Original research

Dissemination of microbiota between wounds and the beds of patients with pressure injuries: a cross-sectional study

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Abstract

Aim Wound infection is a life-threatening complication of pressure injuries (PIs) and is not yet completely preventable. This study aims to explore the dissemination of microbiota between PIs and hospital beds using a culture-independent methodology. This serves as the first step towards developing a new intervention to prevent wound infection.

Methods A cross-sectional study was conducted at a long-term care hospital on patients aged ≥65 years with PIs. The microbiota of wounds, skin and beds were identified using 16S ribosomal RNA (rRNA) gene sequencing analysis. Zero-radius operational taxonomic units (zOTUs) were used for confirming dissemination which indicates bacteria possessing identical sequences within the V3–V4 region of the 16S rRNA gene.

Results Ten PIs were analysed in this study. All individuals had zOTUs common to samples from their wound, skin and bed (median: 194, interquartile range [IQR]: 121–320). Furthermore, the bed samples were classified into the same clusters as the wound samples from eight sites.

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Conclusion Our study is the first to quantitatively show the dissemination of microbiota between PIs and patients' beds using culture-independent analysis. Preventing the dissemination of bacteria to beds may be an effective therapeutic strategy for the prevention of wound infection.

Introduction

Pressure injuries (PIs) are localised injuries to the skin and/or underlying tissue caused by sustained pressure[1], with a reported prevalence of 9–14.5% in the elder care setting[2–4]. Although their prevalence is declining due to advances in prevention and treatment[5], up to 30% of PIs develop infections, which is a life-threatening complication[6–8]. Therefore, controlling wound infections is a crucial intervention after the development of PIs.

Most patients with PIs are compromised hosts due to old age and malnutrition. Thus, treatment directed at improving the overall condition of the patient (e.g., nutritional support) is provided to treat those with PIs. Additionally, breaking the route of transmission of bacteria by using dressings is recommended[1] because the region around the sacrum is susceptible to faecal bacterial contamination. Other bacteria from the peri-wound skin may also pose a potential source of infection[9], a bacterial bioburden which is reduced by cleansing these areas. However, these multifaced interventions have not made wound infection completely preventable.

We focused on hospital beds as the microclimate around patients is conducive to bacterial growth[10]. Furthermore, many patients with PIs are immobile and bed-bound, and PIs are exposed to external bacteria because they are treated as open wounds. Therefore, the bacteria found on sheets and blankets are more likely to colonise the wound. In addition, a previous study of the bed environment of patients with PIs found that the temporal change in the composition of bed microbiota was greater in patients with a PI than in those without[11]. This suggests that the presence of a PI is likely to affect the patient's microbiome and the dissemination of microbiota may occur between PIs and patients' beds. However, no studies have yet quantitatively demonstrated bacterial dissemination of microbiota with the beds of patients with PIs, and no interventions for beds have been implemented.

Traditionally, culture methods have been used to detect bacteria. However, 99% of microbes in the environment, including beds, cannot be cultivated[12]. Therefore, comprehensive investigation of bacteria has been difficult. Recent innovations in sequencing technology have gradually improved culture-independent bacterial identification methods based on bacterial DNA sequences. This study analysed the microbiota of wounds and patients' beds by detailed analysis of the 16S ribosomal RNA (rRNA) bacterial gene. This is the first step towards understanding the dissemination of microbiota from PIs and the development of a new intervention for the prevention of wound infection.

Materials and methods

Ethics

The study protocol was approved by the ethics committee of the Graduate School of Medicine, The University of Tokyo (Approval No. 11812) and performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants enrolled in this study.

Study design and setting

This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement. A cross-sectional study was conducted at a long-term care hospital in Ishikawa Prefecture, Japan, between October and November 2018. At this hospital, patients are bathed and sheets are changed once a week, clothes are changed twice a week, and incontinence pads are changed three times a day as part of routine care. Sheets, clothes and diapers may also be changed as needed. Standard wound care is provided according to national guidelines[13].

Participants

All the participants were recruited during routine rounds by the PI team. The participants were hospitalised patients aged 65 years or older. The inclusion criterion was the presence of d2, D3, D4, D5 or unstageable PIs determined using the DESIGN-R® tool[14]. With this tool, PIs are divided into six categories as follows: d1 for persistent redness; d2 for lesions that extend into the dermis; D3 for lesions extending into the subcutaneous tissue; D4 for lesions extending to the muscle, tendon or bone; D5 for lesions extending into the articular or body cavity; and DU for unstageable PIs due to necrotic tissue that completely covers the wound bed. Patients with deteriorating systemic condition were excluded. Wounds too small to collect swab samples or located on the toes were also excluded. In addition, we excluded wounds with biofilm because previous studies indicated that the presence of biofilm affected the microbiota composition[15].

Data collection

The presence of biofilm was detected by the wound blotting method[16]. In this method, polysaccharides in the exudate are collected by attaching a nitrocellulose membrane to the wound surface, and biofilms are visualised by staining with alcian blue. The membrane was obtained and stained as previously described[17]. The presence of biofilm was evaluated by a researcher who was blinded to wound outcome. Patients' demographic characteristics (age, gender, body mass index, disease, Braden Scale score[18], degree of independence and mattress type), location of PIs, treatment, dressing type and duration of PIs were collected from medical records. The DESIGN-R® was scored by a nurse expert in wound care management to assess the severity of PIs.

Sample collection

The samples were collected from three sites – the wound, bed and skin. All samples were obtained using a flocked swab

(Puritan, Guilford, ME) soaked in saline with 0.1% Tween-20. Wound samples were collected by swabbing a 1x1cm square at the centre of the wound bed using the Levine technique before wound cleansing[19]. The patients' bed samples, defined as the environment formed by the body of the patient, sheet and blanket in this study, were obtained from sheets around the buttock. Skin samples were taken from the patients' back skin to examine commensal skin bacterial communities of patients. Samples of the bed and skin were collected from a 4.4×4.4cm square area on the surface by swabbing twice with the Z stroke technique[20]. Sampling was conducted 3–6 days after changing sheets in order to collect comparable levels of bacterial contamination among patients. The swab samples were stored at –80˚C until DNA extraction.

Next-generation DNA sequencing

Genomic DNA was isolated from swab samples using a QIAmp DNA Mini Kit (Qiagen N.V., Venlo, Netherlands) as previously described[21]. To determine the copy number of the 16S rRNA gene, real-time polymerase chain reaction (PCR) was conducted using universal primer pairs and a universal probe. All reactions were performed using the AriaMx Real-Time PCR System (Agilent Technologies, Santa Clara, CA).

The 16S rRNA gene from each DNA sample was amplified using first PCR primers (Forward: 5'-ACACTCTTTCCCTA CACGACGCTCTTCCGATCT-CCTACGGGNGGCWGCAG-3'; Reverse: 5'-GTGACTGGAGTTCAGACGTGTGCTCTTCCGAT CT-ACTACHVGGGTATCTAAKCC-3') with Ex Taq® Hot Start Version (TaKaRa Bio Inc., Shiga, Japan) using a thermal cycler (GeneAtlas G02; Astec Co., Ltd., Fukuoka, Japan). Cycling conditions were: 94˚C for 2 minutes; followed by 30 cycles at 94˚C for 30 seconds, 50˚C for 30 seconds, 72˚C for 60 seconds and 72˚C for 5 minutes. The PCR amplicons were purified using Agencourt AMPure XP (Beckman Coulter, Inc., Brea, CA) according to the manufacturer's instructions. The second round of PCR was performed with the purified PCR products as template. Cycling conditions were: 94˚C for 2 minutes, followed by 10 cycles at 94˚C for 30 seconds, 55˚C for 30 seconds, 72˚C for 30 seconds and 72˚C for 5 minutes. After the purification of the amplicons and quantification of the DNA concentration with a Qubit® dsDNA HS Assay Kit (Thermo Fisher Scientific Inc., Waltham, MA) using AriaMx Real-Time PCR System, an equimolar mixture of all the amplicons was outsourced (FASMAC Co., Ltd., Kanagawa, Japan) for Illumina Miseq 16S amplicon sequencing.

Microbiome analysis

First, raw pair-end sequences were filtered using Sickle (version 1.3)[22] on the basis of the Q score and combined using PANDAseq (version 2.11)[23]. Second, the chimeric sequences were eliminated by USEARCH (version 8.0.1623_i86linux64)[24], based on the chimera-checked operational taxonomic units (OTUs) GreenGene database (version 13.8)[25]. Finally, the non-chimera sequences were filtered by size (>300 bp accepted) followed by analysis with Qiime (version 1.9.1)[26].

The 16S rRNA gene amplicon analysis was performed as follows: sequences were first assigned to OTUs at 97% and 100% similarity using the 'pick_de_novo_otu.py' command. Then, the OTUs at 100% similarity were analysed as zero-radius OTUs (zOTUs)[27]. When the same zOTU was detected in different microbiota samples, we assumed bacterial dissemination between these samples because zOTUs indicate bacteria with identical sequences within the V3–V4 regions. The number of zOTUs common to an individual's wound, skin and bed samples was calculated. The OTUs clustered using a threshold of 97% sequence identity were used to confirm the composition of the microbiota. Samples with unassigned microbes for over 15% of the microbiome were excluded from further analyses.

The zOTUs were used for the evaluation of alpha and beta diversity. For the alpha diversity, the samples were rarefied with a depth of 14,000 (minimum read number among all samples) followed by calculation of the Shannon diversity index. The rarefaction curves from the Shannon index were used to examine whether the microbiota of the samples was sufficiently characterised. To assess beta diversity, the Bray–Curtis distance for all combinations of different sampling sites was generated. To confirm the validity of zOTU similarity as a test for bacterial dissemination, the unweighted pair group method with arithmetic mean (UPGMA) and principal coordinated analysis (PCoA) plots were performed using the Bray–Curtis distance. The UPGMA is commonly used to construct a phylogenetic tree from a distance matrix and was plotted using R with the 'vegan package' in order to classify the bacterial samples. In addition, PCoA plots were used to confirm whether the wound, skin and bed microbiota were classified into the same cluster.

Results

Participants and wound characteristics

Ten PIs from eight patients were analysed in this study (Figure 1). The characteristics of the study participants are summarised in Table 1. The median age was 85 and the median Braden Scale score was 13 points. Six participants (75.5%) were classified as rank C based on their degree of independence (bed-bound). PI characteristics are shown in Table 2. The median DESIGN-R® total score is 4 points (interquartile range [IQR]: 4–4 points). All PIs were assessed as d2 (superficial), n0 (no necrotic tissue) and i0 (no signs of inflammation). The median of the duration from sheet change to sampling was 4 days (IQR: 3–4 days).

Bacterial diversity in bed, wound and skin samples

The relative abundance was calculated from the OTUs cluster using a threshold of 97% sequence identity. The dominant genera in bed samples were *Corynebacterium* (27.0%), followed by *Staphylococcus* (26.7%). *Staphylococcus* (44.6%) and *Corynebacterium* (35.2%) were dominant in wound samples. *Corynebacterium* (28.7%) and *Staphylococcus* (24.5%) were also dominant in skin samples.

A total of 625,472 zOTUs were identified from 26 samples. The median number of zOTUs collected from bed, wound and skin samples was 25,987 (IQR: 22,383–29,932), 22,364 (IQR: 18,898–24,752) and 28,148 (IQR: 27,183–37,414), respectively. The median alpha diversity for the bed, wound and skin microbiome was 11.23 (IQR: 11.01–11.42), 10.26 (IQR: 9.71–10.52) and 11.38 (IQR: 11.16–11.57), respectively. Rarefaction curves of the Shannon index reached a plateau, which indicated that sequencing sufficiently characterised the microbiota (data not shown).

zOTUs common to microbiotas within an individual

All PIs had zOTUs common to the bed and wound (median: 352, IQR: 281–648), the bed and skin (median: 577, IQR: 241–1,398) and wound and skin (median: 1,243, IQR: 1,145–1,406). All wounds had zOTUs common to the bed, wound and skin samples (median: 194, IQR: 121–320) (Figure 2).

Confirmation of the zOTU similarity as a test for bacterial dissemination

Wound and bed samples were classified into the same clusters in eight of the ten study wounds. Furthermore, in three of those eight wounds, the skin samples were also classified into the same clusters. *Klebsiella* was the dominant genus in Cluster 1; *Staphylococcus* or *Corynebacterium* were the dominant genera in Cluster 2; and Cluster 3 was made up of commensal skin bacteria including *Staphylococcus*, *Corynebacterium* and *Propionibacterium* with high diversity (Figure 3). We also assessed the validity of zOTUs for evaluating the dissemination using a PCoA plot. Figure 4 shows that the samples within the same cluster of UPGMA were plotted closely.

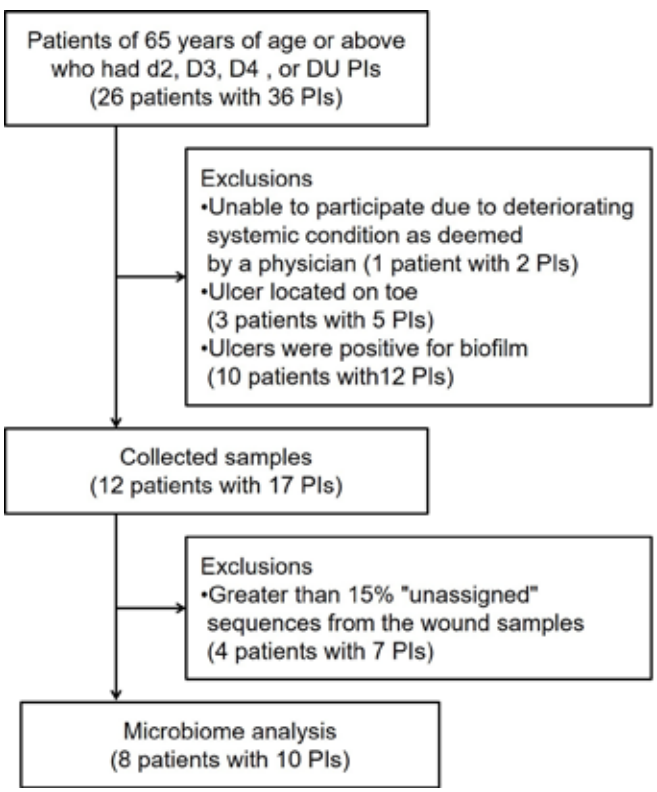

Figure 1. Exclusion criteria for participating in the study

Discussion

We explored the microbiota of PIs, back skin and patients' beds by using 16S rRNA gene analysis. All wounds had several zOTUs common to several microbiota. These microbiota were classified into the same cluster by UPGMA and samples within the same cluster were plotted close to one another for the PCoA. This is, to the best of our knowledge, the first study to quantitatively describe the dissemination of microbiota between wounds and patients' hospital beds. The result of this study highlights the need for an approach that evaluates the patients' beds to prevent wound infection.

When the rarefaction curves of the Shannon diversity index reached a plateau it indicates that the sequencing depth of all samples was reasonable, and the sequencing results sufficiently characterised the microbiota, thus confirming the internal validity of sampling and sequencing in this study. In addition, we evaluated the bacterial dissemination

Table 1. Characteristics of participants (n=8)

Characteristics	n or median	% or IQR
Age (years)	85	(80–85)
Gender (male)	7	(87.5)
BMI (kg/m²)	15	(14.6–17.4)
Disease		
Cerebrovascular disease	7	(87.5)
Respiratory disease	7	(87.5)
Dementia	4	(50.0)
Braden scale score	13	(9.5–15)
Degree of independence		
Rank B	2	(25.0)
Rank C	6	(75.0)
Mattress type		
Air mattress	7	(87.5)
Foam	1	(12.5)
Red blood cells (million cells/μl)	3.51	(3.01–3.93)
White blood cells (thousand cells/μl)	6.8	(5.5–14.9)
Total protein (g/dl)	6.1	(5.4–6.9)
Albumin (g/dl)	2.9	(2.8–3.0)

using zOTUs defined by a 100% 16S gene sequence identity threshold of the V3–V4 region. The traditional OTU clustering method with a threshold of 97% sequence identity is inadequate for assessing bacterial dissemination. Sequences differing by even one nucleotide (i.e. 99.2% similarity) significantly decreases the degree to which dynamic similarity is observed between two individuals in correlated sequences in comparison to when 100% sequence identity is used[28]. Several approaches have been proposed to improve the resolution of the 16S data analysis beyond 97% similarity[27,29,30]. zOTUs were used in this study also to address problems inherent to the standard clustering method. Further, molecular typing has previously been used to evaluate dissemination[31]; however, this method cannot identify the dissemination of microbiota because it only detects target bacteria. Moreover, the validity of zOTUs for evaluating bacterial dissemination was confirmed in this study by the clustering of microbiota based on the UPGMA tree and PCoA. Thus, the method using zOTUs is suitable for assessing the dissemination of microbiota.

The most abundant genus in patients' beds was *Corynebacterium* (27.0%) followed by *Staphylococcus* (26.7%), which are commonly found on the skin[32] and PI[33]

Table 2. PI characteristics (n=10)

Characteristics	n or median	% or IQR
Location		
Sacrum	3	(30.0)
Coccyx	2	(20.0)
Greater trochanter	1	(10.0)
Heel	1	(10.0)
Others	3	(30.0)
Duration (weeks)	10	(2–42)
Dressing type		
Silicone gel	3	(30.0)
Soft silicone faced polyurethane foam	3	(30.0)
Hydrocolloid	1	(10.0)
Gauze only	2	(20.0)
Other	1	(10.0)
Ointment type		
Sucrose, povidone-iodine	1	(10.0)
Non-used	9	(90.0)
Frequency of changing dressing (per week)	2	(2–7)

microbiota. Previous studies of hospital beds focused on pathogens that cause nosocomial infection. Malnick et al. report that *Enterococcus faecalis,* coagulase-negative *Staphylococcus* and *Bacillus* were detected on sheets after they were used overnight[10]. Moreover, it has been suggested that contaminated hospital linens contribute to *Bacillus cereus* bacteraemia outbreaks[34]. Our findings show that not only pathogens but also resident skin, gut and oral bacteria are present in the microbiota of patients' beds and suggested the need for a comprehensive search of microbiota to understand the dissemination between PIs and patients' beds.

All PIs had zOTUs common to the bed, wound and skin microbiota, even though the wound location varied. Due to the immobility of many PI patients, patients receive repositioning care by healthcare workers for pressure redistribution[1]. Therefore, the area of the sheets that is in contact with the wound changes continuously, presenting an opportunity for bacterial dissemination to occur. Additionally, bacteria can be transmitted via healthcare workers[35,36], and airborne bacteria significantly increases during dressing changes for burn patients[37]. Daily wound care by healthcare workers may therefore contribute to bacterial dissemination to patients' beds. In addition, bacterial attachment between the wound and the bed during wound care might be re-disseminated to the wound as the patient continues to lie on the bed. This indicates that, no matter how well the wound is cleansed, if there are infectious bacteria present in the bed sheet, the bacteria may re-disseminate to the wound and cause infection. Thus, to establish a new intervention to prevent wound infection whilst the patient remains in bed, a longitudinal study in a larger cohort is needed to clarify the direction of this bacterial dissemination. Furthermore, animal studies should be conducted to confirm whether interventions in the external environment can alter the wound microbiota.

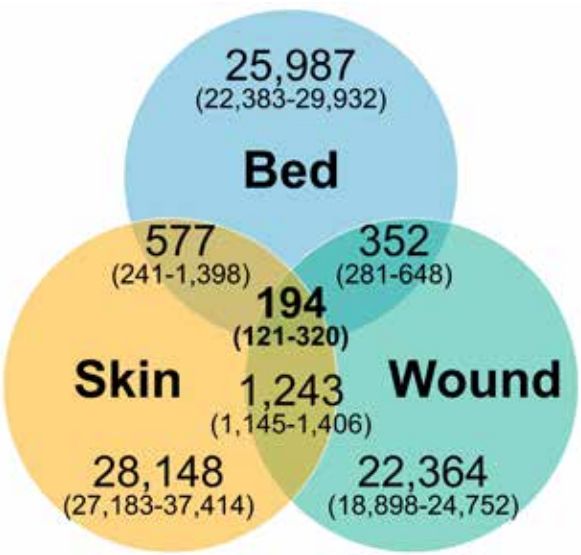

Figure 2. Venn diagram of common zOTUs showing median number of zOTUs (IQR)

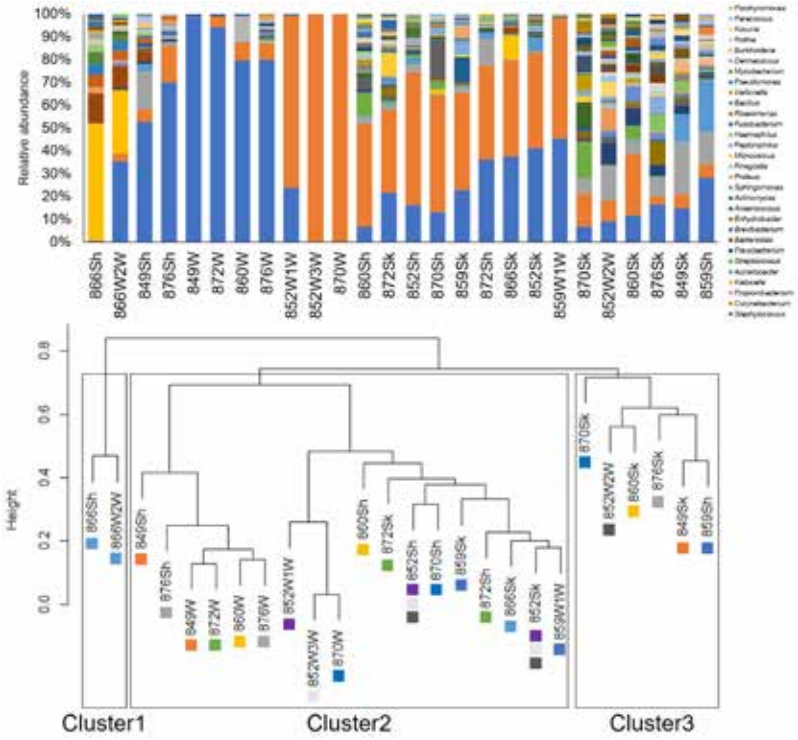

Figure 3. Relative abundance of bacteria classified at the genus level and UPGMA tree

OTUs defined by a 97% identity threshold of the 16S gene sequences, distinguished at the genus level, and used for calculating the relative abundance.
Colour-coded square indicates ID of wounds
W: Wound, Sh: Bed, Sk: Skin

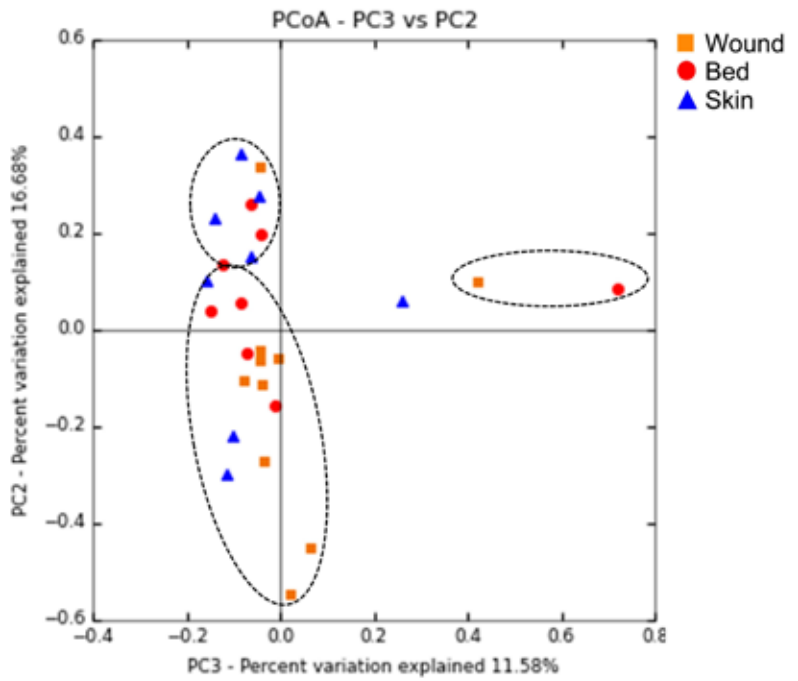

Figure 4. PCoA of microbiome community similarity as measured by Bray–Curtis distance

Ovals indicate the cluster of UPGMA. Red, orange and blue indicate the data collected from the bed, wound and skin, respectively.

Limitations

The present study has several limitations. First, we could not investigate bacterial dissemination of deep wounds due to the presence of biofilm. Therefore, the degree of dissemination for deep wounds is not clear. Second, we could not verify the relationship between bacterial dissemination and wound outcome because no wounds in the study had symptoms of inflammation. A longitudinal study is thus needed to clarify the causal relationship between bacterial dissemination in hospital beds and wound infection. Third, most participants in this study were immobile and spent 24 hours a day in bed. Therefore, further studies are required to generalise our findings for individuals who are not bed-bound.

In conclusion, the present cross-sectional study was performed to examine the dissemination of microbiota between PIs and hospital beds using a culture-independent methodology. Our results show the bacterial dissemination in all wounds, and further studies are required to clarify the impact of bacterial dissemination in order to develop intervention strategies against the dissemination of microbiota and subsequent infections.

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Conflict of interest

The authors declare no conflicts of interest.

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