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Retrospective Study

Microbial Pathogens Involved in Peritoneal Dialysis-Related Infections

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Abstract

Introduction: Peritoneal dialysis (PD) is an alternative strategy of the management of end-stage renal failure However, it is associated with a high risk of infection of the peritoneum, subcutaneous tunnel and catheter exit site. If peritonitis occurs, it may require removal of the catheter and temporary or permanent transfer to hemodialysis. The aim of this work is to study the bacteriology and antibiotic resistance of peritoneal dialysis catheters infections diagnosed in the microbiology laboratory of the Hassan II University Hospital in FEZ.

Patients and Methods: We conducted a retrospective study over a period of four years, from August 2019 to August 2022, carried out at the microbiology laboratory of the Hassan II University Hospital in Fez. All PD patients followed in the nephrology department were included. For the patients who presented a peritonitis, a tunnel or catheter exit site infection, we analyzed: age, sex, types of kidney disease, white blood cell count, leukocyte count, gram stain, pathogen and its sensitivity to antibiotics.

Results: The analysis of samples from dialysis patients showed predominance of gram-positive *cocci* (58.24%) compared to gram-negative *bacilli* (37.36%) and yeasts (4.4%). We identified (52.74%) isolates as *S. aureus*, followed by *Enterobacteriaceae* (30.76%), non-fermenting gram-negative *bacilli* 6.6%, *streptococci* 5.5% and yeasts (4.4%). The most common species among gram-positive *cocci* are *staphylococci* with a predominance of *S. aureus* (91.66%) followed by coagulase-negative *staphylococci* (8.34%). *Enterococcus faecalis* represent 5.5% of germs. Among *Enterobacteriaceae, E. coli* is the predominant species (60.71%) followed by *Klebsiella* spp 35.71% then *Citrobacter* spp 3.58%. Among the non-fermenting Gram-negative *bacilli*, *Pseudomonas aeruginosa* is the most prevalent (83.33%). *Candida albicans* represents 4.4% of all germs. Gram-positive bacteria were susceptible to the glycopeptide family.

Conclusion: Most cases of peritoneal dialysis catheter-related infections in our study are caused by community - acquired bacteria. However, 4.39% of the isolated bacteria were multidrug resistant, which can limit available treatment options for these patients.

Keywords: *Infectious Peritonitis; Peritoneal Dialysis; Catheters; Bacteria*

Introduction

Peritoneal dialysis (PD) is an extra-renal purification technique that involves using the peritoneal membrane as a natural filter. The dialysate is infused into the peritoneal cavity through a catheter that is placed into the abdominal cavity. It constitutes an alternative in the strategy of management of chronic end-stage renal failure due to its multiple advantages, namely the prolonged preservation of residual renal function, the patient's autonomy, its simplicity and its lower cost.

However, it is the mode of purification of less than 1% of patients with end-stage renal failure in Morocco and only 10% of patients in most countries. The limited use of this technique could be explained by the risk of this process associated infections. Indeed, one of the dreaded complications is the occurrence of infectious peritonitis. A contamination during infection of the catheter exit site is thought to result from translocation of bacteria across the gut wall imposing a removal of the catheter and require temporary or permanent transfer to hemodialysis.

Aim of the Study

The aim of this work is to study the bacteriology and antibiotic resistance of peritoneal dialysis catheters infections diagnosed in the microbiology laboratory of the Hassan II University Hospital in FEZ.

Patients and Methods

We conducted a retrospective study over a period of four years, from August 2019 to August 2022, carried out at the microbiology laboratory of the Hassan II University Hospital in Fez. All PD patients followed in the nephrology department were included. For the patients who presented a peritonitis, a tunnel or catheter exit site infection, we analyzed: age, sex, types of kidney disease, white blood cell count, leukocyte count, Gram stain, pathogen and its sensitivity to antibiotics.

Peritoneal fluid sampling is performed when clinical signs appear: hyperthermia or abdominal pain and the presence of cloudy peritoneal fluid. The analysis of the samples includes a macroscopic examination, from which the color, consistency, appearance, odor and turbidity are assessed, followed by a microscopic examination performed on a graduated hematimeter (Malassez cell), allowing the counting of the different types of cells (leukocytes, red blood cells) in elements per unit volume (/mm^3, /ul), the observation is done by optical microscope using a x40 magnification lens. The leukocyte formula is done from the centrifugation pellet after staining with Methylene Blue and May Grunwald Giemsa (MGG). The search for the pathogen is done on the pellet by Gram staining.

The culture of the sample is done by plating on Columbia agar with 5% sheep Blood, chocolate agar with polyViteX and on a BHI broth (Brain Heart Infusion) and were incubated at 35°C in a 5% CO_2 enriched atmosphere and kept 48h in the oven before being declared negative. As well as on liquid mediums (aerobic/anaerobic blood culture broth). BACTEC bottles were incubated at 37°C for one week. Once detected positive, the broths will be transferred to selective and enriched solid agars.

The antibiogram is performed by diffusion method on Mueller-Hinton agar or by automated method (BD-Phoenix® 100) with an interpretative reading according to the recommendations of the antibiogram committee of the French Society of Microbiology (CASFM). The results are classified as: S (sensitive), I (intermediate) or R (resistant).

Testing for methicillin resistance is performed using a cefoxitin disc (30 µg) on Mueller Hinton agar after 24 hours of incubation at 37°C.

Extended-spectrum beta-lactamase (ESBL) screening is performed by the synergy test (champagne cork appearance), using the amoxicillin+clavulanic acid disc (30 µg) and a ceftazidime disc (30 µg).

The diagnosis of infectious peritonitis is retained if at least two of the following three criteria are met:

1) Abdominal pain or cloudy peritoneal fluid.
2) Leukocytes counts above 100 per mm^3 in the drainage fluid with a neutrophil count > 50%.
3) Positive bacteriological culture and/or positive microscopic examination.

An infection of the subcutaneous path of the PD catheter (tunnelitis) was sought with a sample taken at the catheter exit site, in case of discharge for bacteriological analysis. The bacteriological examination on catheters begins with the collection of the catheter in 1 ml of physiological serum, without unblocking the lumen, with agitation for 1 minute. Dilutions of the pathological product are made at 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}. Inoculation of pure broth and dilutions is done in star or rake, on fresh blood agar (COS), chocolate agar (PVX) and on EMB medium with incubation for 48 hours at 35°C in aerobic conditions. The identification and the antibiogram are done according to the isolated germs, hence the need to count the colonies. If there is more than one bacterial population, the number of CFU is determined for each bacterium. Catheter colonization is considered if CFU > 1,000/ml.

The sampling of catheter exit site is done by swabbing, with prior cleaning and necessitates two swabs for direct examination and culture. The direct examination after Gram staining allows to appreciate the importance of the polynuclears as well as the monomicrobial or polymicrobial aspect of the sample.

The culture requires the use of specific culture medium. Different atmospheres (aerobic, anaerobic, $C0_2$) are required for incubation.

Results

195 specimens were received including: 160 peritoneal punctures, 20 PD catheters, 15 catheter exit site specimens, from 60 patients. The average age was 41 years. The sex ratio was 1.2. The predominant etiology was chronic interstitial nephropathy. 91 punctures were culture negative and 69 were culture positive. The mean white blood cell count for positive cultures was 3008 WBC/mm^3 for positive cultures and 3015 WBC/mm^3 for negative cultures. 15 PD catheters and 7 catheter exit sites were culture positive (Table 1).

Sampling	Positive culture		Negative culture	
	Number	Frequency	Number	Frequency
Peritoneal punctures (n = 160)	69	43%	91	57%
PD catheter (n = 20)	15	75%	05	25%
Catheter exit site (n = 15)	07	46.66%	08	53,34%
Total (n = 195)	91	35.16%	104	64.84%

Table 1: *Distribution of microbiological culture according to the type of sampling.*

The culture was positive for the same germs on the catheter in 19.04% of cases and for the same germs at the catheter exit site in 14.28% of cases. The analysis of the samples from dialysis patients showed a clear predominance of gram-positive *cocci* (58.24%) compared to gram-negative *bacilli* (37.36%) and yeasts (4.4%). First, we find *staphylococci* with a frequency of 52.74% followed by *Enterobacteriaceae* 30.76%, non-fermenting gram-negative *bacilli* 6.6%, *streptococci* 5.5% and yeasts at 4.4%. The most common species among gram-positive *cocci* are *staphylococci* with a predominance of *S. aureus* (91.66%) followed by coagulase-negative *staphylococci* (8.34%). *Enterococcus faecalis* represent 5.5% of germs. Among *Enterobacteriaceae, E. coli* is the predominant species (60.71%) followed by *Klebsiella* spp 35.71% then *Citrobacter* spp 3.58%. Among the non-fermenting gram-negative *bacilli, Pseudomonas aeruginosa* is the most prevalent (83.33%). *Candida albicans* represents 4.4% of all germs (Table 2).

Regarding the antibiotic-resistance of our isolates, *Staphylococcus aureus* were resistant to penicillin G in 100% of cases, to fusidic acid in 26.19% of cases, to levofloxacin in 07.14% of cases. Resistance to gentamicin, erythromycin, lincomycin is about 04%. Resistance to Sulfamethoxazol+trimethoprim was about 4.76%. All *S. aureus* were sensitive to vancomycin and teicoplanin. Only one case of methicillin resistance (MRSA) among 44 *S. aureus* was found (2.27%) (Figure 1).

			N	% of bacterial species
GNB N = 34	*Enterobacteriaceae* N = 28	*E. coli*	17	42,42%
		Klebsiella spp	10	27,27%
		Citrobacter spp	01	18,18%
	Non -fermenting N = 6	*Pseudomonas aeruginosa*	06	100 %
Gram+cocci N = 53	*Streptococcus* N = 5	*Enterococcus faecalis*	05	100 %
	Staphylococcus N = 48	*S. aureus*	44	91,66%
		Coagulase negative staphylococci	04	08.34%
Yeast N = 4	*Candida* N = 4	*Candida albicans*	04	100 %

***Table 2**: Distribution according to bacterial species.*

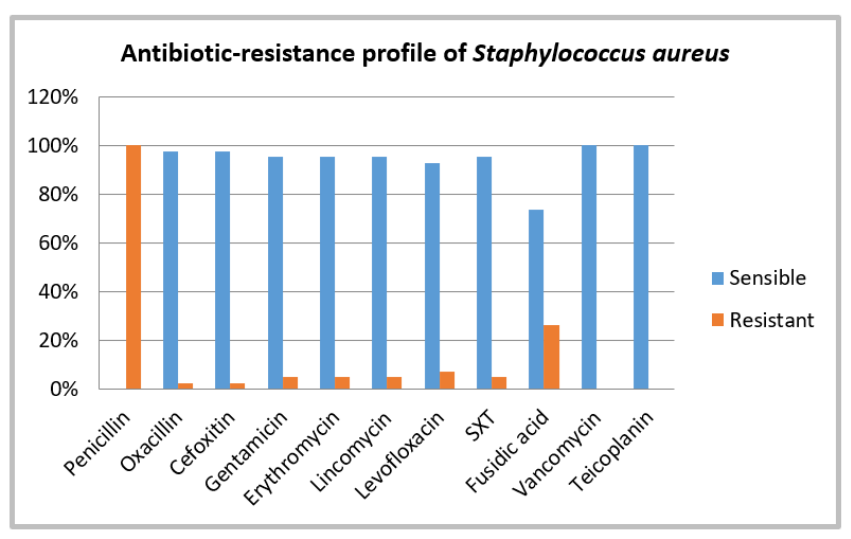

***Figure 1:** Antibiotic-resistance profile of Staphylococcus aureus.*

A high rate of resistance to amoxicillin (71.43%) was observed in *Enterobacteriaceae*, followed by ticarcillin 66.66%, amoxicillin + clavulanic acid and ticarcillin + clavulanic acid which have resistance rates of about 43.48%. Resistance to ciprofloxacin and trimethoprim-sulfamethoxazole are about 24% and 36%. In this study, *Enterobacteriaceae* are all susceptible to colistin (Figure 2).

Regarding multidrug-resistant bacteria (MDR), we report the presence of one case of methicillin resistance (MRSA) among 44 *S. aureus* (2.27%), the presence of an ESBL (extended spectrum beta-lactamase) producing *E. coli* (3.57%) and a carbapenemase producing *Klebsiella pneumonia* (3.57%). Ceftazidime resistance in *Pseudomonas aeruginosa* was about 33.33% (Table 3).

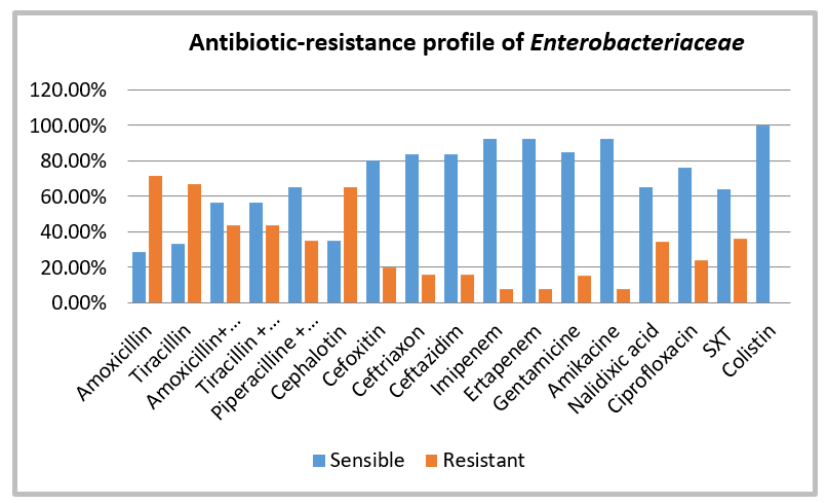

Figure 2: *Antibiotic-resistance profile of Enterobacteriaceae.*

MRB	Number	Frequency
ESBL producing Enterobacteriaceae "extended-spectrum beta-lactamase"	01	3,57%
Carbapenemase-producing *K. pneumoniae*	01	3.57%
Pseudomonas sp Ceftazidime Resistant	02	33.33%
Methicillin-resistant *Staphylococcus aureus* (MRSA)	01	2.27%

Table 3: *Phenotypic analysis of multidrug-resistant bacteria (MRB).*

Discussion

The frequent use of inert materials, such as peritoneal dialysis catheters, can increase the likelihood of biofilm formation on these materials. In the context of peritoneal dialysis, the presence of biofilm on the catheter can predispose the patient to infections. This nutrient-rich environment encourages the growth of bacteria that become resistant to antibiotics. The ability of bacteria to disperse from the biofilm and disseminate across the organism can lead to serious septic states: Peritoneal dialysis-related peritonitis. Microbial contamination of the dialysate in dialysis machines can occur through various routes, including endoluminal and patient-related factors such as airborne or manuported transmission (a lack of asepsis or by accidental disconnection during a PD treatment). In exceptional cases, an intra-abdominal infectious focus, such as appendicitis or diverticulitis, can lead to an infection in the surrounding tissues, including catheter exit site skin and subcutaneous tunnel when the catheter is present.

Our results are consistent with the findings of previous studies, which report a predominance of gram-positive bacteria [1], with a predominance of *Staphylococcus aureus* (48.35%), similar to the results of a Moroccan study and a Tunisian study [2,3]. Compared to the studies of Lioussfi and al and Laurain and al who reported a predominance of coagulase negative *Staphylococcus* (CNS) [4,5].

SA peritonitis may lead to abdominal complications and early catheter removal in patients undergoing peritoneal dialysis and, in some cases, transitioning to hemodialysis [6]. Regarding the antibiotic- resistance of our isolates, the resistance of *Staphylococcus aureus* to penicillin G was very high, this result confirms the findings of previous studies which announced that the secretion of penicillinases is present in 70 to 90% of *S. aureus* [7]. Penicillin G resistance also implies resistance to aminopenicillins, carboxypenicillins and ureidopenicillins. In an Algerian study, among patients undergoing peritoneal dialysis, 3.44% of them were diagnosed with an infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA) [8]. In an American study, the MRSA rate was 4.2% [9]. Our MRSA rate is therefore lower than all these studies (2.27%). The dissemination of MRSA strains in the hospital environment can pose a significant risk to patients, especially those who are immunocompromised, such as dialysis patients. The *staphylococci* isolated in the study were susceptible to the glycopeptide family, similar to the results of an Algerian study [8] and a French study on peritoneal dialysis fluids [10]. Glycopeptides are often used as a treatment option for severe methicillin-resistant staphylococcal infections, they are also used in patients who are allergic to beta-lactams [11].

The gram-negative rate observed in our study is 37.36%, this result is close to Persy and al and Imane and al studies [1,2], but different from the study of Ammari and al in Algeria [8], which reported a rate close to 50%. Gram-negative *bacillus* peritonitis can have different causes, including contamination, catheter exit site infection, or transmural migration related to constipation or colitis. The most frequent germs are *Escherichia coli* (found in 60.71% of cases of *Enterobacteria* in our series), *Klebsiella* sp. and *Proteus* sp [12]. The resistance of *Enterobacteriaceae* to amoxicillin except natural resistance is very high (71.43%) compared to the French study 46% [10] and the Algerian study 53.33% [8]. The production of beta-lactamases confers to the bacteria a resistance to amoxicillin and other beta-lactam antibiotics; The association with a beta-lactamase inhibitor "clavulanic acid" protects the beta-lactam antibiotics from inactivation. In our study, the resistance rate is still high (43.48%), even with the combination of amoxicillin and clavulanic acid. Moreover, we note the presence of an ESBL (extended-spectrum beta-lactamase) producing *Enterobacterium* (*E. coli*) (3.57%) and a carbapenemase producing *Klebsiella pneumoniae* (3.57%). If we compare ESBL with the two other studies already mentioned, we find high frequencies compared to ours, which are 15.38% and 8% [8,10]. On the other hand, carbapenemases were not reported in these studies. As for non-fermenter, gram-negative *bacilli* (BNF), six *Pseudomonas aeruginosa* infections, including two catheter infections, two catheter exit sites infections and two peritonitis were reported. Resistance to ceftazidime was about 33.33%. *Pseudomonas aeruginosa* peritonitis is usually severe and associated with peritoneal dialysis catheter infection; in fact, according to some studies, *Pseudomonas* is frequent in countries where catheter exit site care is performed more than twice a week, using non-sterile wound cleansers and Mupirocin application at the exit sites [13].

The catheter exit site should therefore always be kept dry and clean. Moreover, we did not detect any cases of *Acinetobacter baumannii* infection. 4% were found to have a fungal infection. Fungal peritonitis is often severe, and leads to death in more than 25% of cases. The catheter must be systematically removed, in order to reduce the risk of death [14].

Our study also reports a high number of aseptic peritonitis (57%), in contrast to the ISPD (The International society for peritoneal dialysis) recommendations, which tolerates a maximum number of culture-negative peritonitis of 20% and a desired level of 10% [5]. The causes were probably prior antibiotic therapy, sampling errors, routing errors, and dialysate processing errors in laboratory settings. In this case, the cultures must be repeated on special media in order to detect atypical germs (yeasts, *Mycobacteria*, *Legionella*, slow growing bacteria, *Campylobacter, Mycoplasma*).

Antibiotic therapy should be initiated promptly and administered first intraperitoneally and then intravenously. The initial treatment should cover both gram-positive and gram-negative bacteria, but should be adapted based on the specific bacteria identified. Rapid exchange of dialysate may help relieve pain, and peritoneal lavage may be used in patients with septic shock and cloudy effluent. Heparin should be administered as long as the drained dialysate remains cloudy. The minimum treatment duration for peritonitis is two weeks, and three weeks for severe infections, regardless of the specific bacteria causing infection. Treatment should be extended by at least one

week after the dialysate returns to normal for coagulase-negative *staphylococci* and for cases of sterile peritonitis (where no bacteria are identified). These recommendations are in line with ISPD guidelines. Treatment decisions should be individualized based on the patient's specific clinical presentation and microbiological results [15].

Conclusion

This study highlighted the spectrum of infections in a selected population of dialysis patients, The samples were mainly contaminated by gram-positive *cocci* dominated by the species *Staphylococcus aureus*, that can lead to significant morbidity and mortality in dialysis patients. Despite the wild phenotype of the majority of our isolates, 4.39% of the isolated bacteria were multidrug resistant, which can limit available treatment options for these patients. The frequency of these infections can be reduced by implementing early prevention such as reinforcing aseptic measures and educating patients and their families about health and hygiene.

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