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Clinical and microbial impact of screening kidney allograft preservative solution for bacterial contamination with high-sensitivity methods

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Summary

The clinical and bacteriological consequences of routinely performing highly sensitive bacterial screening of kidney transplant preservation solution (PS) are not known. To evaluate the clinical and microbiological impacts of this strategy, we retrospectively analyzed 200 consecutive kidney allograft recipients from March 2009 to February 2011 for whom PS samples were routinely screened. PS were inoculated into aerobic and anaerobic blood culture bottles, as well as blood agar plates. A rectal swab for extended-spectrum β-lactamase-producing Enterobacteriaceae (EBSL-PE) faecal carriage was also routinely obtained from each patient at admission and every 7 days until hospital discharge. In addition, a standard culture of drain fluid was collected on the day after kidney transplantation. Complete samples and cultures of PS were performed in 165 cases (82.5%), and 62 (37.6%) had positive blood culture results. The most frequent microbial agent isolated was coagulase-negative staphylococci (51.8%). Of these 62 positive samples, only seven (11.3%) were confirmed to contain the same organism by the standard culture method. Drain fluid and PS culture positivity with the same microorganism occurred in only two patients. Of the 62 patients with positive PS cultures, 26 (41.9%) received pre-emptive antibiotic therapy initiated within 48 h post-transplant. During the hospitalization period, patients with a positive PS culture, regardless of whether they received pre-emptive antibiotic therapy, did not exhibit any invasive infections (urinary, blood, peritoneal or wound) related to the microorganisms isolated in the PS. Patients with positive PS cultures who were treated with antibiotic therapy acquired significantly more colonizing ESBL-PE than patients who did not receive antibiotics (53.8% vs. 16.6%; $P = 0.01$); these patients also developed more clinical infections related to the ESBL-PE (23.1% vs. 5.2%; $P < 0.01$). The use of antibiotics for patients with positive PS cultures was an independent risk factor for ESBL-PE acquisition in both univariate and multivariate analyses. In conclusion, the use of more sensitive culture methods increases the rate of bacterial contamination of PS and is associated with an increased prescription of antibiotics and increased ESBL-PE carriage and related infections. Therefore, the systematic use of PS blood bottle cultures in kidney transplantation may have no benefit and might increase the rate of ESBL-PE emergence.

Introduction

Transplanted organs facilitate the transmission of microbes from organ donors to kidney transplant recipients (KTR). Infections in the early post-transplant period are generally donor- or recipient-derived (colonization, viremia and candidemia) or associated with the technical complications of surgery, which produce mucocutaneous barrier injuries, devitalized tissue hematomas and effusions [1,2]. PS contamination is a common cause of early post-transplant infection, with a reported incidence of 2.2–38.7%, and may be a major cause of early morbidity after transplantation. However, prophylactic antibiotic therapy seems to prevent early post-transplant infection [3–5].

Consequently, strategies for the prevention of infection caused by PS contamination include pretransplant screening of donors for potential PS contamination and preventive antibioprophylaxis. A consensus has emerged that contamination of renal allografts by microorganisms other than skin flora need to be treated pre-emptively before the onset of clinical manifestations. Indeed, contamination with skin flora does not pose a risk to the graft. Consequently, expert opinions recommend the treatment of the recipient when organisms are isolated in organ PS [6].

However, protocols for screening organ or tissue donors for infectious risks are inconsistent and vary according to the type of allograft, national standards and the availability of screening assays. For example, the French National Agency for Transplantation recommends performing systematic bacterial analysis of PS samples using high-sensitivity methods (two blood culture bottles) and one blood agar plate as a standard culture [7]. The clinical and microbial impacts of such a strategy combining systematic and highly sensitive screening of all PS in the context of kidney transplantation followed by pre-emptive antibiotherapy, if deemed necessary, is not known.

The aim of this longitudinal study was to determine the prevalence of PS contamination using systematic and high-sensitivity culture methods and to discuss the clinical and bacteriological significance of positive PS cultures.

Materials and methods

Two hundred consecutive kidney transplantations with a deceased donor that were performed at the Necker hospital between March 2009 and February 2011 were retrospectively analyzed. Data were collected from medical and microbiological records. Age, gender, immunosuppressive regimen, total length of hospitalization, duration of ureteral and urethral catheterization, post-transplant use of antibiotics, acute rejection, dialysis or plasmapheresis requirements and surgical complications were collected for each patient.

Surgical procedure

The transplant procedure involved an extraperitoneal Reisberg incision, and the graft vessels were anastomosed with the common or external iliac vessels. Urological anastomosis was pyelo-ureteral, and urinary anastomosis was protected with a double-J stent for 3 weeks, except in three cases. Each patient's bladder was catheterized for 3–5 days. Aspirative drains were placed in the perirenal region and maintained for at least 2 days. Antibiotic prophylaxis included 2 g amoxicillin–clavulanic acid before the surgical procedure, followed by prophylactic trimetoprim–sulfamethoxazole at 80/400 mg per day over a period of 6 months for pneumocystis prevention.

Immunosuppressive regimen

Patients at low immunological risk received basiliximab as an induction, as well as steroids, cyclosporine or tacrolimus and mycophenolate mofetil. High-risk patients received intravenous antilymphocyte immunoglobulins, steroids, high-dose intravenous immunoglobulins, tacrolimus and mycophenolate mofetil.

Bacteriological procedures

Preservation solution samples were routinely collected at the time of allograft preparation and were analyzed in a local microbiology laboratory for bacterial and fungal contamination. Each sample was inoculated in one aerobic and one anaerobic blood culture bottle (BacT/ALERT®, BioMerieux Craponnes, France), as well as on agar plates (standard culture method). Blood culture bottles were incubated at 35 °C for 5 days, and standard cultures were incubated at 35 °C for 10 days. One day after transplantation, a sample of drain fluid was sent to the local microbiology laboratory and analyzed for bacterial and fungal contamination using standard culture methods.

Rectal swab specimens were routinely obtained from each patient at admission and every 7 days until hospital discharge for extended-spectrum ß lactamase-producing Enterobacteriaceae (EBSL-PE) faecal carriage screening. Swabs were plated on selective chromogenic medium for ESBL-PE screening (chromID ESBL Agar, BioMerieux). The double-disc synergy test was used to detect the presence of ESBL-PE.

Statistics

Statistics were performed using Statview® version 5.0 (SAS Institute Inc., Brie Comte Robert, France). Categorical variables are summarized as percentages, and continuous variables are summarized as the mean and SD. Categorical variables were compared using the chi-squared test, and the

Mann–Whitney test was used for continuous variables. For logistic regression, predictors with a $P < 0.10$ were included in the model. A two-sided $P < 0.05$ was considered to be statistically significant.

Results

The demographical characteristics of the patients are listed in Table 1.

Complete (culture and agar) PS cultures were adequately performed in 165 cases (82.5%). PS samples were missing in 6% of the cases, and 23 PS samples were only cultured on blood agar plates (11.5%). Sixty-two of the 165 complete PS samples (37.6%) were positive in blood culture bottle analysis at a mean of 22.2 h of culture (range: 10.1–51.8) after inoculation. Forty-three (69.4%) positive PS samples were monomicrobial, and 19 (30.6%) were multimicrobial. The organisms isolated from PS samples are summarized in Table 2, and coagulase-negative staphylococci were the most frequently isolated microbial agent (51.8%), followed by Gram-negative organisms. Of the 62 positive samples, only nine (14.5%) were positive in standard culture analysis, and detection of the same organism as that observed in the blood culture bottle analysis occurred in only seven of these cases (*Escherichia coli*, three; *Hafnia alvei*, two; *Candida albicans*, two). None of the observed cases yielded positive PS cultures by the standard method without also demonstrating positivity by the high-sensitivity method. Drain fluid cultures performed the day after transplantation were positive in only five patients (2.5%). A positive drain fluid culture together with a positive PS culture of the same microorganism occurred in only two patients, and candida albicans was identified in both instances.

Of the 62 recipients with positive PS cultures determined using high-sensitivity methods, 26 (41.9%) received antibioprophylaxis based on clinician's assumption. There was no formal or institutional consensus for starting pre-emptive antibiotic therapy, and the choice was left at the discretion and the convictions of the doctor. Antibioprophylaxis was initiated as soon as a patient's blood bottle culture was positive (i.e., within 48 h after transplantation) and was adapted to the resistance profile of the microorganism identified. In 20 cases (76.9%), this therapy was prescribed for more than 7 days. No invasive infections (urinary, blood, peritoneal or wound infection) related to the microorganism isolated in the PS samples occurred in the patients, regardless of pre-emptive antibiotic therapy. Overall, these results indicate that: (i) PS culture with high-sensitivity methods increases the rate of PS microbial contamination diagnosis compared with classical culture methods, (ii) the incidence of the clinical complications of PS contamination is negligible (in our case series) and (iii) antibioprophylaxis does not impact the clinical outcome of PS contamination.

To evaluate the bacteriological impact of the strategy aimed at prescribing antibiotics when PS contamination is diagnosed, we analyzed the emergence of ESBL-PE in rectal swab samples (faecal carriage). ESBL-PE colonization occurred in 47 patients (23.5% of the 200 KTR population), and the two most frequently colonizing ESBL-PE were *E. coli* (34%) and *Enterobacter cloacae* (51%) (Table 3). Patients with positive PS cultures who received antibiotic therapy acquired significantly more colonizing

Table 1. Demographical and transplant characteristics of the cohort.

	n = 200
Age (years)	52.3 ± 14.1
Sex ratio (M/F)	1.27 (112/88)
First kidney transplantation, *n* (%)	153 (76.5)
Diabetes mellitus, *n* (%)	28 (14)
Immunological high risk, *n* (%)	89 (44.5)
Hospitalization duration, days	23.7 ± 15.8
Urethral catheterization, days	7.7 ± 6.6
Ureteral catheterization, days	23.7 ± 15.9
Acute rejection (%)	20 (10)
Post-transplant dialysis requirement (%)	58 (29)
Post-transplant plasmapheresis requirement (%)	39 (19.5)
Post-transplant use of rituximab (%)	37 (18.5)
Post-transplant urinary obstruction (%)	30 (15)
Surgery*(%)	29 (14.5)
Post-transplant use of antibiotics (%)	119 (59.5)
Antibiotherapy, days	6.9 ± 7.6
Colonization with MRB before transplantation (%)	7 (3.5)

Continuous variables are expressed as mean ± SD.
MRB, multiresistant bacteria.
*Re-intervention or ureteral catheterization (anterograde or retrograde) for urine drainage.

Table 2. Organisms isolated from the 62 positive preservation solution (PS).

Organisms*	Number of isolates from PS cultured in blood culture bottles
Gram-negative bacilli, *n* (%)	16 (19.3)
Escherichia coli	8 (9.6)
Hafnia alvei	3 (3.7)
Others	5 (5.9)
Gram-positive cocci, *n* (%)	58 (69.9)
Coagulase-negative staphylococci	43 (51.8)
Staphylococcus aureus	6 (10.3)
Streptococcus	6 (10.3)
Others	3 (5.1)
Gram-positive bacilli, *n* (%)	6 (7.2)
Lactobacillus sp.	2 (33.3)
Corynebacterium sp.	2 (33.3)
Others	2 (33.3)
Yeasts, *n* (%)	3 (3.6)
Candida albicans	3 (4.8)

*19 were multimicrobial.

Table 3. Multidrug resistant bacteria isolated in anal swab.

Organisms	Number of isolates from rectal swab (n = 47)
Enterobacter cloacae, *n* (%)	24 (51)
Escherichia coli, *n* (%)	16 (34)
Citrobacter freundii, *n* (%)	2 (4.2)
Klebsiella pneumoniae, *n* (%)	4 (8.4)

Table 4. Analysis of the risk factors for ESBL-PE colonization.

	Group 1 (n = 47)	Group 2 (n = 153)	*P*
Mean age (years)	53.5 ± 12.9)	51.0 ± 14.5	NS
Sex ratio (M/F)	1.24 (26/21)	1.28 (86/67)	NS
Diabetes mellitus before transplantation, *n* (%)	7 (14.3)	21 (13.7)	NS
Immunological high risk, *n* (%)	26 (55.3)	62 (40.5)	0.07
Hospitalization duration, days	31.9 ± 18.0	21.1 ± 14.2	<0.0001
Urethral catheterization, days	9.7 ± 7.9	7.1 ± 6.0	0.02
Ureteral catheterization, days	30.4 ± 15.0	30.1 ± 13.0	NS
Acute rejection, *n* (%)	5 (10.6)	15 (10.2)	NS
Post-transplant dialysis requirement, *n* (%)	19 (40.4)	39 (26.5)	0.07
Post-transplant plasmapheresis requirement, *n* (%)	17 (36.2)	22 (14.9)	0.001
Post-transplant use of rituximab, *n* (%)	14 (29.8)	23 (15.5)	0.03
Post-transplant urinary obstruction, *n* (%)	14 (29.8)	16 (10.9)	0.002
Surgery, *n* (%)	10 (21.3)	19 (12.9)	NS
High steroids doses, *n* (%)	8 (17.0)	27 (18.2)	NS
Use of antibiotics for positive culture of PF, *n* (%)	14 (29)	12 (8)	<0.0001
Use of antibiotics other than for PF, *n* (%)	27 (57.4)	74 (55.7)	NS
NODAT, *n* (%)	8 (17.0)	7 (4.8)	0.006

Continuous variables are expressed as mean ± SD.

ESBL-PE than patients with positive PS cultures who did not receive antibiotic therapy [14/26 (53.8%) vs. 6/36 (16.6%), $P = 0.01$]. The patients with positive PS cultures who received antibiotic therapy developed more clinical infections (mostly urinary tract infections) related to ESBL-PE than the patients with positive PS cultures who were not treated with antibiotic therapy (23.1% vs. 5.2%; $P < 0.01$). The vast majority of ESBL-PE clinical infections were urinary tract infections (more than 80%).

To test whether the use of antibioprophylaxis for PS positive cultures was independently associated with ESBL-PE colonization, we compared the following two groups of patients: group 1 (n = 47) included patients for whom ESBL-PE colonization occurred during hospitalization and group 2 included patients who did not exhibit ESBL-PE colonization (n = 153). Univariate analysis indicated that the length of hospitalization, urological complications and procedures, post-transplant diabetes, antibiotic therapy use and plasmapheresis use were associated with a higher prevalence of ESBL-PE carriage (Table 4). The multivariate analysis indicated that the use of antibiotics for positive PS cultures and the administration of plasmapheresis were independent risk factors of the independent factors associated with ESBL-PE colonization (OR: 5.2, range 2–13.7, and 7, range 1.5–33.3) (Table 5).

Table 5. Multivariate regression model of the predictive factors for extended-spectrum β lactamase-producing Enterobacteriaceae colonization.

	OR	95% confidence interval	*P*
Use of antibiotics for positive culture of PS	5.2	2.0–13.7	0.0008
Post-transplant plasmapheresis requirement	7.0	1.5–33.3	0.014

Discussion

Our results indicate that the use of high-sensitivity culture methods for the detection of PS microbial contamination increases the prevalence of PS contamination; however, the clinical complications associated with PS contamination were not observed in our series of 200 consecutive patients. We also demonstrate that this strategy may increase the prescription of antibiotic therapies, which are clearly associated with the emergence of ESBL-PE.

There is no consensual definition of bacterial PS contamination in kidney transplantation. Previous studies have reported PS contamination in between 2.2% and 38.7% of tested samples [3–5]. This variation could be explained by the lack of a proper definition and the use of various culture methods. A higher incidence of positive PS cultures (28%) was found in a study of 114 allografts reported by Buchholz *et al.*, which reflected the use of a proactive approach to isolate the organisms; this approach facilitated the isolation of many microbes that were present at low numbers [8]. This finding corroborates our results, which revealed that the use of high-sensitivity culture methods with blood culture bottles led to increased PS contamination with a very high crude incidence of PS culture contamination (37.6%). However, when we take into account the cases that were positive for the same microbial agent in the two different culture methods, the incidence is lower (7/165, 4.2%). Therefore, the use of more sensitive culture

methods might overestimate the real incidence of PS contamination. The high proportion of skin flora microorganisms, such as Gram-positive cocci, found in the blood culture bottles indicated that in the majority of the cases of positive PS cultures, the contamination occurred during the inoculation process of PS into the blood culture bottles and was not attributable to infected PS.

Except in the cases of fungal contamination, for which the initiation of a specific treatment is recommended as soon as possible given the life-threatening consequences of such infections [9], the management of microbial PS contaminations is not universally accepted. Furthermore, data are lacking regarding the consequences of bacterial PS contamination and the appropriate antibiotic therapy strategy. In a meta-analysis of 1264 renal graft recipients, skin contaminants were identified in 67% of kidney allografts and were not associated with increased morbidity, irrespective of the antibiotic treatment strategy [3].

Whereas skin flora contaminants do not seem to be associated with an increased risk of complications, PS contamination with coliform agents is associated with serious complications. In a large study of 638 KTR, the incidence of PS contamination was 9.1%, and seven of the nine recipients with coliform PS contamination required antibiotics. In addition, three of the nine patients lost their grafts [3].

Our results indicate that a pre-emptive antibiotherapy based on the positivity of blood culture bottles is associated with the emergence of ESBL-PE in this patient population. The increasing prevalence of infections caused by ESBL-PE is of major concern in the hospitalized patient population [10]. KTR are at high risk for ESBL-PE colonization as an incidence of ESBL-PE infection of 20% has been reported [11]. In a study of 417 renal transplant recipients, 11.8% ESBL-PE incidence has been reported. Previous use of antibiotics, post-transplant dialysis requirement and post-transplant urinary obstruction were independent variables associated with ESBL-PE infection. These infections were associated with significant morbidity after renal transplantation. Our study confirmed these results and found that patients treated with antibiotics for PS contamination had significantly greater rates of ESBL-PE colonization associated with infections than did patients who did not receive antibiotic therapy during hospitalization.

There are few data regarding the significance of the ESBL-PE carriage in KTR populations. Although ESBL-PE colonization in neutropenic patients with haematological malignancies does not appear to have a significant clinical relevance [12], our data indicate that ESBL-PE is a risk factor for ESBL-PE infection. Notably, ESBL-PE colonization may persist over a prolonged period, and this persistence must be considered in the treatment of KTR [13].

In conclusion, the use of more sensitive culture methods overestimates the rate of PS contamination and is associated with increased antibiotic therapy and the emergence of ESBL-PE colonization and related infections.

Authorship

DB, CL and MFM: designed the study. DB: collected and analyzed the data. DB, AS and RSS: performed research. JRZ and OL: provided microbiological data and advices. NP and DB: wrote the article.

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