

## Research Article

# Effect of virulent anaerobes on the outcome of peritoneal dialysis associated peritonitis

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### ABSTRACT

**Background:** Anaerobic bacteria do not account much of peritoneal dialysis (PD) associated peritonitis; hence most of the reports do not discuss this issue. In the present study we analyze PD patients who developed anaerobic peritonitis.

**Methods:** In between January 2000 to August 2015, we tried to analyze PD patients who have developed only anaerobic peritonitis as a single pathogen, the virulence and impact of those virulent anaerobes on the outcome.

**Results:** In our retrospective study we identified 42 episodes of anaerobic peritonitis which include 36 patients of single episodes and 6 patients of two episodes. The microscopic examination of dialysate pellet revealed 55.6% gram negative bacilli, 36.1% gram positive cocci and 8.3% gram positive bacilli. Culture examination revealed 47.3% *Bacteroides* species, 16.7% *Fusobacterium* species, 13.7% *Lactobacillus acidophilus*, 16.7% *Peptostreptococcus anaerobius*, 2.8% *Peptococcus* and 2.8% *Propionibacterium* acnes. On analyzing the outcome data through chi test, we inferred that *Bacteroides fragilis* (36.1%) was the major cause of death among gram negative anaerobes while among gram positive anaerobes *peptostreptococci* (16.7%) was the major cause of death in majority of patients. Statistically significant association was found between virulent anaerobes and their outcome.

**Conclusion:** Our results revealed that among both in gram negative anaerobes *bacteroides fragilis* and in gram positive anaerobes *peptostreptococci* were the major cause of death in PD associated anaerobic peritonitis. Hence prompt identification of virulent anaerobes may alarm us for early and judicious management to reduce morbidity, mortality and technique failure in PD associated anaerobic peritonitis.

**Keywords:** End stage renal disease, CAPD, Virulence, Anaerobes

### INTRODUCTION

End stage renal disease (ESRD) is associated with a state of immune dysfunction characterized by immunodepression that likely contributes to the high prevalence of infections.<sup>1</sup> Continuous ambulatory peritoneal dialysis (CAPD) is an important treatment option for patients with end-stage renal disease. Although, the rate of peritonitis has declined in recent years because of significant technical improvements in CAPD techniques, peritonitis remains the leading cause

for discontinuation of CAPD. Alteration in peritoneal defences may increase the risk of peritonitis in patients undergoing CAPD.<sup>2</sup> Impaired maturation of thymus lymphocytes, seen in PD subjects, also leads to a disabled immune response and infection susceptibility.<sup>3</sup> Anaerobic peritonitis is a serious complication of peritoneal dialysis but previous reports on this aspect are limited only to small single centre studies.

According to the guidelines of International Society of peritoneal dialysis (ISPD) anaerobic associated PD

peritonitis episodes were discussed mainly with in the poly microbial peritonitis sections and surgical interventions were recommended if anaerobic pathogens co-exist with other enteric microorganisms.<sup>4</sup> However, not all anaerobic PD associated peritonitis episodes occur with intra-abdominal catastrophe.<sup>5</sup> Issues pertaining to peritonitis from a single anaerobic pathogen also remained unexplored, and reports on anaerobic PD associated peritonitis were extremely sparse. Therefore, this retrospective study analyzed the PD patients who have developed only anaerobic peritonitis as a single pathogen, the virulence and impact of those virulent anaerobes on the outcome.

### METHODS

It is a retrospective study involving patients undergoing CAPD at our centre who developed PD associated peritonitis over a period of 16 years, from Jan 2000 to March 2015.4 Out of 402 patients, 245 (60.94%) ESRD patients developed bacterial peritonitis, 65 (16.16%) patients developed fungal peritonitis and 92 (22.90%) patients were found culture negative.<sup>6</sup> Out of 245 patients, 202 (82.44%) patients developed aerobic peritonitis, single patient (0.42%) suffered with mycobacterium infection and 42 (17.14%) patients developed anaerobic peritonitis. Aerobic, mycobacterium, polymicrobial and fungal peritonitis were excluded from the analysis due to their different outcomes.

As per the peritoneal dialysis related infection recommendations published by ISPD in 2010,<sup>4</sup> the patient's exchange bags, containing effluent dialysate were received in the microbiology laboratory for macro level examination, microscopic examination and culturing simultaneously. From these bags, 100 ml of fluid was withdrawn with a sterile needle and syringe under aseptic conditions. The fluid was centrifuged in sterile tubes at a rate of 3000 g for 15 minutes and supernatant was discarded, leaving 0.5 ml. In the centrifuged deposit, 10 ml of sterile distilled water was added together and the mixture was shaken vigorously on vortex for 30 sec. This mixture was then divided into 4 parts of 1 ml, 3 ml, 3 ml and 3 ml each. 1 ml was further divided for staining characteristic like gram stain, Z.N. stain, and lacto phenol cotton blue film, while 3 ml in FA bottle for isolation of aerobes, 3 ml in FN bottle and remaining 3 ml in MP bottle for the isolation of anaerobes and mycobacterium respectively. These three inoculated bottles were further incubated in BactAlert 3D system following standard protocols. The isolated pathogens were re-examined microscopically to ensure the staining and morphologic characteristic of organism. Further identification was done at species level by the Vitek-2 system.<sup>7-8</sup>

Statistical analysis was performed using chi square test and contingency coefficient. Data were expressed as

mean±standard deviation. Statistical significance was defined at a p value of 0.05.

### RESULTS

During the period from January 2000 to June 2015, 402 ESRD patients were initially on CAPD. The total number of episodes of bacterial peritonitis during the entire period was 245, out of these 42 patients developed anaerobic peritonitis. These 42 patients include 36 patients of single episode and 6 patients of 2 episodes. The average rate of anaerobic peritonitis for the entire period was.<sup>3</sup> 77 episodes/CAPD year. Their base line and demographic data were described as follows. Out of total anaerobic peritonitis population male was 37.1% and females were 63.9%, the mean age of the study population was 54.11±8.300, the mean duration on CAPD before development of anaerobic peritonitis was 21.01±8.045 months and predominant causes of ESRD in this group were diabetic nephropathy (41.7%), glomeulonephritis (35.6%), hypertension (14.4%) and others were (8.3%).

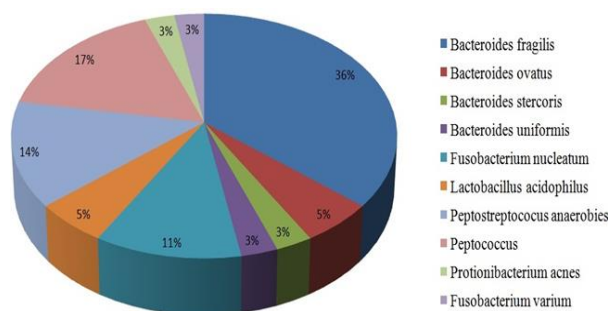


Figure 1: Spectrum of anaerobes.

The microscopic examination of dialysate pellet revealed 55.6% gram negative bacilli, 36.1% gram positive cocci and 8.3% gram positive bacilli. Culture examination revealed 47.3% bacteroides species, 16.7% *Fusobacterium species*, 13.7% *Lactobacillus acidophilus*, 16.7% *Peptostreptococcus anaerobies*, 2.8% *Peptococcus* and 2.8% *Propionibacterium acnes*. Among genus *Bacteroides*, *Bacteroides fragilis* (36.1%) was the most frequent species isolated (Figure 1).

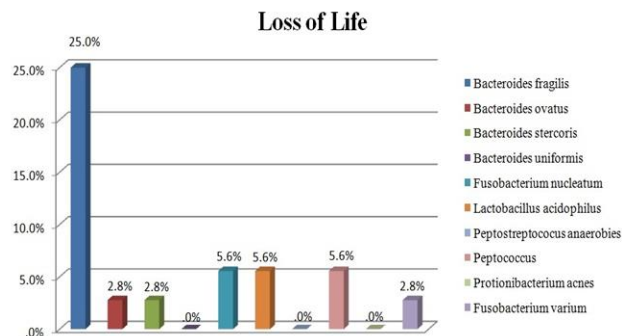


Figure 2: Statistically significant outcome (loss of life) due to anaerobes.

The impact of anaerobes on their outcome revealed that 25.0% loss of life and 5.6% hospitalization were more significant with *Bacteroides fragilis* while 5.6% loss of life was found to be frequent in *Fusobacterium nucleatum* (Table 1). On analyzing the outcome data obtained from all the 36 single episodes of PD associated anaerobic peritonitis through chi test, we inferred that

*Bacteroides fragilis* (36.1%) was the major cause of death among gram negative anaerobes while among gram positive anaerobes peptostreptococci (16.7%) was the major cause of death in majority of patients (Figure 2). Statistically significant association was found between virulent anaerobes and their outcome at 0.05 level.

**Table 1: Association between virulent anaerobes and outcome.**

Anaerobes	Gram	Outcomes					Total	df	Pearson Chi-square
		Loss of life	Loss of catheter	Hospitalization	Change of modality	Cure			
<i>Bacteroides fragilis</i>	GNB	9 25.0%	1 2.8%	2 5.6%	0 0.0%	1 2.8%	13 36.1%	1	5.629*
<i>Bacteroides ovatus</i>	GNB	1 2.8%	1 2.8%	0 0.0%	0 0.0%	0 0.0%	2 5.6%		
<i>Bacteroides stercoris</i>	GNB	1 2.8%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	1 2.8%		
<i>Bacteroides uniformis</i>	GNB	0 0.0%	0 0.0%	0 0.0%	0 0.0%	1 2.8%	1 2.8%		
<i>Fusobacterium nucleatum</i>	GNB	2 5.6%	1 2.8%	0 0.0%	0 0.0%	1 2.8%	4 11.1%		
<i>Fusobacterium varium</i>	GNB	2 5.6%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	2 5.6%		
<i>Lactobacillus acidophilus</i>	GPB	0 0.0%	2 5.6%	1 2.8%	1 2.8%	1 2.8%	5 13.9%		
<i>Peptostreptococcus anaerobies</i>	GPC	2 5.6%	0 0.0%	0 0.0%	0 0.0%	4 11.1%	6 16.7%		
<i>Peptococcus</i>	GPC	0 0.0%	0 0.0%	0 0.0%	0 0.0%	1 2.8%	1 2.8%		
<i>Propionibacterium acnes</i>	GPB	1 2.8%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	1 2.8%		
Total		18 50.0%	5 13.9%	3 8.3%	1 2.8%	9 25.0%	36 100.0%		

\* Significant at 0.05 level

## DISCUSSION

There is a wealth of evidence that disorders of both innate and adaptive immune systems contribute to an increased rate of infections in the course of ESRD. Functional abnormalities of monocytes, neutrophils, and dendritic cells are directly linked with infection risk in this patient population.<sup>3,8-11</sup> Anaerobes are the most predominant components of the normal human skin and mucosal membrane flora hence are the common cause of bacterial infections of endogenous origin. These infections contribute significantly to morbidity and mortality prompting us an early diagnosis and treatment.<sup>12</sup>

It is well known that most of the reports on PD associated peritonitis do not discuss about anaerobes therefore, dedicated description of anaerobic peritonitis features lack in literature. The potential factors associated with anaerobic peritonitis in PD patients might be a recent exposure to antibiotics, failure of technique sterility, transmural migration, intra-abdominal events and baseline diagnosis<sup>8</sup> but another route of infection in PD peritonitis

is ascending infection from the pelvic organs in females.<sup>13</sup>

Our observations revealed 3.77 anaerobic episodes/CAPD year while according to ANZDATA registry, anaerobic peritonitis accounts for only 0.1% of single pathogen peritonitis over a period of 6 years. In USA and Canada, anaerobic peritonitis constitutes <1% of all episodes. Another study reported 1.59% anaerobic peritonitis.<sup>5</sup>

In our case series, out of 36 single episodes, 47.3% *Bacteroides* species, 16.7% *Fusobacterium* species, 13.7% *Lactobacillus acidophilus*, 16.7% *Peptostreptococcus anaerobies*, 2.8% *Peptococcus* and 2.8% *Propionibacterium acnes* were isolated. Hence, here in for the first time in the world we are reporting largest case series on PD associated anaerobic peritonitis. Chao et al reported *Bacteroides fragilis* in 4 episodes, *Lactobacillus* species in 3 episodes, *Bacteroides thetaiotaomicron* in 2 episodes, and *Peptostreptococcus* species in 1 episode.<sup>5</sup>

The factors that determine the outcome of an anaerobic infection are the balance between the bacterial and the host factors. The bacterial factors include the inoculum size, the virulence and the synergistic potential of the infecting organisms, while the opposing host factors include the host defense, breaks in the anatomic barriers and reduction in the oxidation reduction potential.<sup>14</sup> The antimicrobial function of peritoneal macrophages and polymorph nuclear cells generally requires the presence of opsonins. A reduction in the levels of immunoglobulin G and G3 has been noted in peritoneal dialysis effluents in comparison with serum, and the concentrations of these crucial opsonizing agents are related inversely to the frequency of peritonitis.<sup>15</sup> Anaerobes can deplete or bind opsonins that bind to aerobes, thus preventing their opsonization; Other important factors that impair host defence mechanisms are low pH and high osmolality of peritoneal dialysis fluid, both can impair polymorph nuclear leukocyte function and antibiotic efficacy and anaerobes suppress the activity of those polymorph nuclear leukocytes, macrophages, and lymphocytes. *Bacteroides fragilis* can also interact with peritoneal macrophages inducing procoagulant activity and fibrin deposition that impairs clearance of the infecting organisms.<sup>16</sup> Neutrophils killing ability can be inhibited by short chain fatty acids produced by *Bacteroides fragilis* and other anaerobic gram-negative bacilli (AGNB).<sup>17</sup>

The impact of anaerobes on their outcome revealed that 25.0% loss of life and 5.6% hospitalization were more significant with *Bacteroides fragilis* while 5.6% loss of life was more frequent in *Fusobacterium nucleatum* as shown in table 1. On analyzing the outcome data obtained from all the 36 single episodes of PD associated anaerobic peritonitis through chi test, we inferred that *Bacteroides fragilis* (36.1%) was the major cause of death among gram negative anaerobes while among gram positive anaerobes *Peptostreptococci* (16.7%) was the major cause of death in majority of patients. Statistically significant association was found between virulent anaerobes and their outcome.

Strains of the *Bacteroides* and *Fusobacterium* produce Superoxide mutase that allows them to survive in oxygenated tissues until the proper reduced conditions are established for their growth.<sup>18-20</sup> They specifically have a polysaccharide lined 'micro-capsule' external to the outer membrane, which can aggregate and form blebs which involved in adhesion, abscess formation, and impaired phagocytosis of microorganisms. *Bacteroides fragilis* produce neuraminidase that can catalyse the removal of the silica acid from host cell surface and from important immunoactive proteins such as IgA and some components of complement and may consequently disrupt important host functions.<sup>21</sup> The induction of endotoxin liberation on exposure to antibiotics was many times higher with *Bacteroides fragilis* than the other anaerobes, which may also help to explain why this

species is particularly associated with clinical infections and high mortality rate.<sup>22</sup>

*Fusobacterium nucleatum* agglutinates human erythrocytes and also binds to collagen,<sup>23</sup> fibronectin<sup>24</sup> and glycosylated protein-rich glycoprotein.<sup>25-26</sup> *Fusobacterium nucleatum* secretes an immunosuppressive protein (FiP) that arrests human T cells in the mid-G1 phase of the cell cycle.<sup>27-28</sup> Krepel et al suggested that peptostreptococcal production of proteolytic enzymes may have an important adjunctive effect on the pathogenesis of certain soft tissue infections.<sup>29</sup> Thus highly complex roles of these virulent strains in pathogenesis of peritonitis increase the morbidity and mortality in PD associated peritonitis.

Our results summarized that among both in gram negative anaerobes *Bacteroides fragilis* and gram positive anaerobes *Peptostreptococci* were the major cause of death in PD associated anaerobic peritonitis. Although the list of virulence factors and anaerobic bacteria is not complete it goes some way to illustrate the plethora of factors and mechanisms that can be involved in PD associated anaerobic peritonitis. Hence prompt identification of virulent anaerobes may alarm us for early and judicious management to reduce morbidity, mortality and technique failure in PD associated anaerobic peritonitis.

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