Predictive factors of outcome following nonfermenting gram-negative bacilli peritonitis in peritoneal dialysis

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Abstract

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

Methods We reviewed the records of 62 new peritonitis episodes caused by NF-GNB 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* (51.6%), *Acinetobacter* (32.2%), and others (16.1%). There was a high (72.9%) 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 (<45%) among the episodes attributable to *Pseudomonas spp, Acinetobacter spp,* and other NF-GNB. Pre-existent exit-site infection was independently associated with nonresolution. No other factor, including biofilm production, was associated with the outcome.

Conclusions Peritonitis due to NF-GNB in PD is a severe infection with a reduced resolution rate, and pre-existent exit site infection negatively influences the chance of resolution. The higher in *vitro susceptibility* of *Pseudomonas* compared to that of other NF-GNB with a similar resolution rate, suggests bacterial virulence factors beyond biofilm and can act in concert, thereby 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, have 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 etiology of PD peritonitis worldwide, while episodes due to gram-negative bacilli (GNB) usually present greater severity and lower resolution rates [9, 10]. Among them, the worst outcomes are reported in infections caused by *Pseudomonas* species and other nonfermenting GNB (NF-GNB) [11-13]. The findings of a large prospective Brazilian cohort showed that *Pseudomonas* spp. etiology is independently associated with the nonresolution of peritonitis [14].

The reasons for the unfavorable evolution of NF-GNB peritonitis are not fully known. Beyond the patient's clinical and demographic characteristics and antibiotic treatment, factors associated with 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 of NF-GNB-induced PD-related peritonitis, highlighting a 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, but such an analysis has not been conducted in Brazilian or Latin American cohorts. Therefore, the present study aimed to investigate whether causative bacterial 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. The exclusion criteria were: episodes with incomplete clinical data, relapse (episode caused by the same

species or a negative culture result within 28 days of completion of antibiotic therapy), recurrence (episode caused by other species within 28 days after starting antibiotic therapy), and repeat episode (episode caused by the same species or after 28 days following completion of antibiotic therapy)

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]. The outcomes were defined as: resolution (disappearance of signs and symptoms within 5 days after the initiation of antibiotic therapy); relapse, 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 nonresolution (catheter removal before the 5th day of treatment, refractory peritonitis, relapse, or peritonitis-related death).

Catheter insertion and care and dialysis procedures

The catheter placements were made under supervision of a senior nephrologist with percutaneous blind insertion of a double cuff straight Tenckhoff catheter using the Seldinger technique. Until 2003, no patients used antibiotic cream application at the catheter exit-site; from 2003 to 2006, we prescribed daily mupirocin cream, and from January 2007 daily gentamicin was prescribed to all incident patients. All patients used a semiocclusive dressing with sterile gauze and microporous adhesive tape.

Until 1999, the CAPD connection systems were the Y set type; the twin bag was introduced in 1999. APD was introduced in 1998, and its indication and prescription were based on clinical criteria or the patient's preference. For both PD modalities, we used standard glucose solutions, with low pH and high glucose degradation product (GDP) levels.

The diagnosis of exit-site and tunnel infection followed International Society for Peritoneal Dialysis (ISPD) criteria Exit-site infection (ESI) is the presence of purulent discharge, with or without erythema of the skin at the catheter-epidermal interface. Tunnel infection is the presence of clinical inflammation or ultrasonographic evidence of collection along the catheter tunnel.

Data collection

We recorded the following information for each case: date, preexistent ESI (ESI diagnosed until four weeks before a peritonitis episode), tunnel infection, topical antibiotic use at the catheter exit-site, initial antimicrobial treatment for peritonitis and adjustments, outcome, treatment time before the peritonitis episode (dialysis vintage) , patient's characteristics (age, sex, race [Caucasian or non-Caucasian], underlying kidney disease, previous peritonitis by other bacteria, PD modality (continuous ambulatory PD or automated PD), and characteristics of the causative germ (species, biofilm production capability, and *in vitro* antibiotic susceptibility).

Culture and storage

After diagnosis, each dialysate sample was processed following the recommendations of the ISPD [23]. Cultures were performed using the Bactec System (Becton Dickinson Company, Sparks, MD) and then seeded onto blood agar if there was signaling the positivity on the cultures bottles.. After isolation, identification, and susceptibility testing, strains were stored at –70°C.

For the present study, the stored samples were reisolated on MacConkey agar plates and reidentified. 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 [25] and by mass spectrometry using MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight) technology (MALDI-ToF VITEK® MS, Brazil) [26].

Microbiological tests

In vitro susceptibility

The *in vitro* susceptibility to amikacin, ciprofloxacin, cefepime, imipenem, and ceftazidime was determined by the minimum inhibitory concentration (MIC)based on gradient diffusion, using the E test ((bioMérieux, Inc., Durham, NC) The proportion of strains susceptible to each drug was defined based on the 2016 Clinical Laboratory Standards Institute breakpoints [27]. When strains presented intermediate MIC values, we considered them resistant.

Biofilm production

The bacterial samples were grown in Tryptic Soy Broth (TSB) (BD™, Le Pont de Claix, France) 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 µ of the bacterial suspension (approximately 108 CFU/mLµ) to each well, except one well that 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 [28]. Then, we classified the biofilm production into one of four categories as previously published [28]: no producer, weak producer, moderate producer, and strong producer. In our study, we opted for a 48 hours method, instead of faster methods, to obtain a more reliable result, as it allows the strains to have enough time for the production of biofilm because some NF-GNB species show slow growth.

Clinical-microbiological associations

Each patient's characteristics, pre-existent ESI, topical antibiotic use at the catheter exit-site, initial treatment for peritonitis, treatment adjustment with two antipseudomonal antibiotics, previous peritonitis by other bacteria, and microbiological characteristics were analyzed regarding their association with the outcome. For this purpose, we classified the outcomes into two mutually exclusive results: resolution or nonresolution.

Statistical analysis

For comparison between frequencies, we used the chi-squared test or Fisher's exact test. Binary logistic regression with a 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 62 index cases of peritonitis caused by NF-GNB from 62 patients (Figure 1). Pre-existent ESI was diagnosed in 16 cases (25.8%): nine were caused by *Pseudomonas aeruginosa*, three by Burkholderia cepacia, one by *Stenotrophomona maltophilia*, one by *Acinetobacter baumanii*, and two by *Corynebacterium* spp. These same organisms were seen in peritoneal effluent culture in 12 patients. For all ESI episodes, we prescribed oral ciprofloxacin as the initial treatment. No tunnel infection was diagnosed by clinical criteria, although we did not perform ultrasonographic evaluation of the subcutaneous catheter tunnel.

Previous peritonitis caused by other bacteria was reported in 23 cases (37.1%). The clinical and demographic data of the 62 patients at the time of their first episode of peritonitis are shown in Table 1.

Figure 1: Flow diagram showing from the total peritoneal-related bacterial peritonitis episodes to index episodes caused by nonfermenting gram-negative bacilli, between June 1997 and December 2015.

Table 1. Characteristics of 62 patients the time of the 1st episode of non-fermenting Gram-negative bacilli peritonitis

Characteristics	Mean ± standard deviation /number (%)
Age (years)	45,4 ± 20.4
0 a 60	44 (70.9)
> 61	18 (29.1)
Gender (male)	35 (55.6)
Caucasians	46 (73.0)
Underlying kidney disease	
Diabetes renal disease	16 (25.8)
Hypertensive nephrosclerosis	20 (32.2)
Glomerulonephritis	7 (11.3)
Autosomal recessive polycystic kidney disease	3 (4.8)
Undetermined and others	16 (25.8)
Topical antibiotic use at the catheter exit-site	
None	27 (43.5)
Mupirocin cream	16 (25.8)
Gentamycin cream	19 (30.6)
PD vintage (months)	15.4 ± 20.5
Dialysis mode	
CAPD	32 (51.6)
APD	30 (48.4)

APD=automated peritoneal dialysis, CAPD=continuous ambulatory peritoneal dialysis

All patients started treatment within 24 hours of the onset of clinical signs or symptoms of peritonitis, based on ISPD guidelines [23]. From 1996 to 2000, the initial antibiotic therapy consisted of intraperitoneal (i.p.) cefazolin (20 mg/kg daily) plus amikacin (2 mg/kg daily). From 2000 to 2005, we used two regimens: the first consisted of i.p. cefazolin (20 mg/kg daily) plus amikacin (2 mg/kg daily) and the second i.p. cefazolin (20 mg/kg daily) plus ceftazidime (1-1,500 mg daily). After 2005, the initial treatment for all was i.p. vancomycin (15–30 mg/kg every 5–7 days) plus amikacin (2 mg/kg daily) . 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 20 cases, cefazolin plus ceftazidime was used in nine cases, and vancomycin plus amikacin was used in 33 cases. After the results of the *in vitro* susceptibility tests, we adjusted the initial treatment in 18 cases, (two antipseudomonal agents in 16, imipenem in one, and cefepime in one patient).

Of the 62 episodes, there was resolution in 20 (32.2%), relapse in six (9.6%), refractory peritonitis in 19 (30.6%), removal of the peritoneal catheter before the 5th day of treatment in 14 (22.6%), and peritonitis-related death in three (4.8%). Regarding the 16 episodes, in which we made adjustment of the initial treatment to two antipseudomonal agents, six (37,7%) progressed to resolution, while both patients treated with imipenem or cefepime, after prescription adjustment evolved to resolution. We did not observed allergic reactions attributable to antibiotics regimens.

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 48 episodes, as it was not possible to recover the other strains.

Table 2. Etiologic spectrum of 62 non-fermenting Gram-negative bacilli peritonitis episode

	Episodes [n (%)]	Recovered isolates [n]
***Pseudomonas* spp.**	**32 (51.6)**	**24**
Pseudomonas aeruginosa	28 (45.2)	22
Pseudomonas putida	2 (3.2)	2
Pseudomonas fluorescens	2 (3.2)	0
***Acinetobacter* spp.**	**20 (32.2)**	**18**
Acinetobacter baumanii	12 (19.3)	11
Acinetobacter haemolyticus	5 (8.1)	5
Acinetobacter lwoffi	1 (1.6)	0
Acinetobacter ursingii	2 (3.2)	2
***Burkholderia* spp.**	**5 (8.1)**	**2**
Burkholderia cepacia	4 (6.4)	1
Burkholderia gladioli	1 (1.6)	1
***Achromobacter* spp.**	**4 (6.4)**	**3**
Achromobacter denitrificans	3 (4.8)	3
Achromobacter xylosoxidans	1 (1.6)	0
***Stenotrophomona* spp.**	**1 (1.6)**	**1**
Stenotrophomona maltophilia	1 (1.6)	1
Total	62 (100)	48

Regarding the etiology and the outcomes of 62 episodes, there was resolution in 10 (31.2%) of the infections caused by *Pseudomonas* species, 8 (44.4%) of the cases caused by *Acinetobacter* species, and 2 (20%) caused by other NF-GNB (p= 0.39).

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

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

	Pseudomonas spp. (n=24)	*Acinetobacter* spp. (n=18)	*Achromobacter* spp.(n=3)	*Burkholderia gladioli* (n=1)	*Burkholderia cepacia* (n=1)	*Stenotrophomonas* spp (n=1)	NF-GNB (n=48)
	Susceptibility n (%)	Susceptibility n (%)	Susceptibility n (%)	Susceptibility n (%)	Susceptibility n (%)	Susceptibility n (%)	Susceptibility n (%)
Amikacin	20 (83.3)[1,2]	7 (38.9)	1 (33.3)	1 (100)	-	-	29 (60.4)
Ciprofloxacin	17 (70.1)[1]	7 (38.9)	2 (66.7)	0 (0.0)	-	-	26 (54.1)
Ceftazidime	21 (87.3)[1]	8 (44.4)	3 (100)	0 (0.0)	1 (100.0)	1 (100.0)	39 (70.8)
Cefepime	20 (83.3)[1,2]	7 (38.9)	1 (33.3)	0 (0.0)	-	-	28 (58.3)
Imipenem	20 (83.3)	16 (88.9)	3 (100)	0 (0.0)	-	-	39 (81.2)

1=p<0.05 vs Acinetobacter spp, 2=p<0.05 vs Achormobacter

Regarding biofilm production, of the 48 samples, 35 (72.9%) produced biofilm. There were 18 strong producers, seven medium producers, and 10 weak producers. The biofilm producers were 22 of the 24 *Pseudomonas* isolates, 11 of the 18 *Acinetobacter* isolates, and one of the three *Achromobacter* isolates. Both *Burkholderia cepacia* and *Burkholderia gladioli* isolates were not producers, while the only isolate of *Stenotrophomonas maltophilia* was a producer. Among the episodes caused by biofilm, there were nine cases with ESI, and among those by nonproducers, there were four (p=0.72).

Factors associated with peritonitis outcome

Univariate analysis

The univariate logistic regression analysis revealed that pre-existent ESI, age, resistance to ceftazidime, and initial treatment with cefazolin plus ceftazidime were associated with a higher risk for nonresolution of peritonitis at a p value <0.20 (Table 4). The other variables (underlying kidney disease, gender, race, PD modality, dialysis vintage, topical antibiotic use, previous peritonitis due to other bacteria, biofilm production, resistance to amikacin, and treatment adjustment with two antipseudomonal agents) did not reach p < 0.20 and were not included in the multivariate model. There was collinearity between resistance to ceftazidime and initial treatment; therefore, it was not possible to include them together in the same regression model.

Multivariate analysis

This analysis showed that only pre-existent ESI was an independent predictor of nonresolution (4). We constructed a second model (model 2), including the covariate treatment and removing bacterial resistance, in which we observed a tendency for the protocol of cefazolin plus ceftazidime (using other initial treatments as a reference) to be associated with nonresolution, retaining pre-existent ESI as a predictor of nonresolution (Table 5).

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

Variable	*p* value (univariate)	*p* value (multivariate)	OR	95% CI
Age (years)	0.135	0.088	1.026	0.977-1.057
Exit-site infection (yes)	0.027	0.021	12.75	1.469-89.757
Resistance to ceftazidime	0.171	0.98	5.50	0.634-47.74

OR= Odds ratio

Table 5. Predictors of non-resolution of non-fermenting Gram-negative bacilli peritonitis in peritoneal dialysis- Logistic Regression Analysis (model 2)

Variable	*p* value (univariate)	*p* value (multivariate)	OR	95% CI
Age (years)	0.135	0.082	1.030	0.996-1.065
Exit-site infection (yes)	0.027	0.017	18.87	1.703-110.76
Initial treatment with cefazolin plus ceftazidime (vs other initial treatments)	0.122	0.097	5.33	0.512-10.63

OR= Odds ratio

Discussion

In human infections, the clinical course and outcome are strongly dependent on the characteristics of the infecting microorganism and the patient's condition. 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 number of virulence factors among *Staphylococcus* aureus lineages, some of which are associated with worse PD-related peritonitis outcome, despite their low resistance rate to methicillin [29,30]. In a previous report on PD-related *Escherichia coli* peritonitis episodes, we did not find any resistant strain to amikacin or ceftazidime used as initial treatment; in contrast, only 48.1% of the episodes progressed to resolution [31]. In the same study, approximately 50% of the isolates were medium or strong biofilm producers, which tended to be associated with nonresolution, but this did not reach statistical significance (p=0.09) [31].

NF-GNB present both a high antimicrobial resistance rate and high production of virulence factors, such as biofilm, as confirmed in the present series, which potentially could explain the low observed resolution rate. In addition, important virulence factors are present in NF-GNB, particularly *Pseudomonas* species, which induce bacterial adhesion, destruction of cell membranes, and inhibition of the macrophage response, in addition to other actions [16-19, 32,33].

Interestingly, we did not find a high resistance rate among episodes caused by *Pseudomonas* species, over 80% of which were susceptible to frequently used antimicrobials such as amikacin and ceftazidime. Even so, the resolution rate of these episodes was just over 30%, such as that observed with peritonitis caused by other NF-GNB, which suggests the influence of other factors on the outcome. Moreover, the adjustment of the initial therapy for antibiotic regimens with two antipseudomonal drugs did not have association with the outcome. On the other hand, over 90% of the *Pseudomonas* isolates were biofilm producers. 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].

The aggressive character of *Pseudomonas* spp. can explain, at least partially, the findings of this and of the two largest studies, which previously described peritonitis caused by these bacteria as the most frequent etiology of peritonitis among NF-GNB. Silva et al. studied 191 episodes of *col*

peritonitis that occurred in Australian patients who 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 an overall primary response rate of 53.6% and complete cure rate of 42.4%. Interestingly, that study showed a decrease in the incidence of germs resistant to ceftazidime and gentamicin over time.

A significant number of NF-GNB-induced peritonitis cases involved *Acinetobacter* species. Of these, the majority were due to *Acinetobacter baumannii*, similar to previous reports [13, 35]. The importance of these bacteria has increased in recent years due to its great capacity to acquire mechanisms of resistance to different classes of antibiotics, great ability to survive and adapt to adverse conditions, and ability to adhere to different surfaces by the formation of biofilms [36]. This series confirms that *Acinetobacter baumannii* is resistant to several antimicrobials, except for imipenem. As expected, over 50% of the strains were biofilm producers. Despite their greater bacterial resistance compared to *Pseudomonas* spp., the resolution rate was similar between them. In this way, we can speculate that virulence factors influenced in the outcome.

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 [37,38]. 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 identification of these species [39]. 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 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 showed that pre-existent ESI was the only independent predictor of nonresolution. The association between ESI and subsequent peritonitis is widely recognized [40]. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most common microorganisms causing ESI, and they can to tunnel along the subcutaneous pathway and lead to peritonitis [40]. On the other hand, only few publications have focused the influence of ESI on peritonitis treatment response [41-43], reporting the presence of this infection is associated with bad outcomes. According to Gupta et al [41] in such cases, antibiotics do not resolve the peritoneal infection, although transient clearing of the effluent may occur.

Of note, in this study the biofilm production did not influence the outcome; however, this result does not rule out the possibility that biofilm , in concert with other virulence factors, may influence the peritonitis evolution.

Our study has several limitations, the most important being the small sample size, aggravated by the impossibility of recovering approximately 20% of the isolates. In addition, we do not have data at this time on the production of virulence factors by bacteria, other than biofilm. Lastly , there is a lack of information on patients' nutritional status and any comorbidity scores. 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*, but 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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