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Original Research Article

Assessment of microbiological profile in peritoneal dialysis in patients with chronic kidney disease

M Sornaranjani[1], CP Ramani[2,*], K Karuppasamy[3]

[1]*Dept. of Microbiology, Government Kilpauk Medical College, Chennai, Tamil Nadu, India*
[2]*Institute of Microbiology, Madras Medical College, Chennai, Tamil Nadu, India*
[3]*Dept. of Orthopaedics, Government Stanley Medical College, Chennai, Tamil Nadu, India*

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ABSTRACT

Background: Peritonitis is a serious complication of peritoneal dialysis (PD). It is the primary reason for hospitalization and switching over to hemodialysis. In the present study, we aimed to determine the microbiological profile of peritoneal dialysis fluid in patients with acute and chronic renal failure and identify these organisms' susceptibility patterns.
Materials and Methods: This cross-sectional study was conducted in the Institute of Microbiology and Institute of Nephrology in the Rajiv Gandhi Government General Hospital from April 2016 to March 2017. A total of 100 patients who were >18 years of age, acute and chronic renal failure patients who underwent Peritoneal Dialysis and patients on Continuous and Intermittent Peritoneal Dialysis were included.
Results: The study population included 100 patients who satisfied the inclusion criteria. 63% were males, and 37% were females. The patients had a mean age of 44.15±13.89. 28 samples were culture positive, out of which 13 (46.4%) were Gram-negative, 10 (35.7%) were Gram-positive and 5 (17.9%) were Fungal isolates. Among them, the majority were Acinetobacter baumannii (20%) and Candida non-albicans (20%), followed by Staphylococcus aureus (16%), Coagulase-negative Staphylococcus (16%), Klebsiella oxytoca (8%), Klebsiella pneumoniae (4%), Enterococcus faecalis (4%).
Conclusion: Peritoneal infections were more common in patients with longer duration of dialysis and diabetes mellitus. CAPD patients were having a higher risk of infections compared to IPD. As the number of infections associated with peritoneal dialysis rises, routine PD fluid microbiological analysis after the procedure will help improve patient care by using appropriate antibiotics as soon as possible before any major clinical problem arises.

1. Introduction

Chronic Kidney Disease (CKD) affects 10% of the world's population, and millions of people die each year due to a lack of affordable treatment options. According to the Global Burden of Disease study ranking in the year 2010, CKD held 18^{th} place in the number of deaths caused globally. WHO reported 58 million deaths worldwide, of which 35 million were due to CKD in the year 2005.[1] Chronic Kidney Disease refers to irreversible kidney impairment that lasts longer than three months and causes a structural and functional abnormality of the kidneys with or without decreased Glomerular Filtration Rate (GFR).[2] In End-Stage Renal Disease (CKD stage V), a complete cessation of effective kidney function and renal replacement therapy like Hemodialysis or Peritoneal-dialysis or Kidney transplantation may be needed.[3] Another condition is called Acute Renal Failure, where the rapid loss of renal function

* *Corresponding author*.
E-mail address: cpramani68@gmail.com (C. P. Ramani).

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leads to a rapid decline in GFR and the everyday rise in serum creatinine and blood urea nitrogen. This condition will be reversed if the underlying causes are resolved.[4]

Peritoneal dialysis is a common and effective type of renal replacement therapy (15-50%). Peritoneal dialysis does not involve direct access to the circulatory system, and the insertion of a peritoneal catheter enables a dialysate solution to be infused into the abdominal cavity.[5] Many modalities exist for Peritoneal Dialysis now, which are broadly classified as continuous or intermittent dialysis. The main complication of Peritoneal Dialysis is peritonitis which arises the need for hospitalization and hemodialysis and also remains the cause of 2 to 25% mortality rates.[6] The incidence of peritonitis in acute dialysis is 0.5% to 4%. Gram-positive bacteria most commonly cause peritonitis. Coagulase-negative Staphylococci (40-65%)is the predominant one, followed by Staphylococcus aureus (10-25%). Gram-negative organisms are associated with 20-30% of all infections among them (7-12%)is more commonly isolated. Fungal infections are responsible for 1% to 15% of peritonitis episodes, the incidence being 0.2-1.7 episodes per 12 patients per month of dialysis. The risk of PD-related infections significantly reduces the longevity of this procedure.

2. Aim

This study aimed to determine the microbiological profile of peritoneal dialysis fluid in patients with Acute and Chronic Renal Failure in a trtiary care hospital and identify these organisms' susceptibility patterns.

3. Materials and Methods

This cross-sectional study was conducted in the Institute of Microbiology and Institute of Nephrology in the Rajiv Gandhi Government General Hospital from April 2016 to March 2017. A total of 100 patients under dialysis who satisfied the inclusion criteria were included in the study. Informed consent was obtained from all the study patients.

3.1. Inclusion criteria

1. Patients> 18 years of age.
2. Acute and chronic renal failure patients who underwent peritoneal dialysis.
3. Patients on continuous and Intermittent peritoneal dialysis.

3.2. Exclusion criteria

1. Patients below 18 years of age.
2. Patients already on antibiotics treated for peritonitis were not included in this study.

Data collection included patient's name, age, IP number, occupation, address, date of admission, clinical diagnosis at admission, presenting complaints, type of dialysis, frequency of dialysis, prior antibiotic therapy, comorbid conditions.

3.3. Sample collection & processing

Under aseptic precautions, the dialysate was collected from the dialysate bag and transported immediately to the laboratory and processed as per standard guidelines.

For cell count investigation, 5 mL of dialysate was examined under the direct microscope. The dialysate was aspirated and centrifuged for 5 minutes at 1500g. The supernatant was then discarded, and a 0.5 ml deposit was taken instead. A sterile distilled water solution of 10 mL was added to the centrifuged deposit and vigorously agitated for 30 seconds. The fluid was centrifuged at 1500g for 5 minutes once more. Potassium hydroxide [KOH mount] mount, Gram's staining, and aerobic bacterial and fungus culture were all done on the deposit. The sediment was incubated at 37°C for 24-48 hours after plating on 5 percent sheep blood agar, chocolate agar, and MacConkey agar. A candle jar was used to incubate chocolate agar plates at 37°C. The sediment was likewise injected and cultured at 37°C in the Brain Heart Infusion broth. The turbidity of BHI broth was evaluated for a week, and culture was performed. The effluent was streaked on two Sabouraud's Dextrose agar plates and cultured for four weeks at 25°C and 37°C, with growth, monitored at regular intervals. According to CLSI recommendations, antimicrobial susceptibility testing was performed using the Kirby Bauer Disc Diffusion method on Mueller Hinton agar. Statistical analysis was carried out using SPSS software.

4. Results

The study population included 100 patients who satisfied the inclusion criteria. Among the total patients, 63% were males and 37% were females. Most of the study patients belonged to the age group of 31-40 years. The patients had a mean age of 44.15±13.89.(Tables 1 and 2)

Table 1: Gender distribution of cases (n=100)

Gender	Number of patients	Percentage
Male	63	63%
Female	37	37%
Total	100	100%

In the present study, 92% of the patients had chronic kidney disease and only 8% had acute kidney injury and 96% were under intermittent peritoneal dialysis and 4% were under continuous ambulatory peritoneal dialysis. (Table 3)

Out of 100 samples, 28 samples were culture positive. Among them, 13 (46.4%) were Gram-negative, 10 (35.7%) were Gram-positive and 5 (17.9%) were fungal isolates.

Table 2: Age wise distribution of the patients (n=100)

Age	No of patients	Percentage
18-30 years	22	22%
31-40 years	25	25 %
41-50 years	21	21%
51-60 years	18	18%
>60 years	14	14%
Total	100	100%

Table 3: Clinical diagnosis of dialysis patients (n=100)

Diagnosis	Number of patients	Percentage (%)
CKD	92	92%
AKI	8	8%
Total	100	100%

Table 4: Mode of dialysis (n=100)

Type of dialysis	Number of patients	Percentage (%)
IPD	96	96.0%
CAPD	4	4.0%
Total	100	100.0%

Figure 1

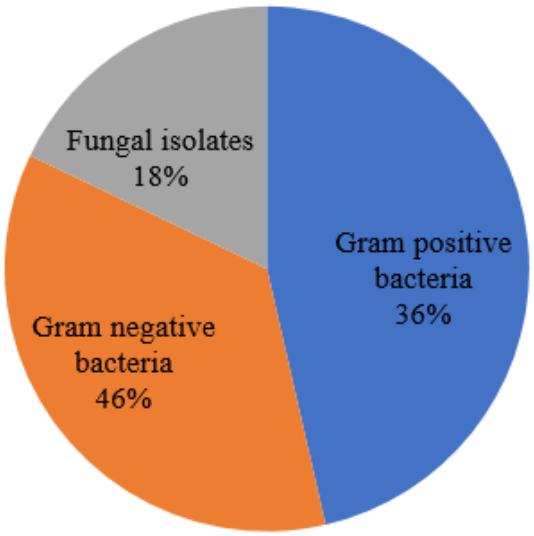

Fig. 1: Distribution of pathogens among dialysis patients (n=28)

Among the 28 culture-positive patients, 89.2% were isolated from Intermittent Peritoneal Dialysis. Among them the majority were Acinetobacter baumannii (20%) and Candida non albicans (20%) followed by Staphylococcus aureus (16%), Coagulase-negative Staphylococcus (16%), Klebsiella oxytoca(8%), Klebsiella pneumonia (4%), Enterococcus faecalis (4%). (Table 5)

Out of 28 isolates, 3 (10.7%) were isolated from Continuous Ambulatory Peritoneal Dialysis patients. The pathogens isolated from CAPD were Klebsiella pneumoniae (33.3%), Pseudomonas aeruginosa (33.3%), Escherichia coli (33.3%).(Table 6)

Table 5: Pathogens isolated from patients with intermittent peritoneal dialysis (n=25)

Isolated pathogens	No. of isolates	Percentage (%)
Acinetobacter baumannii	5	20%
Staphylococcus aureus	4	16%
Staphylococcus epidermidis	4	16%
Escherichia coli	3	12%
Klebsiella oxytoca	2	8%
Klebsiella pneumoniae	1	4%
Enterococcus faecalis	1	4%
Candida non-albicans	5	20%

Table 6: Pathogens isolated from patients with continuous ambulatory peritoneal dialysis (n=3)

Isolated pathogens	No of isolates	Percentage (%)
Klebsiella pneumoniae	1	33.3%
Pseudomonas aeruginosa	1	33.3%
Escherichia coli	1	33.3%

Out of 28 culture-positive patients, 10 patients (35.7%) had peritoneal infection symptoms, and 18 patients (64.2%) had no symptoms. In 72 culture-negative patients, 12 patients (16.6%) had peritoneal infection symptoms, and 60 patients (83.3%) did not have any symptoms. (Table 7) Out of 100 patients who underwent dialysis, the majority, 23% were diabetic, followed by 3%, had cardiac failure, 1% had decompensated liver disease and 6% patients had all these diseases.

Table 7: Frequency of symptoms of peritonitis with culture positivity

Culture	Symptoms of peritonitis	Number of patients	Percentage (%)
Positive (n=28)	Present	10	35.7%
	Absent	18	64.2%
Negative (n=72)	Present	12	16.6%
	Absent	60	83.3%

*p-value-0.0001

The antibiotic sensitivity patterns of the culture organisms are tabulated in Tables 8, 9 and 10. 25% of Staphylococcus aureus and 25% of Coagulase-negative Staphylococcus aureus were Methicillin-resistant. All Methicillin-resistant Staphylococcus species (n=2) were sensitive to Vancomycin E-Strip test. MIC for both isolates were less than two. mecAgene was detected in Methicillin-Staphylococcus species and OXA23 gene was detected in MBL producing Acinetobacter baumannii by polymerase chain reaction.

Table 8: Antimicrobial susceptibility pattern of gram-positive isolates (n=10)

Antibiotics	Coagulase Negative Staphylococcus	Staphylococcus aureus	Enterococcus feacalis
Penicillin	(1)25%	0(0%)	1(100%)
Ampicillin	_	_	1(100%)
Amikacin	(3)75%	4(100%)	1(100%)
High Level Gentamicin	_	_	1(100%)
Erythromycin	4(100%)	4(100%)	1(100%)
Trimethoprim sulfamethoxazole	2(50%)	4(100%)	0(0%)
Ciprofloxacin	1(25%)	2(50%)	0(0%)
Clindamycin	4(100%)	4(100%)	1(100%)
Cefoxitin	2(50%)	3(75%)	_
Vancomycin	1(100%)	1(100%)	_
Linezolid	4(100%)	4(100%)	1(100%)
Chloramphenicol	4(100%)	4(100%)	1(100%)

Table 9: Antibiotic susceptibility pattern of gram negative isolates (n=13)

Antibiotics	Escherichia coli	Acinetobacter baumannii	Klebsiella pneumoniae	Klebsiella Oxytoca
Amikacin	2(75%)	2(40%)	2(100%)	2(100%)
Ampicillin	3(75%)	_	_	_
Trimethoprim sulfamethoxazole	1(25%)	2(40%)	2(100%)	2(100%)
Ciprofloxacin	1(25%)	1(20%)	1(50%)	_
Cefotaxime	3(75%)	_	1(50%)	1(50%)
Ceftazidime	_	1(20%)	_	_
Imipenem	4(100%)	4(80%)	2(100%)	2(100%)
Piperacillin Tazobactam	4(100%)	4(80%)	2(100%)	2(100%)
Tetracycline	4(100%)	4(80%)	2(100%)	2(100%)
Colistin	_	1(100%)	_	_

Table 10: Antifungal susceptibility testing of candida species

Candida Species	Fluconazole	Itraconazole	Amphotericin B	Clotrimoxazole	Ketoconazole	Nystatin
Candida tropicalis (n=2)	50%	100%	100%	100%	50%	50%
Candida glabrata (n=1)	100%	100%	100%	100%	100%	100%
Candida parapsilosis (n=2)	50%	100%	100%	100%	100%	100%

5. Discussion

In patients with Acute and Chronic Renal Failure, peritoneal dialysis is an excellent treatment choice. Peritonitis is the most common complication of peritoneal dialysis, and it continues to be a reason for hospitalization, catheter removal, peritoneal dialysis discontinuation and switch to hemodialysis. In bacterial peritonitis, mortality rates range from 2% to 25%, and in fungal peritonitis, mortality rates range from 5% to 53%. As a result, a routine PD effluent culture should be performed after the procedure to help detect patients at risk of developing peritonitis at an early stage. This can extend the longevity of peritoneal dialysis.

100 patients who underwent intermittent and continuous peritoneal dialysis were included in the present study. Among dialysis patients, 24% were diabetics, 12% had cardiac failure, 6% had coronary artery disease, 2% had decompensated liver disease and 56% were without any comorbid conditions. Among the culture-positive cases, 78.6% were males and 21.4% were females. Most of them were in the age group of 51-60 years which is similar to the study by Sharon J et al. in 2009.[7] This could be due to the high prevalence of immunosuppression and end-stage renal disease in elderly patients.

Among the dialysis patients, 92% had chronic kidney disease and only 8% had acute kidney Injury. Culture

positivity was seen in 30.4% of CKD patients. This indicates that the risk of getting the infection is high in patients who had been in dialysis for a longer duration. Among the culture-positive cases, 26.04% were in intermittent peritoneal dialysis and 75% Continuous ambulatory peritoneal dialysis. The results were similar to Sharma et al. and Soham Gupta et al., who showed 30% culture positivity among IPD patients and 60% among CAPD patients.[8] This variation may be due to faulty sterile technique and increased number of exchanges in CAPD compared to IPD.

Clinical peritonitis was seen in 7% of patients, similar to Bonnie et al. 11%.[9] Meanwhile, 15% showed culture-negative though the cell count was >100/μl, correlated well with the similar studies where 10-50% culture-negative was reported.[10] This non-specific rise in effluent cell count may be due to other extra peritoneal infections like exit site infection, abdominal surgery and diverticulitis. Culture negative peritonitis may be due to constant flow of dialysis fluid into and out of the peritoneal cavity diluting the microbial density to be low or due to infection with fastidious organisms like fungi, mycobacteria, Legionella, Campylobacter, Ureaplasma species, Mycoplasma or enteroviruses or noninfectious causes like chemical irritation (by icodextrin), chylous ascites or effluent eosinophilia.[11,12]

Among the culture-positive cases, 46.4% were Gram-negative bacteria and Gram-positive bacteria constitute 35.7%. Similar findings were reported by Verbrugh et al. in 1984 showed Gram-negative bacteria as the predominant isolates.[13] The study by Prasad N et al. also reported that 60% were Gram-negative and only 30% were Gram-positive organisms.[14]

In the present study, Acinetobacter baumannii constitutes 20% in intermittent peritoneal dialysis cases; similar findings were observed in the study by Sharma et al., in which they showed 21.5% of Acinetobacter baumannii.[8] This may be due to the hygiene breaks and failure to perform sterile exchange procedures. But in Gram-positive isolates, Staphylococcus aureus was 16% in the present study which correlated well with the studies of Sharma et al.Staphylococcus aureus constitutes 25%. Infection with occurs due to touch contamination of catheter infection, or the patient may be a nasal carrier of Staphylococcus aureus techniques.[15]

17.9% of fungal pathogens were isolated in our study. The study by KV Kumar et al. also reported 14% fungal pathogens.[16] All isolates were found to be Candida non-albicans. Similar findings were reported by Jasmin Levallois et al., showing Candida non-albicans as the predominant causative agent. Change in the epidemiology of fungal isolates from Candida albicans to Candida nonalbicans occurs in the post prophylactic era and after usage of fluconazole. Several of these non-albicans Candida species (e.g., C. glabrata and C. krusei) exhibit resistance to traditional triazole antifungals like fluconazole, and may also demonstrate cross-resistance to newer triazoles.[17]

Among the Staphylococcal isolates, 25% each of Staphylococcus aureus and Staphylococcus epidermidis were found to be methicillin-resistant. The source of MRSA could be community-acquired or hospital-acquired. The lattof hospital staff. Therefore, the crucial strategy in avoiding this is through hand disinfection and the therapeutic regimen includes mupirocin nasal ointment combined with parenteral vancomycin administration. Klebsiella species and 25% of Escherichia coli were ESBL producers. Similar findings were also observed in the Kashinath Prasad study, which showed 54.3% were ESBL producers were associated with the development of ESBL production. Therefore, prevention by judicious use of antibiotics and infection prevention measures like hand wash, proper exit site care will be the most efficacious step.[18] antifungal susceptibility of Candida isolates, resistance to Fluconazole, Itraconazole and Ketoconazole are more common than Amphotericin B and Nystatin.

6. Conclusion

In this cross-sectional study, peritoneal infections were more common in patients with longer dialysis duration and diabetes mellitus. CAPD patients were having a higher risk of infections compared to IPD. This can be prevented by advising the patients to maintain proper glycemic control and follow sterile techniques during exchanges. Acinetobacter baumannii was the most common Gram-negative pathogen, which can be decreased by re-education and sterility maintenance. Routine PD fluid microbiological analysis after a peritoneal dialysis procedure will help improve patient care by using appropriate antibiotics as soon as possible before any major clinical problems arise.

7. Conflict of Interest

The authors declare that they have no conflict of interest.

8. Source of Funding

None.

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Author biography

M Sornaranjani, Assistant Professor

CP Ramani, Professor

K Karuppasamy, Assistant Professor

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