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Original Article

Diversity changes of microbial communities into hospital surface environments

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ABSTRACT

Previous works have demonstrated considerable variability in hospital cleanliness in Japan, suggesting that contamination is driven by factors that are currently poorly controlled. We undertook 16S rRNA sequence analysis to study population structures of hospital environmental microbiomes to see which factor(s) impacted contamination. One hundred forty-four samples were collected from surfaces of three hospitals with distinct sizes ("A": >500 beds, "B": 100–500 beds, "C": <100 beds). Sample locations of two ward types (Surgical and Internal) included patient room bed table (multiple) (4BT), patient overbed table (multiple) (4OT), patient room sink (multiple) (4S), patient room bed table (single) (SBT), patient overbed table (single) (SOT), patient room sink (single) (SS), nurse desk (ND), and nurse wagon (NW). Total DNA was extracted from each sample, and the 50 samples that yielded sufficient DNA were used for further 16S rRNA sequencing of hospital microbiome populations with cluster analysis. The number of assigned bacterial OTU populations was significantly decreased in hospital "C" compared to the other hospitals. Cluster analysis of sampling locations revealed that the population structure in almost all locations of hospital "C" and some locations in the other hospitals was very similar and unusually skewed with a family, *Enterobacteriaceae*. Interestingly, locations included patient area (4OT, 4BT, SBT) and nurse area (ND), with a device (NW) bridging the two and a place (4S and SS) shared between patients or visitors. We demonstrated diversity changes of hospital environmental microbiomes with a skewed population, presumably by medical staff pushing NWs or sinks shared by patients or visitors.

1. Introduction

CDC guidelines on controlling hospital-acquired infections (HAIs) prioritize environmental cleanliness, while recommending precautions for contact between patients and medical staff. In particular, the guidelines promote cleanliness of high-contact surfaces such as machines placed around patients [1]. Bacterial contamination of environmental surfaces is the leading cause of HAIs. Although extensive measures to promote cleanliness are routinely undertaken, HAIs are a persistent problem [2–8]. Thus, many hospitals implement monitoring of the effectiveness of cleaning procedures, with visual assessment of surfaces, assessment of residual fluorescent dye after cleaning, determination of aerobic colony-forming units, or detection of ATP on surfaces [9].

Previous assessments of hospital cleanliness in Japan, using ATP bioluminescence and stamp agar methods, revealed bacterial contamination to be highly variable and independent of time or ward type [10]. No significant relationship was observed between the number of patients or medical personnel in the hospital and organic or microbiological contamination [10]. However, the physical properties of the surfaces that were sampled had a strong effect on contamination [11]. These findings suggest that numerous currently uncontrolled factors contribute to HAIs in Japanese hospitals [10,11].

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Amplicon sequencing, targeting the hypervariable region of the bacterial small-subunit ribosomal RNA gene (16S rRNA), is currently a widely used technique to study environmental microbiomes. Data can be used quantitatively and qualitatively to compare environments, as well as provide direct diagnostic information that can inform therapeutic decisions [12,13]. Microbiome analysis also has been applied in a wide range of research fields, including medicine, as well as ecology of indoor and marine environments and urban public spaces such as subways [14–18]. Thus, microbiome analysis is likely to provide pertinent data in attempts to understand the factors controlling hospital cleanliness and HAIs.

In the present study, we undertook amplicon sequencing to visualize and compare microbial population structures in three different hospitals, which differed in bed numbers.

2. Materials and methods

2.1. Hospitals

Because of their different sizes, three hospitals were selected, which have distinct numbers of beds (hospital "A": >500 beds, hospital "B": 100–500, hospital "C": <100). Both hospitals "A" and "B" are general hospitals with 30 and 21 clinical departments, respectively, while hospital "C" specializes mainly in lung cancer treatment (including antibiotic treatment to prevent infectious diseases after surgery) with only 5 clinical departments. Because of distinct treatment policies, "Surgical" and "Internal" wards were selected for sampling locations. For each of the wards, mean hospitalization periods (days)/patient numbers are as follows: hospital "A": 24 (Surgical 33.2, Internal 14.8)/49.5 (Surgical 50.1, Internal 48), hospital "B": 15 (Surgical 16.3, Internal 13.7)/34.7 (Surgical 36.9, Internal 32.5), and hospital "C": 12.3 (Surgical 10.8, Internal 13.7)/21.1 (Surgical 18.5, Internal 23.4). These hospitals are located in the Hokkaido-Tohoku area in Japan. Hospital features with relative ratios of patients with lung cancer are summarized in Table 1. The numbers of patients with lung cancer were obtained from data published as the hospital index, and the relative ratio of patients with lung cancer among all patients was then estimated. Cleaning was similar in all hospitals and followed CDC guidelines [1]. Cleaning was performed daily in several locations, as shown in Fig. 1, according to the following protocol. In brief, floors were cleaned with disinfectant (Fig. 1, asterisks). The overbed table or bed frame was wiped with detergent and then treated with disinfectant (Fig. 1, arrowheads). The handrail or window frame was also cleaned with detergent only (Fig. 1, arrows).

2.2. Sampling locations and swab collection

A total of 144 samples were collected from hospital environments. As mentioned above, sampling locations belonging to two ward types [Surgical (Su) and Internal (In)] included patient room bed table (multiple) (4BT), patient overbed table (multiple) (4OT), patient room sink (multiple) (4S), patient room bed table (single) (SBT), patient overbed table (single) (SOT), patient room sink (single) (SS), nurse desk (ND), and nurse wagon (NW). Samples were collected from these environmental surfaces on weekday mornings. Sterile cotton swabs dipped in sterile saline were wiped over 400 cm^2 at each location. To prevent contamination from hands, disposable sterilized plastic gloves were used for all sample collections.

2.3. DNA extraction

Each cotton swab was intensely vortexed in sterile saline, and the resulting suspension was then centrifuged, yielding pellets that were used for total genomic DNA extraction. Extraction was performed using the QIAamp DNA kit (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions. DNA was eluted in 50 μl of the elution buffer supplied with the kit, and stored at −20 °C until use. In addition, all laboratory procedures were conducted in a safety cabinet with filtered airflow to prevent cross-contamination.

2.4. Quality checking of extracted DNA

The quality of extracted DNA from each sample ($n = 144$) was confirmed by PCR amplification using specific primers that target the 16S rRNA gene of *Mycobacterium* sp., a species that inhabits most indoor and outdoor environments, including pond water and soil [18]. Band densities of electrophoresed PCR samples on 2% agarose gels were quantified using ImageJ software. DNA samples from which *Mycobacterium* sp. 16S rRNA could not be PCR amplified ($n = 94$) were omitted from the following amplicon sequence analysis because of insufficient DNA concentrations.

2.5. Amplicon sequence analysis

Analysis of samples that passed the quality check ($n = 50$) was performed according to the following general protocols. First, DNA amplicons were amplified by specific primer sets that target the V3–V4 region of SSU rRNA (supplied by Hokkaido System Science Co., Ltd.); PCR products were resolved on 2% agarose gels and then purified. After indexing PCR adaptor sequences had been added to the amplicons, a DNA library for each sample was constructed, with a constant volume of amplicon solution (*i.e.*, concentrations were not adjusted), to permit comparisons between samples of read numbers produced by the Illumina 16S Metagenomic Sequencing Library Preparation kit (Illumina). Libraries were sequenced on the MiSeq Illumina sequencer platform, producing 300-bp paired-end reads. QIIME was used to process raw reads, with quality scores >30, and perform OTU (Operational Taxonomic Units) clustering. OTU annotation was based on BLAST analysis with a baseline of >90% similarity. Metagenomic analysis including quality filtering, OTU production, taxonomic classification, and phylogeny generation was all conducted by Hokkaido System Science Co., Ltd. Cluster

Table 1
Hospital's size and summary.

Hospital speciality/total bed number)	Number of clinical department	Ward	Bed number per ward	Average hospitalization periods (days)	Patient numbers per ward	Relative ratio of patients with lung cancer[a]
A (General hospital/500–1000)	30	Surgical	60	33.2	50.1	1
		Internal	60	14.8	48	1
B (General hospital/100–500)	20	Surgical	46	16.3	36.9	0.3
		Internal	40	13.7	32.5	0.5
C (Lung cancer treatment/less than 100)	5	Surgical	24	10.8	18.5	4.5
		Internal	25	13.7	23.4	2.4

[a] Ratio vs. Hospital A: patient numbers with lung cancer were obtained from data published as hospital index.