialysis Outcomes and Practice Patterns Study participating sites

		Culture-negative peritonitis		
Variable	All facilities	Incidence rate, median (IQR), episodes/yr	*P* value for incidence rate difference	Incidence rate ratio (95% CI)
Facilities, No.	22			
Facility characteristics				
Hospital category				
Small (<120 beds)	2 (9)	0.23 (0.16–0.29)	0.03	1.82 (1.04–3.05)
Medium (120–500 beds)	7 (32)	0.10 (0.05–0.12)	0.35	0.82 (0.52–1.27)
Large (>500 beds)	13 (59)	0.10 (0.07–0.13)		1.00 [Reference]
PD unit larger than 100 cases				
Yes	13 (59)	0.10 (0.07–0.12)	0.03	0.66 (0.45–0.98)
No	9 (41)	0.10 (0.05–0.24)		1.00 [Reference]
Bangkok metropolitan region				
Yes	5 (23)	0.05 (0.04–0.10)	0.18	0.67 (0.34–1.22)
No	17 (77)	0.11 (0.08–0.16)		1.00 [Reference]
Facility age >10 years				
Yes	6 (27)	0.08 (0.04–0.10)	0.28	0.77 (0.46–1.24)
No	16 (73)	0.12 (0.07–0.16)		1.00 [Reference]
Availability of nephrologist				
Yes	19 (86)	0.10 (0.06–0.13)	0.02	0.58 (0.37–0.95)
No	3 (14)	0.16 (0.12–0.29)		1.00 [Reference]
Networking in peritonitis treatment				
Yes	11 (50)	0.11 (0.08–0.13)	0.4	0.86 (0.58–1.26)
No	11 (50)	0.08 (0.04–0.18)		1.00 [Reference]
Laboratory characteristics				
Laboratory capacity				
Anaerobic facility	2 (9)	0.07 (0.04–0.10)	0.45	0.44 (0.01–2.52)
Polymerase chain reaction	10 (45)	0.11 (0.04–0.12)	0.76	1.06 (0.71–1.56)
MIC (automated or E-test)	14 (64)	0.10 (0.05–0.13)	0.3	0.82 (0.56–1.22)
Lab workload, c/s specimens/d				
Small (<50 specimens/d)	2 (9)	0.23 (0.16–0.29)	0.02	1.99 (1.07–3.62)
Medium (50–200 specimens/d)	13 (59)	0.10 (0.06–0.12)	0.93	1.02 (0.65–1.63)
Large (>200 specimens/d)	7 (32)	0.10 (0.04–0.13)		1.00 [Reference]
Providing 24-hour service				
Yes	5 (23)	0.12 (0.10–0.13)	0.34	1.24 (0.76–1.95)
No	17 (77)	0.10 (0.06–0.15)		1.00 [Reference]
Lab capability in organism identification				
Aerobic (minimum)	20 (91)	0.10 (0.06–0.13)	0.01	0.51 (0.31–0.87)
Anaerobic	2 (9)	0.07 (0.04–0.10)	0.45	0.44 (0.01–2.52)
Fungus	1 (5)	0.04 (0.04–0.04)	0.3	0.36 (0.01–2.05)
Mycobacterium	6 (27)	0.12 (0.05–0.24)	0.03	1.66 (1.00–2.61)
Standard operating procedure				
Specimen processing	21 (95)	0.10 (0.06–0.15)	0.72	1.37 (0.37–11.49)
Microbial identification	17 (77)	0.10 (0.06–0.13)	0.07	0.68 (0.45–1.05)
Antibiotic susceptibility	20 (91)	0.10 (0.06–0.13)	0.01	0.51 (0.31–0.87)
Lab certification				
CAP or ISO 15189 with LA	6 (27)	0.11 (0.10–0.24)	0.05	1.50 (0.99–2.23)
LA	18 (82)	0.10 (0.06–0.12)	0.01	0.57 (0.37–0.89)

CAP, College of American Pathologist; CNR, culture-negative rate; c/s, culture; E-test, epsilometer test; IQR, interquartile range; ISO, International Organization for Standardization; LA, local accreditation; MIC, minimum inhibitory concentration; PD, peritoneal dialysis.
Values are represented as number (%), unless otherwise specified.

Our result affirms the existence of such variation and demonstrates the association of such variations and the CNP rate. Exposure to over-the-counter antibiotics before taking the PDE culture might be another explanation for the high CNP rate in our study. Over-the-counter antibiotics are known to reduce a culture yield of bacteria by half[26] and are commonly misused in Thai culture. However, only 16% of the participants with peritonitis in our series had exposure to a previous antibiotic within 1 month before the episode.

To minimize CNP rates, the ISPD guidelines updated in 2016[1] recommend that bedside inoculation of 5 to 10 ml of PDE in 2 (aerobic and anaerobic) blood culture bottles for bacterial culture should be preferred (Level 1C), and the inoculated bottles should arrive at the laboratory within 6 hours. If this is not possible, the

inoculated culture bottles should ideally be incubated at 37 °C.[1] However, the present study showed that center adherence to this guideline was generally slight. No facilities used anaerobic broth to inoculate PDE. Three-quarters of local laboratories provided microbiologic services only during working hours, indicating that the inoculated bottles would be stored at room temperature (6 centers) or 4 °C (1 center) for more than 6 hours if the specimens were collected after-hours, thereby compromising culture-positive yields of the local laboratories. Refrigeration or freezing may kill or retard the growth of some microorganisms.[27]

ISPD also recommends that the solid media should be incubated in aerobic, microaerophilic, and anaerobic environments.[1] However, only 2 laboratories (9%) delivered full microbiology laboratory capability, defined as the availability of an anaerobic incubator, proper specimen preparation stations, and appropriate media, including those required for cultivating and isolating obligated anaerobes.

Molds, environmental yeasts, and mycobacteria were only detected by the central laboratory but not at all by local laboratories, confirming the latter's limited capacity to cultivate these fastidious organisms. This is further supported by the finding that only 2 (9%) and 6 laboratories (27%) had complete equipment, reagents, and media to culture molds and mycobacteria, respectively. Thirteen laboratories (59%) had no availability of fungal-specific media and instead relied on bacterial broth for capturing fungus. Unfortunately, the automated blood culture systems can detect the growth of most pathologic *Candida* isolates, but not filamentous mold and non-*Candida* yeast.[28] Three laboratories(14%) used Mycosel medium, consisting of soybean meal digest, dextrose, chloramphenicol, and cycloheximide, which is recommended for the isolation of dimorphic fungi and dermatophytes but selectively excludes saprophytes, which are the most common cause of filamentous mold peritonitis.[29] Five facilities (23%) used SDA. Most fungal opportunists and pathogenic fungi (both filamentous fungi and yeasts) can grow well on SDA and potato dextrose agar (PDA); however, they need a specific temperature to grow. Typically, molds actively multiply at room temperature (22 °C to 25 °C), whereas yeasts require temperature at 28 °C to 30 °C or 37 °C (for dimorphic fungi).[29,30] Some fungal strains may grow poorly or fail to grow on SDA, including *Aspergillus niger*, *A sulphureus*, *A versicolor*, *Penicillium corylophilum*, *P expansum*, *Fusarium oxysporum*, *Histoplasma capsulatum*, and many strains of *Nocardia asteroides*.[30,31] These strains need alternative agars for growth, including sheep blood agar, nutrient agar, tryptic soy agar, and PDA.[31]

All facilities but 1 used only 35° to 37 °C incubation temperatures for cultivating bacteria and fungus, such that filamentous mold would likely not be detected. The ISPD guideline states that special culture techniques may be considered for the isolation of unusual organisms (e.g., mycobacteria, filamentous fungus, and other fastidious bacteria) if PDE yields no growth after 3 days and the infection has not resolved[1]; however, no details are provided regarding these special culture techniques. A future revision of the guidelines concerning fastidious pathogen identification is warranted.

In particular, specimens should be inoculated and cultivated in media and conditions that promote the growth of unusual pathogens (particularly molds and mycobacteria) if an episode of CNP is refractory to antibiotics. To fully assess filamentous fungal pathogens, appropriate fungal media should be selected, and inoculated agar should be incubated under 2 temperature conditions (room temperature and 35 °C–37 °C). To increase the yield of mycobacterium detection, specific automated mycobacterium broth (BACTEC 460, MGIT 960, MB/BacT) recommended by the World Health Organization is preferred.[32] Furthermore, inoculation and pathogen isolation should be handled by an experienced, accredited laboratory with a clearly documented standard operating procedure.

Discordant culture results between the central laboratory and local laboratories were observed in 8 cases of culture-positive peritonitis, mostly *Streptococcus* spp. A falsely interpreted hemolytic pattern of streptococci between partial (alpha, including viridans group streptococci and *S pneumoniae*) and no hemolysis (gamma, including group D and mutans group streptococci) groups was commonly observed in our study. The degree of partial hemolysis can vary widely with the growth medium used to cultivate the organism and the incubation temperature.[33]

Automated biochemical methods (VITEK-2, BD Phoenix system, etc.) also had limited capacity in the identification of streptococci, particularly viridans group streptococci, because the systems do not have all streptococci species represented in their databases.[34] Compared with biochemical methods as the reference technique, 10% of the streptococci showed disagreement between the automated biochemical technique and the reference method,[35] and the discordance was higher in viridans group streptococci species (53%).[36] Falsely subclassified streptococci might not only affect clinical outcomes of peritonitis treatment but was also a proxy of a problem in microbiologic diagnosis in Thailand.

The strength of our study is the use of systematic, comprehensive, and prospective data collection according to robust PDOPPS methodology, which permitted detailed analysis of the associations between

center and laboratory characteristics and practices with culture yield in PD-related peritonitis. Additionally, the study performed paired cultures of the first cloudy bag in both central and local laboratories, thereby permitting a head-to-head comparison of laboratory competency concerning microorganism identification from the same specimen.

However, some limitations are worth mentioning. Firstly, the number of patients with paired PDE samples, the number of Thai participating facilities was relatively small, and the number of large specimen exclusion was large, such that a much larger study would be required to establish other limitations in microbial identification of local laboratories confidently.

Secondly, the results of this study may not be generalized to PD populations outside Thailand in whom the incidence of peritonitis with fastidious organisms is appreciably lower.

Thirdly, the high detection rate of environmental mold raises a concern of specimen contamination during bag transfer. However, our study was well conducted with an orientation provided at all sites on how to collect and handle the specimens with aseptic techniques and conditions. Of interest, colonization of the fungus was observed inside the PD catheter collected from most of the cases with filamentous fungus, and subsequently, cultivation of the removed catheter confirmed its presence, thereby supporting its role as a genuine pathogen. We also tested the fungal cell wall in the PDE and used polymerase chain reaction to confirm cases as true positives.

CONCLUSION

CNP rates in Thailand were high. There were large variations in center and laboratory capacities, capabilities, and practices, which were associated with the likelihood of culturing and correctly identifying organisms responsible for causing PD-associated peritonitis. In particular, deviation from ISPD guidelines was associated with CNP rates. Center and laboratory practices should be standardized in line with ISPD guidelines, and all laboratories should receive proper accreditation. Continuous quality improvement processes should be introduced to ensure that these processes are sustained, and patient-important peritonitis outcomes are improved.

DISCLOSURES

TK has received consultancy fees from Visterra Inc. as country investigator and received speaking honoraria from AstraZeneca and Baxter Healthcare. JP has received speaking honoraria from AstraZeneca, Baxter Healthcare, DaVita Healthcare Partners, Fresenius Medical Care, Dialysis Clinics Incorporated, and Satellite Healthcare, and has served as a consultant for Baxter Healthcare, DaVita Healthcare Partners, Fresenius Medical Care, and liberDi. DJ has previously received consultancy fees, research grants, speaker’s honoraria, and travel sponsorships from Baxter Healthcare and Fresenius Medical Care, consultancy fees from AstraZeneca and AWAK, speaker’s honoraria from Ono, and travel sponsorships from Amgen. All other authors have no financial conflicts of interest to declare. All funders and pharmaceutical companies did not have any role in the study design, data collection, data analysis, data reporting, and the decision to submit for publication.

ACKNOWLEDGMENTS

Authors would like to acknowledge contributions of the staffs, nurses, and all investigators who work at participating Thailand PDOPPS centers, including Professor Kearkiat Praditpornsilpa, MD, Chulalongkorn University, Bangkok, Thailand; Areewan Cheawchanwattana, PhD, Khon Kean University; Ms. Piyaporn Towannang and Kanittha Triamamornwooth, MPham, King Chulalongkorn Memorial Hospital, Bangkok, Thailand; Mrs. Nisa Thongbor, Department of Medicine, Sunpasitthiprasong Hospital, Ubon Ratchathani, Thailand; Ms. Nipa Aiyasanon, Division of Nephrology, Department of Internal Medicine, Siriraj Hospital, Bangkok, Thailand; Mrs. Donkum Kaewboonsert, and Mrs. Pensri Uttayotha, Department of Medicine, Phayao Hospital, Phayao, Thailand; Wichai Sopassathit, MD, and Mrs. Salakjit Pitakmongkol, Division of Nephrology, Department of Internal Medicine, Pranangklao Hospital, Nonthaburi, Thailand; Ussanee Poonvivatchaikarn, MD, and Mrs. Bunpring Jaroenpattrawut, Nephrology Clinic, Nakhon Pathom Hospital, Nakhon Pathom, Thailand; Somphon Buranaosot, MD, Sukit Nilvarangkul, MD, and Ms. Warakoan Satitkan, Bangkok Metropolitan Administration General Hospital, Bangkok, Thailand; Wanida Somboonsilp, MD, Pimpong Wongtrakul, MD, Ms. Ampai Tongpliw, and Ms. Anocha Pullboon, Continuous Ambulatory Peritoneal Dialysis (CAPD) Clinic, Chaoprayayomraj Hospital, Suphanburi, Thailand; Ms. Montha Jankramol, Division of Nephrology, Department of Internal Medicine, Taksin Hospital, Bangkok, Thailand; Mrs. Apinya Wechpradit, Renal Unit, Department of Internal Medicine, Udonthani Hospital, Udon Thani, Thailand; Ms. Chadarat Kleebchaiyaphum, Nephrology Division, Department of Internal Medicine, Chaiyaphum Hospital, Chaiyaphum, Thailand; Wadsamon Saikong, MD, Mrs. Worauma Panya, and Mrs. Siriwan Thaweekote, CAPD Clinic, Mukdahan Hospital, Mukdahan, Thailand; Sriphrae Uppamai, MD, Jarubut Phisutrattanaporn, MD, and Mrs. Sirirat Sirinual, Department of Internal Medicine, Sukhothai Hospital, Sukhothai, Thailand; Setthapon Panyatong, MD, Puntapong Taruangsri, MD, Mrs. Boontita

Prasertkul, and Ms. Thanchanok Buanet, Kidney Center, Department of Internal Medicine, Nakornping Hospital, Chiang Mai, Thailand; Mrs. Panthira Passorn, Dialysis Unit, Sawanpracharak Hospital, Nakhon Sawan, Thailand; Mrs. Rujira Luksanaprom, Department of Medicine, Trang Hospital, Trang, Thailand; Angsuwarin Wongpiang, MD, and Ms. Metinee Chaiwut, CAPD, Pong Hospital, Phayao, Thailand; Mrs. Ruchdaporn Phaichan, Department of Medicine, Chaophya Abhaiphubejhr Hospital, Prachin Buri, Thailand; Peerapach Rattanasoonton, MD, and Mrs. Wanlaya Thongsiw, Department of Medicine, Trat Hospital, Trat, Thailand; Narumon Lukrat, MD, and Mrs. Sayumporn Thaitrng, CAPD Unit, KhueangNai Hospital, Ubon Ratchatani, Thailand; Mrs. Yupha Laoong, and Mrs. Niparat Pikul, Division of Nephrology, Department of Medicine, Amnatcharoen Hospital, Amnatcharoen, Thailand; and Mrs. Navarat Rukchart, Mrs. Korawee Sukmee, and Mrs. Wandee Chantarungsri, Kidney Diseases Clinic, Department of Internal Medicines, Songkhla Hospital, Songkhla, Thailand. This study was supported by Rachadaphiseksompot Endowment Fund, Chulalongkorn University, Thailand (CU-GRS_61_06_30_01); National Research Council of Thailand, Thailand (6/2562); and Thailand Research Foundation, Thailand (IRG5780017). TK is a current recipient of the National Research Council of Thailand.

DJ is supported by an Australian National Health and Medical Research Council Practitioner Fellowship.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Characteristics and outcomes of patients with local negative cultures.

Supplementary Questionnaire: Survey of PD Fluid Sampling and Microbiological Laboratory Culture Practices

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