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BRIEF REPORT

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Impact of antifungal treatment on *Candida–Pseudomonas* interaction: a preliminary retrospective case–control study

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Abstract *Objective:* A pathogenic interaction between *Candida albicans* and *Pseudomonas aeruginosa* has recently been demonstrated. In addition, experimental and clinical studies identified *Candida* spp. tracheobronchial colonization as a risk factor for *P. aeruginosa* pneumonia. The aim of this study was to determine the impact of antifungal treatment on ventilator-associated pneumonia (VAP) or tracheobronchial colonization due to *P. aeruginosa*. *Design and setting:* Retrospective observational case–control study conducted in a 30-bed ICU during a 1-year period. *Patients and methods:* One hundred and two patients intubated and ventilated for longer than 48 h with tracheobronchial colonization by *Candida* spp. Routine screening for *Candida* spp. and *P. aeruginosa* was performed at ICU admission and weekly. Antifungal treatment was based on medical staff decisions. Patients with *P. aeruginosa* VAP or tracheobronchial colonization were matched (1:2) with patients without *P. aeruginosa* VAP or tracheobronchial colonization. In case and control patients, risk factors for *P. aeruginosa* VAP or tracheobronchial colonization were determined using univariate and multivariate analyses. *Results:* Thirty-six patients (35%) received antifungal treatment. Nineteen patients (18%) developed a *P. aeruginosa* VAP or tracheobronchial colonization, and all were successfully matched. Antifungal treatment [31% vs 60%; $p = 0.037$, OR (95% CI) = 0.67 (0.45–0.90)], and duration of antifungal treatment (7 ± 11 vs 14 ± 14 days; $p = 0.045$, in case and control patients respectively) were significantly associated with reduced risk for *P. aeruginosa* VAP or tracheobronchial colonization. Antifungal treatment was the only variable independently associated with *P. aeruginosa* VAP or tracheobronchial colonization (OR = 0.68, 95% CI = 0.49–0.90, $p = 0.046$). *Conclusion:* In patients with *Candida* spp. tracheobronchial colonization, antifungal treatment may be associated with reduced risk for *P. aeruginosa* VAP or tracheobronchial colonization.

Keywords *Pseudomonas aeruginosa* · *Candida spp.* · Antifungal treatment · Interaction · Ventilator-associated pneumonia · Tracheobronchial colonization

Introduction

Ventilator-associated pneumonia (VAP) occurs in a considerable proportion of patients undergoing mechanical ventilation and is associated with substantial morbidity, two-fold mortality, and excess cost [1]. Several studies have identified *Pseudomonas aeruginosa* as the most frequent microorganism in patients with VAP [2]. Tracheobronchial colonization and duration of mechanical ventilation are the two most important risk factors for VAP [3]. Although risk factors for VAP and tracheobronchial colonization are probably similar, outcomes of patients with VAP and those with tracheobronchial colonization are clearly different [3, 4].

According to the results of a recent experimental study, there is a pathogenic interaction between *Candida albicans* and *P. aeruginosa*, suggesting that *P. aeruginosa* could be more virulent in the presence of *C. albicans* [5]. Other experimental studies identified physical, chemical, and environmental similarities between the two pathogens [6, 7]. Moreover, a recent clinical study identified *Candida* spp. tracheobronchial colonization as an independent risk factor for *P. aeruginosa* VAP [8]. Therefore we conducted this retrospective case–control study to determine the impact of antifungal treatment on *P. aeruginosa* VAP or tracheobronchial colonization in patients with *Candida* spp. tracheobronchial colonization.

Patients and methods

This observational retrospective case–control study was conducted in a 30-bed medical and surgical intensive care unit (ICU) from January 2004 to January 2005. No informed consent was required by the Institutional Review Board because of the retrospective and noninterventional design of the study.

All patients intubated and ventilated for longer than 48 h who had tracheobronchial colonization by *Candida* spp. were eligible for this study. Patients with *P. aeruginosa* VAP or tracheobronchial colonization diagnosed before or at the same time as *Candida* spp. tracheobronchial colonization were excluded. Patients were identified using the electronic files of mycology laboratory. Data collection was based on retrospective chart review.

All patients were screened on endotracheal aspirate for *Candida* spp. and *P. aeruginosa* at ICU admission and weekly thereafter. In addition, other microbiologic examinations were performed according to patient status. Antifungal treatment and its duration were based on medical staff decisions.

Definitions

Tracheobronchial colonization was defined as positive respiratory specimen culture. VAP was defined by the presence of new or progressive radiographic infiltrate associated with two of the following criteria: temperature $> 38.5\,^{\circ}C$ or $< 36.5\,^{\circ}C$, leukocyte count $> 10{,}000/\mu l$ or $< 1{,}500/\mu l$, purulent tracheal aspirate; and positive ($\geq 10^6$ cfu/ml) endotracheal aspirate. Only VAP episodes occurring more than 48 h after the commencement of mechanical ventilation were taken into account.

Matching criteria

Cases were patients with *P. aeruginosa* VAP or tracheobronchial colonization; controls, patients without *P. aeruginosa* VAP or tracheobronchial colonization. Every case was matched with two controls according to all the following criteria: (1) duration of mechanical ventilation before first positive respiratory specimen for *P. aeruginosa* (controls $\geq$ cases), (2) admission category (medical/surgical), (3) immunologic status, and (4) date of ICU admission when more than two potential control patients were available.

Statistical analysis

Qualitative variables were compared using the χ^2 test or Fisher's exact test where appropriate. The Mann–Whitney U-test was used to compare quantitative variables.

To determine variables associated with *P. aeruginosa* VAP or tracheobronchial colonization, cases were compared with controls by univariate and multivariate analyses.

Please see the electronic supplementary material (ESM) for additional information.

Results

One hundred and seventeen patients were eligible. Fifteen patients were excluded because *P. aeruginosa* VAP or tracheobronchial colonization was diagnosed before or at the same time as *Candida* spp. tracheobronchial colonization.

Mycology results and antifungal treatment

130 *Candida* spp. were isolated in the 102 study patients. *C. albicans* was the most frequently isolated species (67%). Thirty-six patients (35%) received antifungal treatment. The mean duration of antifungal treatment was 13 ± 12 days. Fluconazole was the most frequently used antifungal (66%). Indications for antifungal treatment included: candidemia ($n = 3$), pneumonia in immunosuppressed patients ($n = 8$), peritonitis ($n = 6$), and preemptive therapy ($n = 19$).

Characteristics of study patients

The characteristics of the study patients are presented in Table 1. The durations of ICU stay and of antifungal treatment were highly colinear ($r = 0.716$).

Risk factors for *P. aeruginosa* VAP or tracheobronchial colonization

Nineteen patients developed a *P. aeruginosa* VAP or tracheobronchial colonization, including 13 patients who received antifungal treatment (7 patients before antifungal treatment, 4 patients during antifungal treatment, and 2 patients after antifungal treatment), and 6 patients who did not receive antifungal treatment. Among the 19 patients with *P. aeruginosa* VAP or tracheobronchial colonization, 10 developed at least one VAP episode, and 9 remained colonized.

The 19 patients with *P. aeruginosa* VAP or tracheobronchial colonization were all successfully matched with 2 control patients each for a total of 38 controls. Results of univariate analysis are presented in Table 2. Multivariate analysis identified antifungal treatment as the only factor independently associated with *P. aeruginosa* VAP or tracheobronchial colonization (OR = 0.68, 95% CI = 0.49–0.90, $p = 0.046$).

Please see the ESM for additional results.

Table 1 Patient characteristics

	Antifungal treatment ($n = 36$)	No antifungal treatment ($n = 66$)	p
Age, years	60 ± 17	58 ± 16	0.286
Male gender	13 (36)	29 (43)	0.290
SAPS II	48 ± 16	49 ± 18	0.796
LOD score	5 ± 3	6 ± 4	0.792
Surgery	9 (25)	10 (15)	0.169
Diabetes mellitus	10 (27)	13 (19)	0.245
Prior antibiotic treatment	19 (52)	20 (30)	0.029*
Immunosuppression	8 (22)	2 (3)	0.003*
Chronic respiratory disease	15 (41)	29 (43)	0.496
Chronic heart failure	8 (22)	12 (18)	0.403
Cirrhosis	4 (11)	2 (3)	0.114
Chronic renal failure	1 (2)	7 (10)	0.154
Cause for ICU admission			
ARDS	5 (13)	2 (3)	0.051
Pneumonia	11 (30)	16 (24)	0.321
CAP	4 (11)	13 (19)	0.204
HAP	7 (19)	3 (4)	0.021*
Acute exacerbation of COPD	8 (22)	16 (24)	0.511
Acute poisoning	3 (8)	4 (6)	0.476
Septic shock	4 (11)	11 (16)	0.328
Congestive heart failure	2 (5)	1 (1)	0.716
Cellulitis	1 (2)	5 (3)	0.306
Others	2 (5)	11 (16)	0.093
During ICU stay			
Days of MV free of *P. aeruginosa*	21 ± 15	13 ± 9	0.003
Number of *Candida*-colonized sites	1.7 ± 1	1.4 ± 0.6	0.099
Duration of MV before *Candida* colonization	4 ± 6	4 ± 6	0.574
Antibiotic treatment	17 (89)	35 (92)	0.545
Antipseudomonal antibiotics	14 (38)	26 (39)	0.566
Non-antipseudomonal 3GC	10 (27)	20 (30)	0.488
Other antibiotics	33 (91)	55 (83)	0.195
Duration of antibiotic treatment, days	24 ± 14	12 ± 8	< 0.001
Duration of MV, days	30 ± 22	16 ± 14	< 0.001
Duration of ICU-stay, days	35 ± 26	18 ± 17	< 0.001
ICU mortality	19 (48)	21 (31)	0.032*

Data are expressed as number (%) or mean ± SD. *SAPS* Simplified Acute Physiology Score, *LOD* logistic organ dysfunction, *ARDS* acute respiratory distress syndrome, *CAP* community-acquired pneumonia, *HAP* hospital-acquired pneumonia, *MV* mechanical ventilation, *3GC* third-generation cephalosporins. *OR (95% CI) = 2.4 (1–5.7), 3.4 (1–12), 5 (1.2–21), 2.3 (1–5.5), respectively, from top to bottom

Table 2 Risk factors for ventilator-associated pneumonia or tracheobronchial colonization related to *Pseudomonas aeruginosa* in univariate analysis

	Cases ($n = 19$)	Controls ($n = 38$)	p
At ICU admission			
Age, years	63 ± 13	59 ± 16	0.402
Male gender	10 (52)	24 (63)	0.315
SAPS II	49 ± 21	49 ± 17	0.803
LOD score	6 ± 4	6 ± 3	0.966
Surgery	3 (15)	6 (15)	0.639
Diabetes mellitus	6 (31)	10 (26)	0.452
Prior antibiotic treatment	7 (36)	20 (52)	0.174
Immunosuppression	2 (10)	4 (10)	0.661
Chronic respiratory disease	8 (42)	22 (57)	0.199
Chronic heart failure	4 (21)	10 (26)	0.464
Cirrhosis	2 (10)	3 (7)	0.545
Chronic renal failure	2 (10)	2 (5)	0.407
Cause for ICU admission			
ARDS	1 (5)	2 (5)	0.712
Pneumonia	6 (31)	13 (34)	0.544
CAP	1 (5)	10 (25)	0.055
HAP	5 (26)	3 (7)	0.072
Acute exacerbation of COPD	5 (26)	11 (26)	0.548
Acute poisoning	2 (10)	1 (2)	0.255
Septic shock	2 (10)	5 (13)	0.571
Congestive heart failure	1 (5)	1 (2)	0.560
Others	2 (10)	5 (13)	0.661
During ICU stay			
Number of *Candida*-colonized sites	1.2 ± 0.6	1.9 ± 1.3	0.121
Antifungal treatment	6 (31)	23 (60)	0.037*
Duration of antifungal treatment, days	7 ± 11	14 ± 14	0.045
Antibiotic treatment	17 (89)	35 (92)	0.545
Antipseudomonal antibiotics	6 (31)	17 (44)	0.254
Non-antipseudomonal 3GC	8 (42)	9 (23)	0.131
Other antibiotics	17 (89)	33 (86)	0.571
Duration of antibiotic treatment, days	13 ± 6	18 ± 14	0.536
Duration of mechanical ventilation, days	16 ± 7	22 ± 15	0.333

Data are expressed as number (%) or mean ± SD. In cases, only exposure to potential risk factors before first positive respiratory specimen for *P. aeruginosa* was taken into account. *SAPS* simplified acute physiology score, *LOD* logistic organ dysfunction, *ARDS* acute respiratory distress syndrome, *CAP* community-acquired pneumonia, *HAP* hospital-acquired pneumonia, *3GC* third generation cephalosporins. *OR (95% CI) = 0.67 (0.45–0.90)

Discussion

Our results suggest that antifungal treatment is associated with reduced risk for VAP or tracheobronchial colonization related to *P. aeruginosa*. To our knowledge, this study is the first to evaluate the impact of antifungal treatment on *Candida* spp. and *P. aeruginosa* interaction. Several studies have identified pathogenic interactions between microorganisms, such as herpes simplex virus and human immunodeficiency virus; influenza virus and *P. aeruginosa*; and *C. albicans* and *P. aeruginosa* [5, 9, 10]. Theses interactions have major environmental and medical consequences. A pathogenic interaction between *C. albicans* and *P. aeruginosa* has been demonstrated in experimental studies. Recent *P. aeruginosa* infection has been identified as a risk factor for fatal candidiasis in burned mice [11]. Molecular studies identified phylogenetic similarities between the two pathogens [5, 6]. The morphology and virulence of *C. albicans* are significantly affected by the presence of *P. aeruginosa* [5]. A cell–cell signaling molecule capable of inhibiting *C. albicans* filamentation is produced by *P. aeruginosa* [5]. *P. aeruginosa* forms a dense biofilm on *C. albicans* filaments and kills the fungus. In contrast, *P. aeruginosa* neither binds nor kills yeast-form *C. albicans*. Several *P. aeruginosa* virulence factors that are important in disease are involved in killing of *C. albicans* filaments [5]. Azoulay et al. [8] recently reported the results of the first clinical study suggesting an interaction between *C. albicans* and *P. aeruginosa*. The authors identified *Candida* spp. tracheobronchial colonization as an independent risk factor for *P. aeruginosa* pneumonia. No cause-and-effect relationship was demonstrated in that study. In addition, *Candida* spp. tracheobronchial colonization and *P. aeruginosa* pneumonia could both be a consequence of prior antibiotic treatment. However, the lack of association with *Staphylococcus aureus* pneumonia, another consequence of antibiotic treatment, indicates that

an association between *Candida* spp. tracheobronchial colonization and *P. aeruginosa* remains plausible. A more recent experimental study has evaluated the impact of *C. albicans* tracheobronchial colonization on the occurrence of *P. aeruginosa* pneumonia [12]. Rate of *P. aeruginosa* pneumonia was significantly higher in rats with *C. albicans* tracheobronchial colonization as compared with rats without *C. albicans* tracheobronchial colonization (33% vs 4%, $p < 0.05$).

Although antifungal treatment was associated with reduced risk for *P. aeruginosa* VAP or tracheobronchial colonization, no significant relationship was found between antifungal treatment and *P. aeruginosa* VAP. However, the small number of patients with VAP precludes a definite conclusion. In addition, a recent meta-analysis outlined the similarity of risk factors for colonization and infection related to multidrug-resistant bacteria [4]. Moreover, our results may provide support for the notion that the interaction between *Candida* spp. and *P. aeruginosa* is at bronchial or biofilm level.

Bacterial biofilm has been demonstrated on inner surface of endotracheal tubes removed from mechanically ventilated patients. Bacterial biofilm may play an important role as a persistent source of infectious material for recurrent episodes of VAP [13]. *Candida* spp. and *P. aeruginosa* are the most common pathogens retrieved from endotracheal tube biofilm and tracheal secretions in patients with VAP [14]. Although all *Candida* spp. were taken into account in our study, previous experimental studies [5, 6, 12] were performed exclusively on *C. albicans.* However, in the study by Azoulay et al. [8] tracheobronchial colonization with any *Candida* spp. was identified as a risk factor for *P. aeruginosa* VAP. Further studies are needed to determine whether *Candida* spp. and *P. aeruginosa* interaction could be influenced by the nature of *Candida* spp.

Inclusion of immunosuppressed patients could be a matter of debate. However, immunologic status was a matching criterion. Future randomized interventional studies on the impact of antifungal treatment on *P. aeruginosa* VAP or tracheobronchial colonization should be conducted in immunocompetent patients.

Our study has several limitations. First, this was a retrospective observational study. Second, some of the trends observed in this study could have reached statistical significance if more patients had been included. In addition, the number of patients needed to demonstrate a beneficial effect of antifungal treatment was not calculated a priori. Third, our study was conducted in a single ICU. Therefore, our results may not be generalizable to other ICUs. Fourth, no information was available on the nature of prior antibiotic treatment or and on the quantity of *Candida* spp. in respiratory specimens. In addition, invasive methods were not used to diagnose VAP. However, quantitative tracheal aspirate culture was used in all patients with a high threshold ($\geq 10^6$ cfu/ml). Postmortem studies showed acceptable overall diagnostic accuracy of quantitative tracheal aspirate compared with bronchoalveolar lavage or protected specimen brush [15]. Finally, antifungal treatment was based on medical staff decisions. Among the 36 patients who received antifungals, 19 patients received preemptive antifungal treatment. This finding is consistent with the results of a recent survey conducted in French ICUs [16]. However, recovery of *Candida* spp. from the respiratory tract of mechanically ventilated patients without risk factors for immunosuppression is common and frequently reflects a tracheobronchial colonization [17, 18]. Some authors suggest that antifungal treatment should be based on the colonization index in these patients [19, 20].

We conclude that in patients with *Candida* spp. tracheobronchial colonization, antifungal treatment is associated with reduced risk for *P. aeruginosa* VAP or tracheobronchial colonization. Prospective randomized studies are required to confirm this result.

References

1. Safdar N, Dezfulian C, Collard HR, Saint S (2005) Clinical and economic consequences of ventilator-associated pneumonia: a systematic review. Crit Care Med 33:2184–2193
2. Sadikot RT, Blackwell TS, Christman JW, Prince AS (2005) Pathogen-host interactions in Pseudomonas aeruginosa pneumonia. Am J Respir Crit Care Med 171:1209–1223
3. Chastre J, Fagon JY (2002) Ventilator-associated pneumonia. Am J Respir Crit Care Med 165:867–903
4. Safdar N, Maki DG (2002) The commonality of risk factors for nosocomial colonization and infection with antimicrobial-resistant *Staphylococcus aureus*, enterococcus, gram-negative bacilli, *Clostridium difficile*, and *Candida*. Ann Intern Med 136:834–844
5. Hogan DA, Kolter R (2002) *Pseudomonas–Candida* interactions: an ecological role for virulence factors. Science 296:2229–2232
6. Hogan DA, Vik A, Kolter R (2004) A Pseudomonas aeruginosa quorum-sensing molecule influences Candida albicans morphology. Mol Microbiol 54:1212–1223
7. Spinelli SL, Malik HS, Consaul SA, Phizicky EM (1998) A functional homolog of a yeast tRNA splicing enzyme is conserved in higher eukaryotes and in *Escherichia coli*. Proc Natl Acad Sci USA 95:14136–14141
8. Azoulay E, Timsit JF, Tafflet M, de Lassence A, Darmon M, Zahar JR, Adrie C, Garrouste-Org M, Cohen Y, Mourvillier B, Schlemmer B (2006) Candida colonization of the respiratory tract and subsequent *Pseudomonas* ventilator-associated pneumonia. Chest 129:110–117

9. Celum CL (2004) The interaction between herpes simplex virus and human immunodeficiency virus. Herpes 11 (Suppl 1):36A–45A
10. Seki M, Higashiyama Y, Tomono K, Yanagihara K, Ohno H, Kaneko Y, Izumikawa K, Miyazaki Y, Hirakata Y, Mizuta Y, Tashiro T, Kohno S (2004) Acute infection with influenza virus enhances susceptibility to fatal pneumonia following *Streptococcus pneumoniae* infection in mice with chronic pulmonary colonization with *Pseudomonas aeruginosa*. Clin Exp Immunol 137:35–40
11. Neely AN, Law EJ, Holder IA (1986) Increased susceptibility to lethal *Candida* infections in burned mice preinfected with *Pseudomonas aeruginosa* or pretreated with proteolytic enzymes. Infect Immun 52:200–204
12. Roux D, Ricard JD, Dreyfuss D, De Prost N, Grossin M, Saumon G (2006) Impact de la colonisation des voies aériennes à Candida albicans sur l'émergence d'une pneumopathie à Pseudomonas aeruginosa chez le rat. Réanimation 15: S43 (abstract)
13. Bauer TT, Torres A, Ferrer R, Heyer CM, Schultze-Werninghaus G, Rasche K (2002) Biofilm formation in endotracheal tubes. Association between pneumonia and the persistence of pathogens. Monaldi Arch Chest Dis 57:84–87
14. Adair CG, Gorman SP, Feron BM, Byers LM, Jones DS, Goldsmith CE, Moore JE, Kerr JR, Curran MD, Hogg G, Webb CH, McCarthy GJ, Milligan KR (1999) Implications of endotracheal tube biofilm for ventilator-associated pneumonia. Intensive Care Med 25:1072–1076
15. Nseir S, Marquette CH (2003) Diagnosis of hospital-acquired pneumonia: postmortem studies. Infect Dis Clin North Am 17:707–716
16. Azoulay E, Cohen Y, Zahar JR, Garrouste-Org M, Adrie C, Moine P, de Lassence A, Timsit JF (2004) Practices in non-neutropenic ICU patients with *Candida*-positive airway specimens. Intensive Care Med 30:1384–1389
17. Wood GC, Mueller EW, Croce MA, Boucher BA, Fabian TC (2006) *Candida* sp. isolated from bronchoalveolar lavage: clinical significance in critically ill trauma patients. Intensive Care Med 32:599–603
18. Eggimann P, Garbino J, Pittet D (2003) Management of *Candida* species infections in critically ill patients. Lancet Infect Dis 3:772–785
19. Piarroux R, Grenouillet F, Balvay P, Tran V, Blasco G, Millon L, Boillot A (2004) Assessment of preemptive treatment to prevent severe candidiasis in critically ill surgical patients. Crit Care Med 32:2443–2449
20. Leon C, Ruiz-Santana S, Saavedra P, Almirante B, Nolla-Salas J, Alvarez-Lerma F, Garnacho-Montero J, Leon MA (2006) A bedside scoring system ("Candida score") for early antifungal treatment in nonneutropenic critically ill patients with *Candida* colonization. Crit Care Med 34:730–737