Predictive Factors of Outcome Following Non-Fermenting Gram-Negative Bacilli Peritonitis in Peritoneal Dialysis

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Research article

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Abstract

Background Peritonitis due to Gram-negative bacilli (GNB), particularly the non-fermenting GNB (NF-GNB) is a serious complication of peritoneal dialysis (PD) with a low-resolution rate. Beyond the patient's conditions, microbiological properties as antimicrobial resistance, biofilm and other virulence factors production could explain the bad outcomes. This study aimed to evaluate the influence of the patient's conditions, microbiological characteristics, including biofilm production, and treatment of peritonitis on the peritonitis resolution.

Methods We reviewed the records of 68 peritonitis episodes that occurred between 1997 and 2015 at a single university center. The influence of microbiological and clinical factors on resolution chance was analyzed by logistic regression.

Results The etiologies were species of Pseudomonas (51.5%), Acinetobacter (30.9%), and others (18.6%). There was a high (75%) proportion of biofilm producers' lineages. The in vitro susceptibility rate of Pseudomonas spp. to amikacin, ciprofloxacin, and ceftazidime was significantly greater than that of Acinetobacter spp. and other species; however, there was a similar low-resolution rate (<40%) among the episodes due to these etiologies. Concomitant ESI and resistance to amikacin were independently associated with non-resolution. No other factor, including biofilm production were associated with the outcome

Conclusions Peritonitis due to NF-GNB in PD are severe infections with reduced resolution rate; bacterial resistance and concomitant ESI negatively influence the chance of resolution. Even though biofilm production has not had influence the outcome, the found of higher in vitro susceptibility of Pseudomonas than other NF-GNB, with similar resolution rate, suggest bacterial virulence factors as biofilm or others can act in concert, worsening the outcome.

Background

Continuous peritoneal dialysis (PD) was introduced in seventies [1,2] and its initial results were compromised by the high incidence of bacterial peritonitis [3, 4]. Since then, technological advances, particularly in disconnection systems and antimicrobial prophylaxis, strongly reduced the incidence of these infections [5, 6]. However, peritonitis remains a serious complication of PD and the main cause of PD failure and is associated with a higher risk of death from all causes and cardiovascular causes [7, 8].

Gram-positive cocci are the main etiologies of PD peritonitis worldwide, while episodes due to Gram-negative bacilli (GNB) usually presents greater severity and lower resolution rate [9, 10]. Among them, the worst outcomes are reported in infections caused by Pseudomonas species and other non-fermenting GNB (NF-GNB) [11-13]. The findings of a large prospective Brazilian cohort showed that *Pseudomonas* spp. etiology is independently associated with the non-resolution of peritonitis [14].

The reasons for the unfavorable evolution of NF-GNB peritonitis are not fully known. Beyond the patient's clinical and demographic conditions and antibiotic treatment, factors associated with the intrinsic bacterial virulence and antimicrobial resistance are possible determinants of worse outcomes [13,15-19].

NF-GNB are ubiquitous and opportunistic microorganisms that are present in nature and the healthcare environment, where they cause different types of infections [20,21]. *Pseudomonas spp*. are the most isolated NF-GNB and of greatest clinical importance. *Pseudomonas* species' virulence factors enable them to invade tissues, proliferate rapidly, generate biofilms, and quickly develop antibiotic resistance and provide those species with great motility [16-19]. *Acinetobacter* species have been an increasing concern in PD due to their alarming rate of antibiotic resistance development; in particular, the *Acinetobacter baumannii* complex [13] can form biofilms and colonize catheters [22].

In turn, only a few studies have reported the factors influencing the outcomes od NF-GNB-induced PD-related peritonitis, highlighting the study by Silva et al [11], who reported that the use of two antimicrobial agents favored positive outcomes in *Pseudomonas*-induced peritonitis.

Jointly analyzing the microbiological properties of the causative organism, patient-related conditions, PD modality, and peritonitis episode characteristics and its treatment can potentially identify the determinants of outcomes in NF-GNB peritonitis and such an analysis has not been conducted in Brazilian or Latin American cohorts. Therefore, the present study aimed to investigate whether causative bacteria characteristics, including the ability to produce biofilm, as well as those of the patient, PD modality, peritonitis episode, and peritonitis treatment influenced the clinical evolution of NF-GNB-induced PD-related peritonitis.

Methods

Study population

In this retrospective study, we reviewed all episodes of PD-related peritonitis caused by NF-GNB that occurred between June 1997 and December 2015 in a single Brazilian university center. Episodes with incomplete clinical data were excluded.

The diagnosis of peritonitis was made when at least two of the following criteria were present: presence of a cloudy peritoneal effluent; abdominal pain; dialysate containing more than 100 leukocytes per ml (at least 50% polymorphonuclear cells); and positive culture of dialysate [23]. A relapse is an episode caused by the same specie or a negative culture result within 28 days of completion of antibiotic therapy. Recurrence refers to an episode that occurs within 4 weeks of completion of therapy for a prior episode caused by a different NF-GNB species. A repeat refers to an episode that occurs after 4 weeks after completion of therapy for a prior episode caused by the same specie [23]. The outcomes were defined as: resolution (disappearance of signs and symptoms within 5 days after the initiation of antibiotic therapy); refractory peritonitis (presence of turbid dialysate after 5 days of treatment with appropriate antibiotics); peritonitis-related death (death of a patient with active peritonitis or the death of a patient who had an episode within the previous 4 weeks) [23]; and non-resolution (catheter removal before the 5th day of treatment, refractory peritonitis, required a second antibiotic regimen, relapse, or peritonitis-related death)

Data collection

We recorded the following information for each case: episode type (new infection, recurrence, repeat, or relapse), concomitant exit-site infection (ESI), antimicrobial treatment, outcome, treatment time to the peritonitis episode, patient characteristics (age, sex, race [Caucasian or non-Caucasian], presence of diabetes, previous peritonitis by other bacteria), PD modality (continuous ambulatory PD or automated PD), and characteristics of the causative germ (specie, biofilm production capability, and *in vitro* antibiotic susceptibility).

Culture and storage

After diagnosis, dialysate sample was processed following the recommendations of the International Society for Peritoneal Dialysis ISPD [23]. Cultures were performed using the Bactec System (Becton Dickinson Company, Sparks, MD) and then seeded onto blood agar. After isolation, identification, and susceptibility testing, strains were stored at –70°C.

For the present study, the stored samples were re-isolated on MacConkey agar plates and re-identified. For this, isolates were Gram stained to confirm purity and determine each isolate's morphology and specific color. Afterward, the isolates were identified by conventional biochemical testing, [24], and by mass spectrometry using MALDI-TOF (Matrix-Assisted Laser Desorption Ionization Time-of-Flight) technology (MALDI-Tof VITEK® MS, Brazil) [25].

Microbiological tests

In vitro susceptibility

The *in vitro* susceptibility to amikacin, ciprofloxacin, cefepime, imipenem, and ceftazidime was determined by the minimum inhibitory concentration (MIC) test. The proportion of strains susceptible to each drug was defined based on the 2016 Clinical Laboratory Standards Institute breakpoints [26]. When strains presented intermediate MIC values, we considered them as resistant.

Biofilm production

The bacterial samples were grown in Tryptic Soy Broth (TSB) at 37°C for 18 hours. To assess the bacterial ability to adhere to abiotic surfaces, we used 96-well polystyrene plates and added 200 µl of TSB and 10 µL of the bacterial suspension (approximately 108 CFU/ml) to each well except that one well was inoculated only with culture medium to be used as a reading standard (blank). The plates were incubated at 37 ° C for 48 hours and then washed with phosphate-buffered saline 4 times to remove non-adherent bacteria. Bacteria that adhered to the abiotic surface were then fixed with formalin (2%) and after 20 minutes, the formalin was removed, and the preparations were washed 4 more times with water. Then, the preparations were stained with a crystal violet solution (1%) for 20 minutes, after which they were washed 3 times with water to remove excess dye. After drying, the dye was solubilized with methanol for 10 minutes and the optical density, measured at 540 nm, was determined [27]. Then, we classified the biofilm production into one of four categories as previously published [27]: no producer, weak producer, moderate producer, and strong producer.

Clinical-microbiological associations

Each patient's characteristics, including episode type, the presence of concomitant ESI, initial treatment, previous peritonitis, and microbiological characteristics, were analyzed regarding their association with the outcome. For this purpose, we classified the outcomes in two mutually exclusive results: resolution or non-resolution.

Statistical analysis

For comparison between frequencies, we used the chi-squared test or Fisher's exact test. Binary logistic regression with backward stepwise procedure was used to determine the independent predictors of outcomes. For this purpose, we first performed a univariate logistic regression analysis to select the variables that would enter the final model, with p> 0.20 as the elimination criterion. Collinearity among variables was tested and, if statistically significant interactions occurred, one of the variables was excluded. A p value < 0.05 was considered significant.

Results

Between June 1997 and December 2015, there were 726 episodes of bacterial peritonitis in 542 PD patients in our center. Of these, 194 (26.7%) were caused by GNB, 70 of which were caused by NF-GNB. Based on the exclusion criteria, we studied 68 cases from 63 patients. Of these, there were 62 new infections, 3 recurrences, 2 repeats, and 1 relapse. Concomitant ESIs were diagnosed in 17 cases (25%): nine were caused by Pseudomonas and eight by other species. Previous peritonitis caused by other bacteria were reported in 27 cases (39.7%). The clinical and demographic data of the 63 patients at the time of their first episode of NF-GNB peritonitis are shown in Table 1.

Characteristics	Mean ± standard deviation /number (%)
Age (years)	45,4 ± 20,4
0 a 60	44 (69.8)
> 61	19 (30.2)
Gender (male)	35 (55.6)
Caucasians	46 (73.0)
Diabetes	17 (27.0)
PD vintage (months)	15.8 ± 20,7
Dialysis mode	
CAPD	33 (52.4)
APD	30 (48.6)
APD=automated peritoneal dialysis, CAPD=continuous ambulatory peritoneal dialysis	

Table 1
Characteristics of 63 patients the time of the 1st episode of non-fermenting Gram-negative bacilli peritonitis
All patients started treatment within 24 hours of the onset of clinical signs or symptoms, based on ISPD guidelines [23]. From 1996 to 2000, the initial antibiotic therapy consisted of intraperitoneal (i.p.) cefazolin plus amikacin. From 2000 to 2005, we used two regimens: the first consisted of i.p. cefazolin plus amikacin and the second i.p. cefazolin plus ceftazidime. After 2005, the initial treatment for all was i.p. vancomycin plus amikacin. When the results of peritoneal effluent culture and *in vitro* susceptibility tests were available, we adjusted the treatment. The duration of antibiotic therapy was at least 21 days [23]. Cefazolin plus amikacin was used in 22 cases, cefazolin plus ceftazidime in nine, vancomycin plus amikacin in 19 and other treatments in 18 (two antipseudomonal agents in 16, imipenem in one, and cefepime in one case).

Of the 68 episodes, there was resolution in 24 (35.3%), relapse in six (8.8%), refractory peritonitis in 21 (30.9%), removal of the peritoneal catheter before the 5th day of treatment in 14 (20.6%), and peritonitis-related death in three (4.4%).

The descriptions of the etiological agents are shown in Table 2. Of the total episodes of peritonitis included in the study, microbiological tests were carried out in 52 episodes, as it was not possible to recover the other strains.

	Episodes [n (%)]	Recovered isolates [n]
***Pseudomonas* spp.**	**35 (51.5)**	**26**
Pseudomonas aeruginosa	31 (45.6)	24
Pseudomonas pútida	3 (4.4)	2
Pseudomonas fluorescens	1 (1.5)	0
***Acinetobacter* spp.**	**21 (30.9)**	**18**
Complex Acinetobacter baumanii	12 (17.6)	11
Acinetobacter haemolyticus	5 (7.3)	5
Acinetobacter lwoffi	2 (3.0)	0
Acinetobacter ursingii	2 (3.0)	2
***Burkholderia* spp.**	**6 (8.8)**	**3**
Complex Burkholderia cepacia	5 (7,3)	2
Burkholderia gladioli	1 (1.5)	1
***Achromobacter* spp.**	**5 (7.3)**	**4**
Achromobacter denitrificans	3 (4.4)	3
Achromobacter xylosoxidans	2 (2.9)	1
***Stenotrophomona* sp.**	**1 (1.5)**	**1**
Stenotrophomona maltophilia	1 (1.5)	1
Total	68 (100)	52

Table 2
Etiologic spectrum of 68 non-fermenting Gram-negative bacilli peritonitis episode
Regarding the etiology the outcomes of 68 episodes, there was resolution in 32.4% of infections caused by *Pseudomonas* species, 39.1% of cases by Acinetobacter species, and 36.4% in peritonitis by other NF-GNB (p= 0.68).

The results of *in vitro* susceptibility are described in the Table 3. *Pseudomonas* species were more susceptible than *Acinetobacter* species to all the tested antimicrobials, except imipenem. *Pseudomonas* species were also more susceptible than *Achromobacter* species to amikacin, ciprofloxacin, and cefepime. Isolates of the *Burkholderia cepacia* and *Stenotrophomonas maltophilia were* tested only for ceftazidime, and all were susceptible.

	***Pseudomonas* spp. (n=26)**	***Acinetobacter* spp. (n=18)**	***Achromobacter* spp.(n=4)**	***Burkholderia gladioli* (n=1)**	***Burkholderia cepacia* (n=2)**	***Stenotrophomonas* spp (n=1)**	**NF-GNB (n=52)**
	Susceptibility n (%)	Susceptibility n (%)	Susceptibility n (%)	Susceptibility n (%)	Susceptibility n (%)	Susceptibility n (%)	Susceptibility n (%)
Amikacin	22 (84.6)[1,2]	7 (38.9)	1 (25.0)	1 (100)	-	-	31 (63.3)
Ciprofloxacin	17 (65.4)[1]	7 (38.9)	2 (50.0)	0 (0.0)	-	-	27 (55,1)
Ceftazidime	24 (92.3)[1]	8 (44.4)	4 (100)	0 (0.0)	2 (100.0)	1 (100.0)	39 (75.0)
Cefepime	21 (80.8)[1,2]	7 (38.9)	1 (25.0)	0 (0.0)	-	-	32 (65.3)
Imipenem	21 (80.8)	16 (88.9)	4 (100)	0 (0.0)	-	-	41 (93.7)
1=p<0,05 vs Acinetobacter spp, 2=p<0,05 vs Achormobacter spp							

Table 3
Non-fermenting Gram-negative bacilli-causing peritoneal dialysis-related peritonitis and their in vitro susceptibility rates

Regarding biofilm production, of the 52 samples, 39 (75%) produced biofilm. There were 20 strong producers, eight medium producers, and 11 weak producers. The biofilm producers were 24 of the 26 *Pseudomonas* isolates, 11 of the 18 *Acinetobacter* isolates, and two of the four *Achromobacter* isolates. Both *Burkholderia cepacia* isolates were producers, one *Burkholderia gladioli* was not, and the only isolate of *Stenotrophomonas maltophilia* was a producer.

Factors associated with peritonitis outcome

Univariate analysis

The univariate logistic regression analysis revealed that concomitant ESI, age, biofilm production (non-producers plus weak producers vs moderate plus strong producers), resistance to amikacin, resistance to ceftazidime, and treatment with cefazolin plus ceftazidime were associated with a higher risk for non-resolution of peritonitis at the level of p <0.20 (Table 4). There was collinearity between resistance to amikacin and resistance to ceftazidime as well as between those two variables and treatment; therefore, it is not possible to include them together in the same regression model.

Multivariate analysis

This analysis showed that only the variables concomitant ESI and *in vitro* resistance to amikacin were independent predictors of the non-resolution (table 4). After replacing resistance to amikacin with resistance to ceftazidime, the association between bacterial resistance and outcome was eliminated (p = 0.99) (model 2). Including the variable treatment and removing the bacterial resistance in the model resulted in a tendency for the protocol cefazolin plus ceftazidime (using other treatments a reference) to be associated with non-resolution (p = 0.074), remaining the concomitant ESI as a predictor of non-resolution (p = 0.023) (model 3).

Variable	*p* value (univariate)	*p* value (multivariate)	OR	95% CI
Age (years)	0.155	0.093	1.026	0.097-1.057
Exit-site infection (yes)	0.029	0.021	7.305	1.342-39.757
Resistance to amikacin	0.191	0.039	4.838	1.084-21.589
No plus weak biofilm producers (vs strong producers plus moderate producers)	0.185	0.183	0.376	0.09-1.587
OR= Odds ratio				

Table 4
Predictors of non-resolution of non-fermenting Gram-negative bacilli peritonitis in peritoneal dialysis- Logistic Regression Analysis.

Discussion

In human infections, the clinical course and outcome are strongly dependent on the characteristics of the infecting microorganism and patient's conditions. In the case of bacteria, despite the indisputable role of bacterial resistance, this does not seem to be the only property influencing the outcomes; this holds true for PD-related peritonitis. Previous publications by our group showed a high arsenal of virulence factors among *Staphylococcus* aureus lineages, some of which are associated with worse PD-related peritonitis outcome, in spite their low resistance rate to methicillin [28,29].

NF-GNB present both, a high antimicrobial resistance rate and a high production of virulence factors, such as biofilm, as confirmed in the present series, which potentially explains the low observed resolution rate. In addition, other virulence factors are present in NF-GNB , particularly *Pseudomonas* species, such as: alginate, which is associated with bacterial adhesion; exoenzyme S, an inhibitor of protein synthesis; hemolytic phospholipase C, which is associated with the destruction of cell membranes and osmoprotection; exotoxin A, which is associated with tissue destruction and inhibition of the macrophage response; alkaline protease, which is associated with tissue damage and inactivation of IgG; elastase, an immunoglobulin degradation factor; and ramnolipids, which are associated with bacterial adhesion [16-19, 30-32].

These findings corroborate the aggressive character of these bacteria and explain, at least partially, the findings of this and of the two largest series, which previously described peritonitis caused *by Pseudomonas* species, i.e., the most frequent etiology of peritonitis among NF-GNB. Silva et al. studied 191 episodes of *col* peritonitis that occurred in Australian patients reported high rates of catheter removal (44%), permanent hemodialysis transfer (35%), hospitalization (96%), and change to a second antibiotic (66%). Lu et al. [12] reviewed 153 episodes of peritonitis

caused by *Pseudomonas* species in Hong Kong, reporting overall primary response rate was 53.6% and complete cure rate was 42.4%. Interestingly, this study showed a decrease in the incidence of germs resistant to ceftazidime and gentamicin over time, which suggests that other factors, may have influenced the outcome.

We showed that concomitant ESI and resistance to amikacin were independently associated with non-resolution, in agreement to found by Krishnan et al [33]. The high rate of this infection (25%) in our sample, can at least partly explain the observed low-resolution rate. Regarding bacterial resistance, a considerable proportion of lineages was resistant to the tested antimicrobials. However, this does not occurred in episodes caused by *Pseudomonas* species, over 80% of which were susceptible to the frequently used antimicrobials as amikacin and ceftazidime, which reinforces the influence of other factors on the outcome. Even so, the resolution rate of these episodes was just over 30%, like observed with peritonitis by other BF-GNB.

Biofilm production is another factor reported as a determinant of the unfavorable response to Pseudomonas infections in PD [11]. We emphasize that antibiotic susceptibility is based on the MIC of the drug for planktonic cells, which are more sensitive to antimicrobials than cells wrapped in biofilms [34]. We identifies a high proportion of biofilm-producing isolates (75%); even though moderate or strong biofilm production were associated with non-resolution in in the univariate analysis, this association was not significant in the multiple regression model. However, this result does not rule out the possibility that its pathogenic action, in concert with other virulence factors, may influence the outcome.

Notably, we observed a greater chance of non-resolution in episodes initially treated with cefazolin plus ceftazidime, in contrast to other treatments. However, this association did not reach statistical significance, likely due to the small number of treated cases (nine). Such a finding, although inconsistent from a statistical point of view, agrees with the ISPD guideline [23], which recommend the use of two antipseudomonal drugs for peritonitis caused by species of *Pseudomonas* and *Stenotrophomonas*.

A significant number of NF-GNB-induced peritonitis cases involved *Acinetobacter* species. Of these, 57.1% were due to *Acinetobacter baumannii*, similar to found by Chao et al. [35], although Li et al. [13] reported higher frequencies. This series confirms that *Acinetobacter baumannii* is resistant to several antimicrobials, except for imipenem, and that this is a major therapeutic challenge.

Other identified germs, such as *Achromobacter* species, lineages of the *Burkholderia cepacia* complex, and *Burkholderia gladioli*, have rarely been described as etiologies of PD-related peritonitis [36,37]. The precise identification of NF-GNB is a challenge for conventional microbiology due to the phenotypic similarity and taxonomic complexity of these agents. Phenotypic tests based on morphology and biochemical characteristics often provide erroneous identifications of these species [38]. In our study, such limitations were minimized with the identification of the isolates by the MALDI-TOF technique, which is used in clinical microbiology to identify bacterial species based on the microorganisms' protein profiles. This identification technique was mentioned in the most recent ISPD guideline on PD-related peritonitis, although at the time of its publication there was insufficient evidence for its recommendation [23].

Our study has several limitations, the most important being the small sample size, aggravated by the impossibility of recovering about 20% of the isolates. However, this is a study of NF-GNB-induced peritonitis as a whole, and therefore allows comparisons between peritonitis episodes due to *Pseudomonas* species and those due to other NF-GNB. In addition, to our knowledge, this is the first study to address the role of biofilm production in the outcomes of NF-GNB-induced PD-related peritonitis. In addition, it revealed novel information about pathogens that cause peritonitis, including those of the genus *Achromobacter*, which suggests that there is some benefit to using new techniques, e.g., MALDI-TOF, to identify bacteria in peritonitis.

Finally, the prevalence of PD-related NF-GNB in our center was similar to that of the Brazilian PD cohort, the largest Latin American cohort of incident PD patients, and again highlighted the severity of these infections.

Conclusions

NF-GNB-induced PD-related peritonitis is a serious infection with a reduced resolution rate. Bacterial resistance the concomitant presence of ESI negatively influence the chance of resolution. Biofilm production was not significantly associated with the outcome, which does not rule out the possibility that it can act in concert with other virulence factors and thus impair the response to antimicrobial therapy. The presence of uncommon etiologies of peritonitis in PD, such as *Achromobacter* species, highlights the need for future studies regarding the clinical behavior of these infections. The considerably high prevalence of multi-resistant *Acinetobacter* species causing PD-related peritonitis raises an alert about care for the prevention and management of these infections.

Declaration

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Authors' contributions

All authors contributed to conception and design of the study. ACMLS wrote the first draft of the manuscript. ACMLS and PB organized the database. PB performed the statistical analysis.

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Availability of data and materials

The datasets that were used for the analysis and the preparation of this manuscript are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

When the patients started their regular PD treatment, they gave written consent for the use of their clinical and laboratory data for research purposes. Therefore, the institutional research ethics committee approved this study (CAAE 64736017.2.0000.5411 statement) and exempted it of any specific written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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