ORIGINAL

Bacterial Contamination of Hemodialysis Devices in Hospital Dialysis Wards

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Abstract : Chronic care patients undergoing hemodialysis for treatment of end-stage renal failure experience higher rates of bloodstream-associated infection due to the patients' compromised immune system and management of the bloodstream through catheters. *Staphylococcus* species are a common cause of hemodialysis catheter-related bloodstream infections. We investigated environmental bacterial contamination of dialysis wards and contamination of hemodialysis devices to determine the source of bacteria for these infections. All bacterial samples were collected by the swab method and the agarose stamp method. And which bacterium were identified by BBL CRYSTAL Kit or 16s rRNA sequences. In our data, bacterial cell number of hemodialysis device was lower than environment of patient surrounds. But *Staphylococcus* spp. were found predominantly on the hemodialysis device (46.8%), especially on areas frequently touched by healthcare-workers (such as Touch screen). Among *Staphylococcus* spp., *Staphylococcus epidermidis* was most frequently observed (42.1% of *Staphylococcus* spp.), and more surprising, 48.2% of the *Staphylococcus* spp. indicated high resistance for methicillin. Our finding suggests that hemodialysis device highly contaminated with bloodstream infection associated bacteria. This study can be used as a source to assess the risk of contamination-related infection and to develop the cleaning system for the better prevention for bloodstream infections in patients with hemodialysis. J. Med. Invest. 66 : 148-152, February, 2019

Keywords **:** *Bacterial infection, Bacterial contamination, Hemodialysis device, Methicillin Resistant Staphylococcus*

INTRODUCTION

Chronic care patients undergoing hemodialysis for treatment of end-stage renal failure experience higher rates of healthcare-associated infection (1-4). In modern hemodialysis, infection is a serious problem that gives rise to higher mortality rates. The relative number of hemodialysis patients and the number of patient deaths from infection are varying increasing in Japan (5). In spite of the improvements to dialysis systems, infection-related causes remain second to cardiovascular events as a cause for mortality among hemodialysis patients (5). The increased risk for contracting healthcare-associated infections among hemodialysis patients is due to their immunocompromised status combined with the prolonged blood exposure during dialysis treatments through the vascular access and extracorporeal circuit (1, 5). Indeed, hemodialysis patients showed a disproportionately large percentage of bloodstream infections compared to peritoneal dialysis patients (6).

During hemodialysis treatment, patients are at risk for both bloodstream infections and localized infections of the vascular access (7). Sources of main bloodstream infections could be water or chemical reagents contaminated with disease-causing microorganisms, such as hepatitis virus (8). On the other hand, sources of localized infection of vascular access usually come from the environment, including contaminated equipment and surfaces in the treatment area or infectious patients that are near patients being treated with hemodialysis devices (1, 9). It is suggested that those sources of localized infection, associated with environmental contamination, closely related with bacterial or bacterial associated endotoxin contamination (10).

Infections in hemodialysis patients are frequently caused by skin bacterial flora such as *Staphylococcus* species (spp.), including

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both highly pathogenic *Staphylococcus* spp. and low pathogenic coagulase-negative *Staphylococcus* (1-4). *Staphylococcus aureus*, in particular, causes multiple infections in humans and induces severe hemodialysis catheter-related infection (11, 12).

The current recommendations to prevent infection of hemodialysis users are to clean and disinfect the external surface of the hemodialysis device after each dialysis session and to perform strategies for ensuring steady cleaning and disinfecting of the internal system (13, 14). In this study, the bacterial contamination level of the dialysis devices, including the dialysis ward or patients' surroundings, were assessed. And identified specific bacterial species isolated from a dialysis device to estimate the risk of bacterial infection. This knowledge can make sure the risk of bacterial infections and contribute to the development of preventive strategies to reduce infection in hemodialysis treatment.

MATERIALS AND METHODS

Protocol for the research

This study is not including any clinical data or patients data. Thus, approved by constituted Ethics Committee of institution was not required. Isolated bacteria were treated in P2 room under the Cartagena Protocol on Biosafety.

Sample collection and processing

All bacterial samples were isolated from hospital dialysis room in Tokushima city. Usually, for the prevention of bacterial diffusion, dialysis wards were properly cleaned with duster after the daily work. The samples were collected after daily work of hemodialysis room, before cleaning. Environmental samples for the isolation of bacteria were collected by sterile swab from the 3-4 dependent dialysis wards in the dialysis room (15). The bacteria swab was suspended in 10 ml phosphate buffered saline (PBS) (pH 7.4). For the cultivation of bacteria, 125 µl of the PBS sample was spread onto a standard method agar plate (peptone 0.5%, yeast extract 0.25%, glucose 0.1% agar powder 1.5%). Isolation of bacteria from hemodialysis devices or patient surroundings was performed based on the swab method and the agarose stamp method, where the agar plate is placed in direct contact with the instruments. Bacteria in bedding, such as a blanket or pillow, were collected onto homemade standard method agar plates by agarose stamp method. For the hemodialysis devices, bacterial contamination was estimated by agarose stamp method with DD Checker (Kyokuto Pharmaceutical Industrial Co., Ltd) standard agar plate. Plates were incubated at 37°C for 2 days, and then picked and further purified on new plates. Each bacterial cell numbers were indicated by colony forming unit (CFU), which number was normalized by area (100 cm^2).

Isolation and species identification

In order to identify the bacterial species, the bacterial strains were grown on blood agar plates at 37°C for 12-24 hours. Gram-stain was performed on isolated colonies. Gram-positive and Gram-negative bacteria were further identified using the BBL CRYSTAL Identification Systems Rapid Gram-positive ID kit or Gram-negative ID kit (BD), respectively (16). The identification code obtained was crosschecked with the BBL CRYSTAL computer codebook Ver.5.4 according to the manufacturer's instructions.

DNA isolation and sequencing

Some colonies, which bacteria could not identify the species by BBL CRYSTAL kit, applied DNA sequencing method. For isolation of the DNA, the bacterial strains were grown on blood agar plates at 37℃ until sufficient cell biomass was obtained. The bacterial cells were suspended in distilled water and boiled at 95℃ for 10 minutes. The boiled bacterial suspensions were centrifuged at 15,000 rpm for 10 minutes. Thereafter, the supernatants were transferred to a new tube and used for PCR amplification. A fragment of the bacterial 16S rRNA gene was amplified with primers 10F (5'-GTTTGATCCTGGCTCA-3') and 800R (5'-TACC AGGGTATCTAATCC-3'). The PCR products obtained were purified using the QIAquick Gel Extraction Kit (QIAGEN). The 16s rRNA genes of the strains were partially sequenced by using BigDye terminator (Applied Biosystem), and then they were analyzed by an ABI 3130 DNA Sequencer (Applied Biosystem).

Sequence analysis

The partial 16S rRNA sequences were analyzed by the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI) and DNA Data Bank of Japan (DDBJ) according to the gene database (17). The bacterial cells were identified according to the sequence identity in those databases (more than 99%).

Screening of Methicillin resistance Staphylococcus spp.

The colonies identified as *Staphylococcus* spp. underwent susceptibility testing by using the Pourmedia MRSA II agar plate (EIKEN CHEMICAL). Isolated *Staphylococcus* spp. were separated on the Pourmedia MRSA II agar plate, cultured for 48 hours, checked cefoxitin (5 mg / mL) resistance, and identified as MRS.

RESULTS

Bacterial load in the hemodialysis ward

First, we checked the load of bacterial contamination in the hemodialysis ward. Samples were collected after a daily work of common day in the hemodialysis ward. Bacterial cell load was indicated by the number of colony forming units (CFU). The amount of bacterial contamination in the hemodialysis ward and patient surroundings are summarized in Table 1. Overall, bacteria were more abundant in the patient surroundings (> 10^2 CFU /

Table 1 Bacterial cell number in hemodialysis ward

Sampling point		Colony number (CFU / 100 cm^2)	sample number
Environment of hemodialysis ward			
Hemodialysis device	Touch screen	ND	(n = 4)
	Side of body	ND	(n = 4)
	Upper surface	ND	(n = 4)
	Tubes	11.6	(n = 4)
Computer	Keyboard	0.2	(n = 2)
	Mouse	2.2	(n = 2)
Floor		226.7	(n = 10)
Door knob		0.0	(n = 2)
Hand-wash station	Outlet	50.0	(n = 1)
	Sink	0.3	(n = 1)
Environment of patient surrounds			
Bed	Head side	1.7	(n = 4)
	Foot side	779.9	(n = 4)
	Coverlet	12.5	(n = 4)
	Blanket	40.0	(n = 1)
	Pillow	30.0	(n = 1)
Bed side	Table	43.8	(n = 4)
	Curtain	26.7	(n = 2)

ND, not detected (Less than 100 cm^2).

100 cm²) than in the hemodialysis ward (< 10^2 CFU / 100 cm²). Furthermore, we could not isolate bacteria from all but one part of the hemodialysis device using the swab method, because of the small number of bacterial contamination.

Bacterial contamination of the hemodialysis device

Next, we attempted to isolate bacterium with the more sensitive agarose stamp method by direct connection with the hemodialysis device. Furthermore, to get a better understanding of the risk for bacterial infection, isolated bacterial colonies were subjected to species identification analysis. A total of 365 bacterial colonies were collected from the hemodialysis devices with the agarose stamp method (Table 2). Of the 365 samples collected, 171 colonies were identified as *Staphylococcus* species (spp.), 95 colonies were *Bacillus* spp., 42 colonies were *Micrococcus* spp., and 14 colonies were *Corynebacterium* spp.. Those four bacterial species accounted for about 90% of bacteria on the hemodialysis device, and all remaining bacterial species were categorized into "others" (containing *Kytococcus* spp., *Gardnerella* spp., *Moraxella* spp., *Roseateles* spp., *Cellulomonas* spp., *Kocuria* spp., *Streptococcus* spp., *Roseomonas* spp., *Escherichia* spp., and *Acinetobacter* spp.). According to the data, *Staphylococcus* was the predominant contaminant of the hemodialysis device.

All species of *Staphylococcus* isolated from the hemodialysis device were checked for methicillin resistance and categorized the coagulase production each *Staphylococcus* spp.. Species of *Staphylococcus* were identified and analyzed characteristic species of *Staphylococcus* spp. (Table 3). Most isolated *Staphylococcus* spp. were coagulase-negative (about 98%), while an alarming number were methicillin-resistant (49%). *Staphylococcus epidermidis* was the most abundant species, which is not surprising since it is normally present on human skin. Only 2% of the bacteria identified were *Staphylococcus aureus*, one of the most important bacteria responsible for hemodialysis infection. Considering the results of the first and second studies, while there are a low number of total bacteria on hemodialysis devices, the species that are present are predominantly *Staphylococcus* with high resistance for methicillin.

Finally, we separated the hemodialysis device into 8 parts (touch screen, pump pit, tube connection, tubes, fixing pole, upper surface, side surface, and back surface) and estimated the distribution of bacterial content and the bacterial composition of each part by agarose stamp method. The bacterial contents were summarized in Table 4, and compered with bacterial distribution in each part (Figure 1). Interestingly, bacterial distribution did not indicate ensure consistency but wide variety each parts. *Staphylococcus* spp., in particular, were the main bacteria found on the touch

Table2 Bacterial species isolated from 3 hemodialysis devices

Species	Colony number	(%)
Staphylococcus spp.	171	46.8
Bacillus spp.	95	26.0
Micrococcus spp.	42	11.5
Corynebacterium spp.	14	3.8
Others	43	11.8
Total	365	100

Table3 Character of Staphylococcus, which isolated from hemodialysis devices

Species	Colony number	(%)	Mechicillin resistance in each species(%)
Staphylococcus epidermidis	72	42.1	53.3
Staphylococcus capitis	36	21.1	45.5
Staphylococcus haemolyticus	21	12.3	90.0
Staphylococcus saprophytics	12	7.0	50.0
Staphylococcus hominis	8	4.7	37.5
Staphylococcus schleiferi	8	4.7	0
Staphylococcus xylosus	6	3.5	100
Staphylococcus aureus	3	1.8	0
Staphylococcus warneri	3	1.8	0
Staphylococcus saccharolyticus	2	1.2	0
Total	171	100	49.2

Table4 Bacterial cell number in hemodialysis devices

Sampling point	Colony number (CFU / 100 cm²)	sample number
body of device		
Upper surface	6.9	(n = 3)
Side surface	2.2	(n = 3)
Back surface	4.4	(n = 3)
Part of device		
Touch screen	14.4	(n = 3)
Pump pit	5.2	(n = 3)
Tube connection	5.3	(n = 3)
Tubes	16.5	(n = 3)
Fixing pole	4.2	(n = 3)

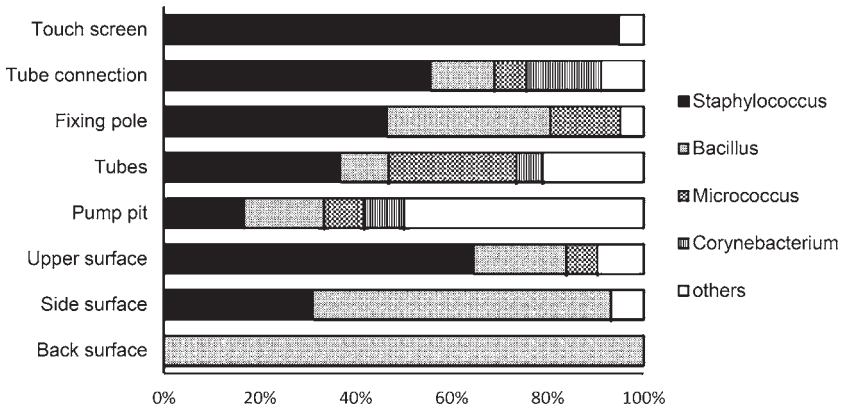

Fig. 1. Bacterial distribution and composition in hemodialysis divices.
Hemodialysis device was separated into touch screen, pump pit, tube connection, tubes, fixing pole, upper surface, side surface, and back surface. And the distribution of bacterial content and the bacterial composition were estimated by agarose stamp method.

screen, tube connection, fixing pole, and upper surface, which are areas frequently touched by healthcare workers. Taken together, these results indicate that the hemodialysis device is partially contaminated with *Staphylococcus* spp. despite a careful cleaning.

DISCUSSION

In our study, in order to elucidate the bacterial mass in the hemodialysis ward, we performed isolation of bacteria. In the first experiment, swab method revealed that bacterial cell numbers in hemodialysis device was lower than environment of patient surrounds or hemodialysis ward. And it bacterial contamination level was lower than detection limit of swab method such as Touch screen, Side of body, and Upper surface of hemodialysis wards (10^3 CFU / 100 cm^2). We found much colony only the Tubes of hemodialysis device. Those data suggest that the body of hemodialysis devices were well cleaned by daily cleaning. Next, we applied stamp method, sensitivity is more higher than swab method, despite the small number of bacteria found on the hemodialysis device (1 CFU/4 cm^2) (Table 1, 4), we also detected *Staphylococcus* species in the highest abundance on the hemodialysis device (Fig. 1). Moreover, we tried to reveal the bacterial distribution into hemodialysis device, and we found localization of the *Staphylococcus* species in hemodialysis, especially on areas of the device that where in frequent contact with healthcare workers such as the touch screen. The commensal skin bacterium *Staphylococcus epidermidis* was found most frequently (Table 3). Among the isolated bacteria, *Staphylococcus* spp. has been previously implicated in hemodialysis-related infection. In particular, the coagulase-positive *Staphylococcus* spp. are a frequent culprit of these infections (1, 2). Another concern is the possible presence of methicillin-resistant *Staphylococcus* spp., which causes serious problems in modern treatment of infection (18, 19). Especially, methicillin-resistant *Staphylococcus aureus* (MRSA) receives special attention in hospital infection (20). This is especially concerning for hemodialysis patients since they have a higher risk for infection with antimicrobial-resistant bacteria because of frequent use of antimicrobials (21, 22). Our data may serve good evidence that bacterial contamination in hemodialysis is partially caused by skin contaminants from the healthcare workers. And it is suggested that, about risk for infection, we need to pay more attention to bacterial localization rather than bacterial mass.

Staphylococcus aureus and *Pseudomonas aeruginosa* are the most important reference bacteria for careful management of the nosocomial infections. Previous studies reported that gram-positive bacteria are still the predominant pathogens isolated from hemodialysis bloodstream infection, such as coagulase-negative *Staphylococcus, S. aureus,* and *Enterococcus* species (1-4). Gram-negative bacteria have also been shown to cause hemodialysis catheter-related bloodstream infection, including *Pseudomonas aeruginosa,* which accounts for 21-43% of the catheter-related infections (1-4). *Candida* species are an infrequent cause of these of infections, accounting for only 1% (1-4, 23). Additionally, drug resistance bacteria, including MRSA, *Pseudomonas aeruginosa, Acinetobacter baumannii,* or ESBL-producing Gram-negative bacteria, causes severe problem in hospital.

It is suggested that the bacteria in hemodialysis device is one of the key factor of blood stream infection. Therefore we checked the bacterial mass in hemodialysis device to monitoring the risk factor of blood stream infection. In our study, we could not detect those catheter-related infections associated bacteria. Also, we could not detect drug resistant bacteria such as ESBL-producing Gram-negative bacteria, MRSA, or *Pseudomonas aeruginosa*. But we found abundant gram-positive, *Staphylococcus* spp. predominantly, bacterial mass in hemodialysis device. Bacterial mass may provide an indication of infection risk in the hospital. Thus, we focused on *Staphylococcus* spp. in this study.

It is therefore important to define risk factors related to hemodialysis infection and to learn how to mitigate them. Risk of infection in hemodialysis patients is mainly attributed to : (A) immunosuppression with compromising renal function, (B) frequent blood exposure during hemodialysis treatments through the vascular access, (C) close proximity to other patients during hemodialysis treatment, and (D) frequent contact with healthcare workers who regularly move between patients and hemodialysis devices. Risk of (A) and (B) are common to all hemodialysis patients, however, the risk of (C) and (D) are closely associated with the level of cleanliness in general hospital wards, and it is suggested that the difference in risk is dependent on the difference in bacterial conditions between countries, hospitals, and wards (9).

Cleaning in health care environments aims to reduce levels of organisms to the point at which they do not pose a cross-contamination risk to patients. However, a previous study reported that when 82% of ward sites were clean visually, just 30% of those sites were considered clean by organism sampling (24). The report suggested that cleaning of a hospital environment may not, therefore, provide a reliable assessment of environmental cleanliness or assess the risk of infection to patients. In prevention of bacterial infectious disease, cleaning is necessary but no longer sufficient in hospitals (25, 26). Cleaning the bacterial mass from the hemodialysis environment and device were helpful for establishing the initial bacterial disinfection system. We believe that the cleaning system should be changed from a visible clean standard to a bacteria specific cleaning system as soon as possible to prevent these life-threatening infections.

Several limitations of this study should be acknowledged. First, the bacterial samples were collected in only 3 hemodialysis devices. Those sampling number is not suffice for the explanation of bacterial mass, and we could not make a statistical comparison because of small sample number. Next, the bacterial samples were corrected in only 2 times, in Japanese winter season. The data may be subject to seasonal influence. But, we collected so large number of bacterial colony, thus, our data may be biologically plausible for the basal or regular bacterial contamination and infectious risk.

Investigation of monitoring the bacterial contamination and the locus of *Staphylococcus* spp. in a hospital environment is therefore warranted. This knowledge may then be used to assess the risk of contamination-related infection. The findings in this report may serve as a baseline to build better prevention strategies for not only bloodstream infections in patients with hemodialysis but also other tunneled catheter therapies.

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CONFLICT OF INTEREST

None to declare.

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