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Association between Antimicrobial Administration and Nasal Carriage of *Pseudomonas aeruginosa* and *Candida* Species in ICU Patients

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Abstract:

Background: Patients admitted to the intensive care unit (ICU) often develop hospital-acquired infections (HAI).

Objective: We started an active surveillance culture (ASC) to monitor bacterial or fungal carriage in the ICU of our hospital and retrospectively evaluated in this study.

Materials and Method: ASC was performed using a nasal swab culture when the patients were admitted to the ICU, regardless of whether the patients had any infectious diseases. If the patients continued to stay in the ICU for the following week or later, ASC was performed regularly once a week until discharge.

Results: When comparing the bacteria isolated from nasal swab cultures at the time of ICU admission, *Pseudomonas aeruginosa*, which sometimes develops drug resistance and can cause HAI, was isolated more frequently from ASC the following factors for increased isolation of *Pseudomonas aeruginosa* week or later. Antipseudomonal penicillin and antifungals were independent risk for increased isolation of *Candida* species. Considering that most ICU patients are administered antimicrobials, it was suggested that using antimicrobials during long-term ICU stays affects the nasal bacterial flora of ICU patients.

Conclusion: ASC would help understand the nasal carriage status and changes in the bacterial flora of ICU patients, supporting taking measures against drug-resistant bacteria.

Keywords: Antimicrobial, *Pseudomonas aeruginosa*, *Candida* species, ICU

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Introduction

Patients admitted to intensive care units (ICU) are relatively immunocompromised due to their severe illness. They are constantly exposed to the risk of infection due to the insertion of various medical devices or in vasive treatments such as surgery. In addition, ICU patients often develop hospital-acquired infections (HAI), including ventilator-associated pneumonia, central venous catheter-associated bloodstream infections, and catheter-associated urinary tract infections. Such severe or life-threatening infectious diseases are common among ICU patients, and most of them are caused by bacterial or fungal infection and require antimicrobial therapy for clinical resolution.[1-4] These patients are prone to opportunistic infections caused by endogenous or environmental bacteria. In addition, antimicrobials are administered to most ICU patients to treat infections or preventive purposes, such as perioperative administration.[5] The Extended Prevalence of Infection in Intensive Care (EPIC II) study, which investigated the status of infections in ICUs in 75 countries, reported that 51% of ICU patients had infectious diseases.[6] In addition, the Japanese Survey of Antimicrobial Use in ICU Patients (JSCRIPT) study, which investigated antimicrobial use in Japan, found that 50.1% of ICU patients had some bacterial infection, and 72.6% were administered intravenous antimicrobials. Empirical antimicrobial treatment was started in 43.1% of patients, and antimicrobials were administered prophylactically to 32.8% of patients.[7] Therefore, the selective pressure by antimicrobial administration increases the risk of ICU patients carrying resistant bacteria. Since ICU patients are always relatively immunocompromised, increasing the pressure of carrying resistant bacteria is a risk factor for transmitting infection to other patients. Active surveillance culture (ASC) is often performed as a screening method for patients with resistant bacteria. We reported that administering antimicrobials was a significant risk factor that affects changes in the nasal bacterial flora of ICU patients.[8] In particular, glucose non-fermenting gram-negative rods (NF-GNR), including *Pseudomonas aeruginosa*, and *Candida* species, increased after admission to the ICU.

In this study, we investigated factors that affect changes in the isolation status of *Pseudomonas aeruginosa* and *Candida* species in the ASC of ICU patients.

Subjects and Method

This study is a retrospective, single center-cohort study and included a total of 815 ASC samples submitted from 366 patients who were admitted to the ICU of Yamagata Prefectural Central Hospital from June 2015 through September 2016. All of the 366 patients underwent ASC by nasal swab culture at the time when admitted to the ICU, regardless of whether they had any infectious diseases (Initial ASC). If the patient continued to stay in the ICU from the following week onwards, ASCs were continued once a week regularly until discharge (Follow-up ASC). Samples were collected from all ICU-admitted patients, with no cases of refusal. There were 364 actual patients who underwent the initial ASC, and 138 actual patients who underwent the follow-up ASC. The culture conditions for ASC were 35°C and 5% carbon dioxide

culture for sheep blood agar medium (Kyokuto Pharmaceuticals, Japan), and 35°C and aerobic culture for BTB agar medium (Kyokuto Pharmaceuticals, Japan), Chromoagar Candida medium (Kanto Chemicals, Japan), and Poremedia MRSA isolation medium (Eiken Chemicals, Japan) for MRSA screening. The cultures were observed after 24 and 48 hours, and the culture-positive bacterial species were identified using a fully automated bacterial testing device, VITEK2 (SYSMEX bioMérieux, Japan). Bacteria or fungi isolated from nasal swab cultures of the patients were counted as one for each strain, including cases in which multiple bacteria or fungi were isolated from the same patient simultaneously. In addition, each case was counted as one even if the culture was negative. The antimicrobial susceptibility of the isolated bacteria was determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

All isolated bacteria were classified according to the time of isolation into two groups: those isolated at the time of ICU admission (Initial ASC group) and those isolated every week after admission (Follow-up ASC group), and the clinical and bacteriological information was evaluated. Data was analyzed using JMP 14 (SAS Institute), with Wilcoxon tests for continuous variables and chi-square tests for non-continuous variables. Multivariate analysis was performed using multiple logistic regression analysis.

This study was reviewed and approved by the Yamagata Prefectural Central Hospital Ethics Committee (approval number: H30-25).

Results

A total of 815 ASC samples submitted from 366 patients who were admitted to the ICU during the period were analyzed. The initial ASC group, in which samples were collected from patients only at admission to the ICU, consisted of 569 samples (364 actual patients). The follow-up ASC group, in which samples were collected from patients in the ICU from the following week onwards, consisted of 246 samples (138 actual patients) (Table 1A and 1B).

When comparing the clinical background of patients in both groups, there were no significant differences in gender and age. Antimicrobials were used in the initial ASC group for 257 out of 364 cases (70.6%). Among these, 57 cases (15.7%) were complicated by infectious diseases. Seven of these 57 cases had antimicrobials administered more than one week before ICU admission, and 15 had antimicrobials administered within one week before admission. The remaining 35 cases had antimicrobials started simultaneously with admission. The 200 cases without infectious diseases had also antimicrobials started simultaneously with admission. While antimicrobials were used in 133 of 138 cases (96.4%), and any infectious diseases in the follow-up ASC group complicated 21 cases (15.2%) (Table 1A). When comparing the proportion of ASC among the isolated strains in both groups, the bacteria which consist of normal nasal flora, such as coagulase-negative Staphylococci (CNS) and Corynebacterium species, accounted for 64.5% (367 strains) in the initial ASC group whereas it was 43.5% (107 strains) in the follow-up ASC group (Figure 1).

Table 1A Patient characteristics

ICU stay	Initial ASC (≤1 week)	Follow-up ASC (>1week)	p-value
Patients, number	364	138	N/A
Gender, male, number (%)	243 (66.8%)	104 (75.4%)	0.059
Age, years, median (IQR)	71 (63-78)	70 (63.8-79)	0.42
ID co-morbidities, number (%)	57 (15.7%)	21 (15.2%)	0.90
ABX use, number (%)	257 (70.6%)	133 (96.4%)	< 0.0001*

ICU: Intensive care unit, ASC: Active surveillance culture, IQR: Interquartile range, ID: Infectious diseases, ABx: antimicrobials
*p < 0.05 as a statistically significant difference

Table 1B ASC profiles

ICU stay	Initial ASC (≤1 week)	Follow-up ASC (>1week)	p-value
ASC samples, number	569	246	N/A
Under ABX use, number (%)	386 (67.8%)	237 (96.3%)	< 0.0001*
Isolated microbes, number (%)	506 (88.9%)	233 (94.7%)	0.0062*

ASC: Active surveillance culture, ICU: Intensive care unit, ABx: antimicrobials
*p < 0.05 as a statistically significant difference

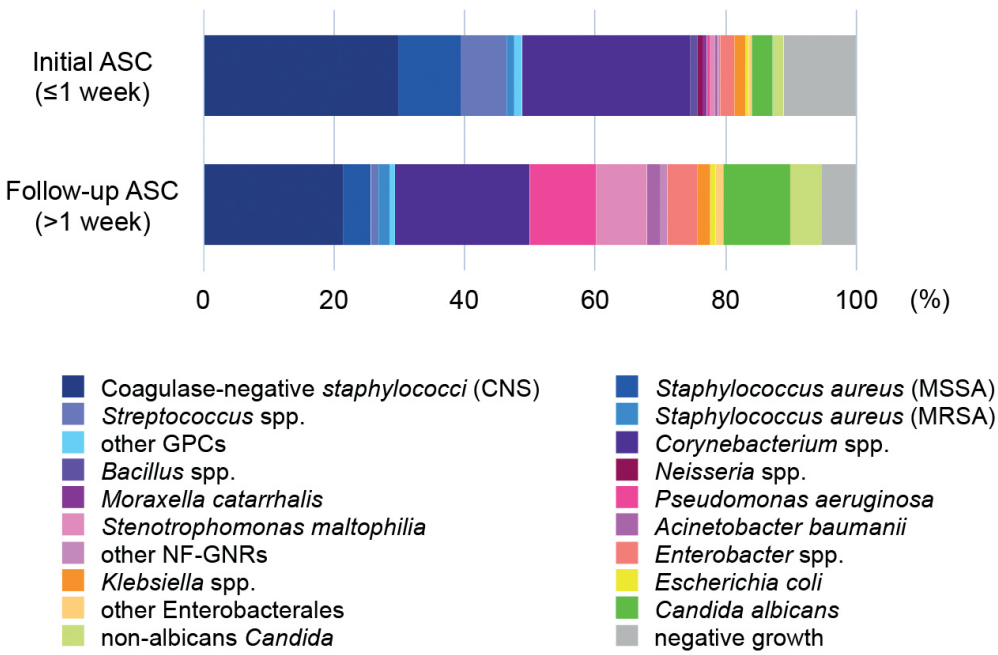

Figure 1 Proportion of isolated bacterial or fungal cultures

ASC: Active surveillance culture, MSSA: Methicillin-sensitive *Staphylococcus aureus*, MRSA: Methicillin-resistant *Staphylococcus aureus*, GPC: Gram positive coccus, NF-GNR: Glucose non-fermenting gram-negative rods, spp: species

In terms of comparing the changes in isolated bacteria or fungi in both the initial ASC group and the follow-up ASC group, *Pseudomonas aeruginosa* was increased from 0.5% (3 strains) to 10.2% (25 strains) (Figure 1). Regarding the drug susceptibility of isolated *Pseudomonas aeruginosa*, the number of strains resistant to antipseudomonal drugs (I or R) was increased from 1 to 12 (Figure 2). In patients who had received some antimicrobials (carbapenems, fluoroquinolones, antipseudomonal penicillin, aminopenicillin, the first-generation cephalosporin, anti-MRSA drugs, and antifungals), the rate of isolation of *Pseudomonas aeruginosa* was increased during ICU stay, regardless of whether the initial ASC or the follow-up ASC (Table 2A). The effect of administration of these antimicrobials on isolation of *Pseudomonas aeruginosa* was examined by multivariate analysis, resulting in administration of antipseudomonal penicillin (odds ratio 5.76) and antifungals (odds ratio 9.75) were independent risk factors for isolation of *Pseudomonas aeruginosa* (Table 2B).

In addition, the number of *Candida* species in the initial ASC group increased from 4.9% (28 strains) to 15.0% (37 strains) in the follow-up ASC group. Of these, the number of *Candida albicans* cases increased from 18 to 25, but the number of non-albicans *Candida* cases did not change significantly, from 10 to 12 (Figure 3). Similarly, we investigated the effect of antimicrobial administration on the isolation of *Candida* species, and the isolation of *Candida* species was higher in patients who had received carbapenems, fluoroquinolones, aminopenicillins, or anti-MRSA drugs. However, no change was observed in patients who had received antipseudomonal penicillin, first-generation cephalosporin, or antifungals (Table 3A). Multivariate analysis showed that the administration of carbapenems was an independent risk factor for the isolation of *Candida* spices (odds ratio 2.11) (Table 3B).

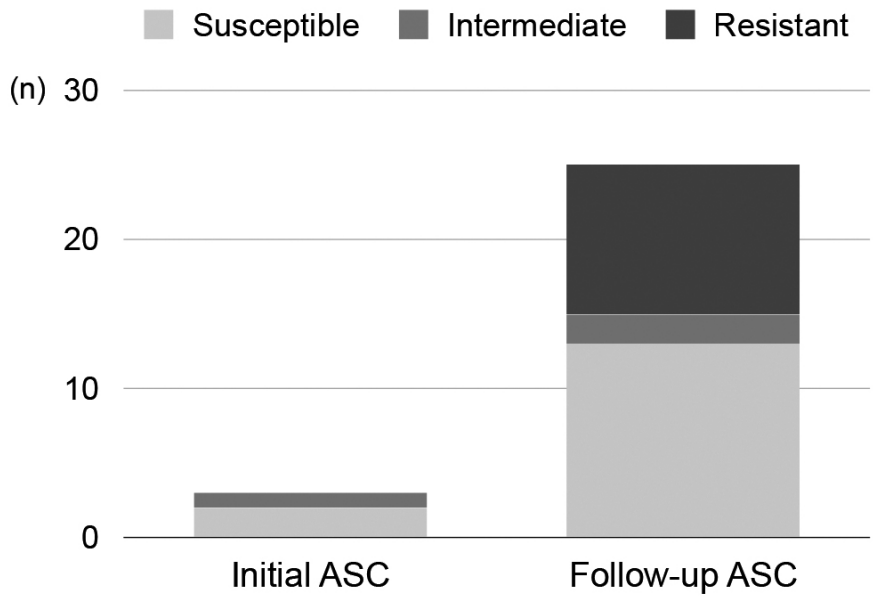

Figure 2 Prolonged ICU stay increased *P. aeruginosa* in ASC
ASC: Active surveillance culture

Table 2A Effect of ABx on *Pseudomonas aeruginosa* isolation in ASC

ASC	*P. aeruginosa*	others	p-value
ASC isolation, number	28	787	N/A
Carbapenems, number (%)	15 (53.6%)	152 (19.3%)	< 0.001*
Fluoroquinolones, number (%)	5 (17.9%)	33 (4.2%)	0.0082*
Antipseudomonal penicillins, number (%)	23 (82.1%)	329 (41.8%)	< 0.0001*
Aminopenicillins, number (%)	16 (57.1%)	145 (18.4%)	< 0.0001*
1st gen. Cephalosporins, number (%)	20 (71.4%)	358 (45.5%)	0.0063*
Anti-MRSA antibiotics, number (%)	6 (21.4%)	68 (8.6%)	0.0437*
Antifungals, number (%)	13 (46.4%)	44 (5.6%)	< 0.0001*
No ABX use, number (%)	1 (3.6%)	191 (24.3%)	0.0027*
Age, years, median (IQR)	71 (66-80)	70 (63-78)	0.3158
Gender, male, number (%)	22 (78.6%)	552 (70.1%)	0.3221

The isolation of *P. aeruginosa* during ICU stay, regardless of whether the initial ASC or the follow-up ASC, were evaluated.
Data was analyzed with Wilcoxon tests for continuous variables and chi-square tests for non-continuous variables.
Clinically important factors were evaluated using univariate analysis, followed by multivariate analysis adjusting for confounding factors.
ABx: antimicrobials, ASC: Active surveillance culture, 1st gen: First generation, MRSA: Methicillin-resistant *Staphylococcus aureus*, IQR: Interquartile range
*$p < 0.05$ as a statistically significant difference.

Table 2B Independent risk factors for isolation of *Pseudomonas aeruginosa* by administration of antimicrobial

	OR	95% C.I.	p-value
Antipseudomonal penicillins	5.76	1.49-23.57	0.0108*
Antifungals	9.75	1.66-89.29	0.0099*

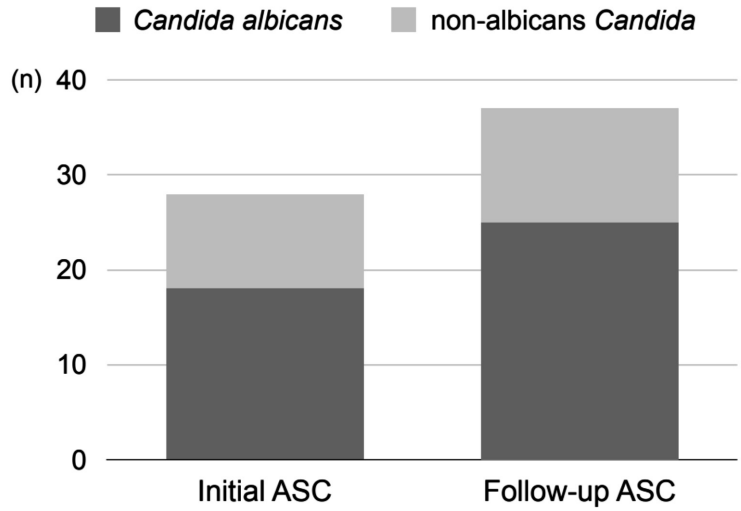

Figure 3 The number of *Candida* species in the initial ASC group increased from 4.9% (28 strains) to 15.0% (37 strains) in the follow-up ASC group

Discussion

Our results showed non-resident intranasal bacteria, such as Enterobacteriaceae, *Pseudomonas aeruginosa* and other NF-GNR, or *Candida* species, were increased in nasal ASC from the week after admission to the ICU. This result suggested that the nasal flora changed due to the extension of the ICU stay. It is known that an extended stay in the ICU increases the risk of acquiring resistant bacteria such as carbapenem-resistant gram-negative bacilli and MRSA.[9,10]

Table 3A Effect of ABx on *Candida* spp. isolation in ASC

	***Candida* spp. (n = 65)**	**others (n = 750)**	**p-value**
Carbapenems	26 (40.0%)	141 (18.8%)	0.0002*
Fluoroquinolones	0 (0%)	38 (5.1%)	0.0109*
Antipseudomonal penicillins	34 (52.3%)	318 (42.4%)	0.1238
Aminopenicillins	20 (30.8%)	141 (18.8%)	0.0273*
1st gen. Cephalosporins	31 (47.7%)	347 (46.3%)	0.8251
Anti-MRSA antibiotics	13 (20.0%)	61 (8.1%)	0.0045*
Antifungals	5 (7.7%)	52 (6.9%)	0.8204

Table 3B Risk factor for *Candida* spp. Isolation

	OR	**95% C.I.**	**p-value**
Carbapenems	2.11	1.10 -3.97	0.025*

Multivariate analysis was performed using multiple logistic regression analysis.
OR: Odds ratio, CI: Confidence Interval
*p < 0.05 as a statistically significant

Regarding the impact of antimicrobial administration on the acquisition of drug-resistant bacteria, there are some reports that antimicrobial administration within the past 3 months is a risk factor for infection with carbapenem-resistant Enterobacteriaceae[11], that administration of carbapenems increases drug-resistant *Pseudomonas aeruginosa*[12], and that antimicrobial administration increases ESBL-producing bacteria.[13] Antimicrobials are administered to most ICU patients admitted for therapeutic or prophylactic purposes, but increased exposure to antimicrobials can disrupt the nasal flora[8] or intestinal flora.[14] ICU patients are generally in critical condition, often in a relatively immunocompromised state, and constantly at risk of developing opportunistic infections, such as NF-GNR, including *Pseudomonas aeruginosa*, Enterobacteriaceae, and yeast-like fungi, including *Candida* species. Many of these bacteria have acquired multiple drug resistance, often developing difficult-to-treat infectious diseases. Considering that the carriage pressure of *Pseudomonas aeruginosa*, especially drug-resistant strain, and *Candida* species increased with prolonged ICU stays, it would be helpful for both appropriate infectious disease treatment and infection control that being aware of the carriage information of patients admitted to the ICU in advance.

Pseudomonas aeruginosa and *Candida albicans* are opportunistic pathogens frequently co-isolated from critically ill patients in ICU.[15,16] Their interactions can exacerbate patient outcomes through various mechanisms.[17] The antimicrobial usage in ICU patients, especially broad-spectrum antibiotics, can disrupt the normal flora, potentially leading to overgrowth and invasion of opportunistic pathogens due to microbial replacement, as well as promoting the emergence of antimicrobial resistance.[18-23]

The primary purpose of ASC is to grasp the patient's bacterial carriage information; however, an increase in the number of specific bacteria detected does not necessarily mean an increase in the number of patients who develop infectious diseases. Furthermore, delays in appropriate infection control measures can directly spread HAI in a limited space such as an ICU. Regular implementation of ASC can monitor changes in the bacterial flora of ICU patients, allowing for early detection of resistant bacteria and the prompt implementation of additional infection control measures such as contact precautions.[24] ASC needs help with the effort required to implement and its cost-effectiveness. Furthermore, there is still much debate about the usefulness of ASC. It is reported that when ASC was implemented in the ICU, the number of MRSA bacteremia, SSI, and VAP in the entire hospital was reduced[25-27], and that ASC was also useful for controlling carbapenem-resistant Acinetobacter species.[28] In contrast, there is a report that ASC was ineffective in suppressing resistant bacterial infections.[29] For this reason, the Society for Healthcare Epidemiology of America (SHEA) guidelines recommend the active implementation of ASC.[30] In contrast, the Centers for Disease Control and Prevention (CDC) guidelines only recommend ASC in patients at high risk of carriage or in emergencies such as outbreaks[31], showing differing opinions on guidelines. Our study showed that administering broad-spectrum antimicrobials and antifungals affects the increase in *Pseudomonas aeruginosa* and *Candida* species in the nasal cavity. Given the recent global demand for measures against antimicrobial resistance (AMR), the significance of implementing ASC is worth enough from the perspective of appropriate use of antimicrobials. In particular, for patients in ICU, where prophylactic antimicrobials are often administered

perioperatively and broad-spectrum antimicrobials are often administered to critically ill patients, understanding the carriage status of resistant bacteria and other bacteria through ASC would help to control appropriate antimicrobial use.

This study has several limitations. First, it was a retrospective study at a single facility. Second, the selection bias of antimicrobials may have contributed to the study results. In addition, this study cannot state whether nasal colonization with *Pseudomonas aeruginosa* or *Candida* species is directly related to the actual onset of HAI.

In our hospital, the ASC has enabled us to grasp the carriage status of ICU patients, and these data improve staff awareness of infectious diseases. Implementing more appropriate and prompt infection control based on objective data will lead to AMR measures in the ICU as well as throughout the hospital.

Conflict of interest

None of the authors have any conflicts of interest to declare.

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