

Article

Evaluation of contaminated bacteria inside door- and conveyor-types of dishwashers used in food service facilities

Urara Ishizaki^{1,2}, Hideki Kohno³, Kazuaki Yoshimune^{2*}

¹Research & Development Laboratories, Lion Hygiene Corporation, Tokyo, Japan.

²Department of Applied Molecular Chemistry, Graduate School of Industrial Technology, Nihon University, Chiba, Japan.

³Hoshi University, Tokyo, Japan.

Dishwashers are one of the sources of bacterial contamination in dishes despite the fact that they comprise extreme environments due to the high temperature, high pH, and the presence of detergents during their operation. In this study, viable bacteria were isolated from 6 door-type and 8 conveyor-type dishwashers that were being used in food service facilities. Each door- and conveyor-type dishwasher was divided into 6 and 9 sections respectively. Thermophilic bacteria, which grow at 60°C, were obtained from 5 sections of door-type dishwashers, but obtained from one section of conveyor-type dishwashers tested. The 16S rRNA sequences of the cultured isolates revealed that fewer Gram-negative bacterial strains were found in door-type dishwashers than in conveyor-type dishwashers. The most frequently identified bacterial species in the samples tested were spore-forming bacteria, such as *Bacillus* and *Anoxybacillus* species. This study highlights the vulnerabilities presented by dishwashers used in food service facilities.

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Key words: dishwashers; bacterial contamination; spore-forming bacteria; Gram-positive bacteria; Gram-negative bacteria.

Introduction

Effective management of food safety in food service facilities is essential for the health of consumers. Individual health is compromised not only by food itself but also by contaminated crockery and cutlery. Dishwasher-based contamination of crockery in a hospital, highlighting the elevated risk of the spread of infection in this setting [1,2]. Contaminated dishwashers by *Legionella* spp. are reported and such dishwashers can be a potential reservoir for the spread of Legionnaires' disease [3]. It is, therefore, necessary to maintain hygiene in dishwashers and the crockery and cutlery washed in them [4]. Despite the fact that dishwashers can be considered extreme habitats for bacteria due to the high temperature,

high pH, and the presence of detergent involved during their operation, bacterial strains have been isolated from dishwashers. Biofilms have been isolated from the rubber seals of household dishwashers and the microbial communities were analyzed using next-generation sequencing of 16S rRNA [5]. The presence of a wide range of bacterial and fungal species [6-8] in the microbial communities is suggested, and the composition of the communities varied between different types of dishwashers [9]. There is, however, no information on the tendency of the bacteria contamination in different types of dishwashers. It is necessary to elucidate the relationship between the bacterial communities and the types of dishwashers for the effective prevention of bacterial contamination. There are two basic types of automatic dishwashers; door-type, with a front door and racks that are fixed during the washing process, and conveyor-type, with a moving conveyor that carries utensils during

*Correspondence author: Kazuaki Yoshimune.

Phone: +81-47-474-2598

Fax: +81-47-474-2579

E-mail: yoshimune.kazuaki@nihon-u.ac.jp

washing. Both types of dishwashers are used in food service facilities in hotels, restaurants, and hospitals. The dishwashers in food service facilities are more hostile toward bacteria than the household dishwashers, as they wash and rinse at higher temperatures and use detergents at higher pH values (pH 10-12). Generally, dishwashers in businesses wash at 60°C for 1 min and rinse at 80°C for 10 s. In contrast, household dishwashers take 20 min for washing using detergents with neutral or weak alkaline pH (pH 7-10) followed by rinsing, and dry at 60°C.

In this study, we analyzed 6 door-type and 8 conveyor-type dishwashers that were in use in food service facilities for the presence of bacteria. We cultured the bacteria at 35°C and 60°C, and analyzed the 16S rRNA sequences of the isolates. Our results provide an understanding of the location of the bacteria within the dishwashers and enable the control of bacterial contamination in dishwashers used in food service facilities.

Materials and Methods

Sampling and cultivation. Microorganisms were collected from 6 door-type and 8 conveyor-type dishwashers that were being used in 5 hotels, 8 restaurants, and 1 hospital. Each door- and conveyor-type dishwasher was divided into 6 and 9 sections respectively, and microorganisms were collected once from each of these sections of door-

(Fig. 1) and conveyor-type (Fig. 2) dishwashers. Microorganisms were collected by swabbing an area 100 cm² with a sterilized pre-moistened cotton plug; the swab was transferred to 10 mL sterilized saline according to the manufacturer’s instructions (Fukifuki Check[®], Eiken Kizai Co., Ltd., Tokyo, Japan). The suspended bacteria were plated on soybean casein digest agar containing lecithin and polysorbate 80 (SCDLP, Nihon Pharmaceutical Co., LTD., Tokyo, Japan) and incubated at 35°C or 60°C for 5 days; the colonies that formed were counted.

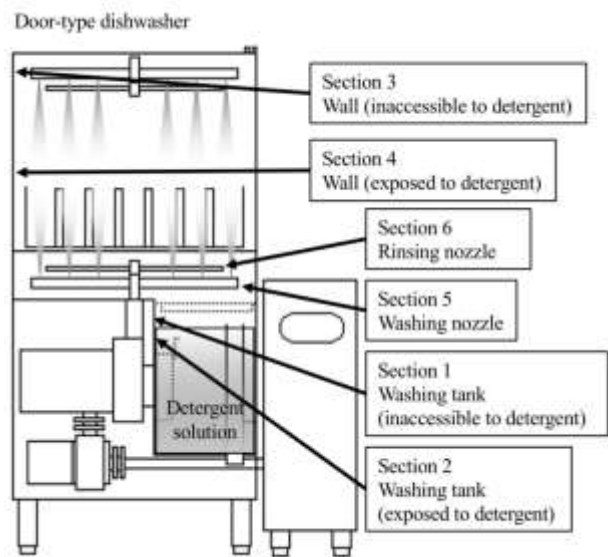


Fig. 1. Diagrammatic representation of the door-type dishwashers. Samples were collected from each section and whether they are exposed to detergent, are labelled in the figures.

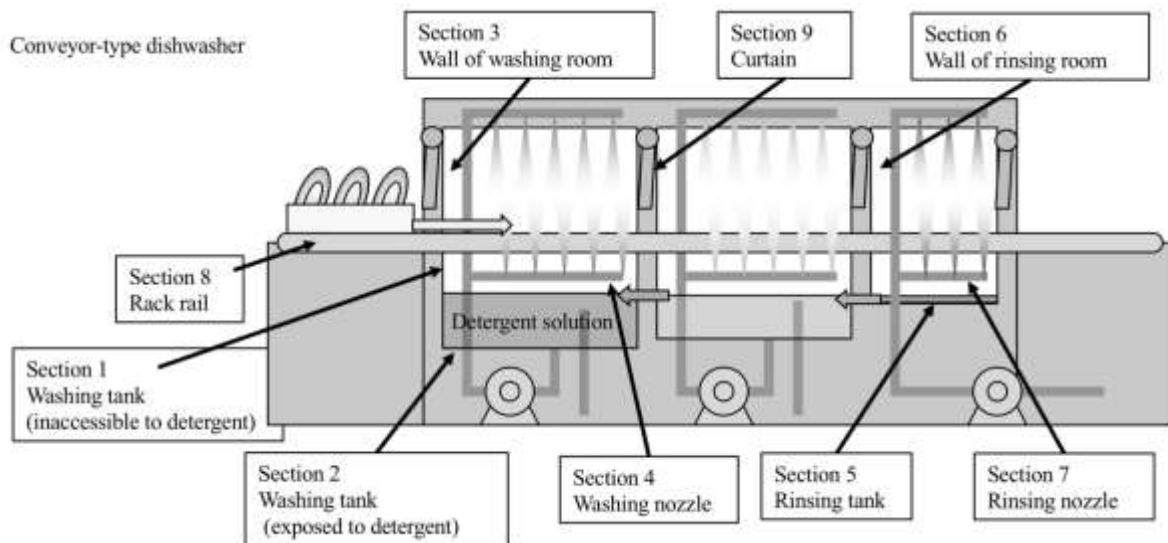


Fig. 2. Diagrammatic representation of the conveyor-type dishwashers. Samples were collected from each section and whether they are exposed to detergent, are labelled in the figures.

PCR and sequencing of the 16S rRNA gene.

A morphologically distinct colony based on colony size, shape and colour from the SCDLP agar plate was used in a cell suspension PCR, without prior DNA purification, to amplify the 16S rRNA gene for that particular bacterial isolate. PCR was performed using the Bacterial 16S rDNA PCR Kit (Takara Bio Inc., Shiga, Japan) according to the manufacturer's instructions. In addition to the single colony, the PCR reaction mixture contained a TaKaRa *Ex Taq*® DNA polymerase and oligonucleotide primers 10F and 800R (Takara Bio Inc., Shiga, Japan). The resultant PCR product was confirmed by agarose gel electrophoresis to be approximately 800 bp in size. The PCR-amplified products were purified and the gene sequences determined by MacroGen Japan Corp. (Kyoto, Japan). The partial 16S rRNA gene sequences were compared with sequences available in the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST), and genus of the isolates were defined by the related bacteria with the similarity more than 98.7 % [10].

Results and Discussion***Viable bacteria were isolated from dishwashers***

Mesophilic bacteria that grow at 35°C were obtained from both door and conveyor-type dishwashers (Table 1). Relatively large numbers of bacteria were isolated from samples taken from the washing tank exposed to detergent than that inaccessible to detergent in door-type dishwashers (Table 1, door-type, Sections 2 and 1 respectively). On the contrary, more bacteria were grown from samples taken from the wall inaccessible to detergent than that exposed to detergent (Table 1, Sections 3 and 4 respectively). These data are unexpected, as the washing tanks that were exposed to detergent should have been decontaminated by the presence of the detergent. It is possible that aerosolized bacteria could be deposited on this surface during the normal operation of the dishwasher and could accumulate in that section over time. The inaccessibility to detergent of the wall also means it is unlikely to be cleaned. Fewer mesophilic bacteria were obtained from the wall that

was exposed to detergent, and both the washing and rinsing nozzles of the door-type dishwashers. It is important to note that the detergents are reused during the washing process whereas fresh water is used to rinse the washed dishes in the door-type dishwasher. It is therefore possible that bacteria could be washed away from the wall, as well as the washing and rinsing nozzles, by the clean rinsing water. The rinsing process may result in the decreased contamination of the door-type dishwasher by bacteria. Thermophilic bacteria, which grow at 60°C, were obtained from most sections of door-type dishwashers (Table 1). Considerable thermophilic bacteria were isolated from the washing tank, which is exposed to detergent, and the wall, which is inaccessible to detergent (Table 1, Section 2 and 3 respectively). These sections in the door-type dishwasher are maintained at higher temperatures due to the rinsing step, usually approximately 80°C, than the conveyor-type dishwashers. In contrast to the door-type dishwasher, thermophilic bacteria were rarely obtained from the conveyor-type dishwasher. Only one thermophilic bacterial strain was isolated from the sample taken from conveyor-type dishwashers (Table 1, Section 4). The environment of the door-type dishwashers and particularly the higher temperatures, may provide optimal growth conditions for thermophilic bacteria.

Classification of the isolated bacteria

A total of 94 strains of viable bacteria were subjected to PCR with primers specific to the 16S rRNA sequence and the sequence analyzed to identify the bacterial genus. *Bacillus* sp. was isolated from almost all the sections of both the door- (Table 2) and conveyor type dishwashers (Table 3) and it was the most abundant isolate in all the samples tested. It is possible that the source of the *Bacillus* sp. may be foods that contain fermented soybean, which is often eaten in Japan. *B. subtilis* has a robust stress response [11] and *B. subtilis* can form thermotolerant spores [12]. Spore-forming bacteria can survive under a wide range of environmental conditions to form biofilms and to form spores which are the most resistant life forms [13]. These features could account for the survival of this strain in the thermophilic environment of the dishwashers. In addition to *Bacillus* sp., two spore-forming thermophilic bacteria (*Anoxybacillus* and

Table 1. Number of viable bacteria collected from dishwashers.

	Section 1		Section 2		Section 3		Section 4		Section 5		Section 6		Section 7		Section 8		Section 9		
	35°C	60°C	35°C	60°C	35°C	60°C	35°C	60°C	35°C	60°C	35°C	60°C	35°C	60°C	35°C	60°C	35°C	60°C	
Door-type																			
A: Bar	3.1	-	6.4	-	4.1	-	2.0	-	3.0	-	-	-	-	-	-	-	-	-	-
B: Cafeteria	-	-	5.3	5.4	4.1	4.6	<1.0	<1.0	2.0	<1.0	2.0	3.8	-	-	-	-	-	-	-
C: Delivery restaurant	3.0	<1.0	2.8	2.7	5.4	2.7	<1.0	2.0	2.0	<1.0	2.3	2.3	-	-	-	-	-	-	-
D: Delivery restaurant	<1.0	2.0	6.0	5.4	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-	-	-	-	-	-	-
E*: Hotel	<1.0	-	-	-	<1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F*: Hotel	-	-	-	-	2.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Conveyor-type																			
G: Cafeteria	4.5	-	-	-	4.5	-	-	-	5.1	-	3.8	-	-	-	3.9	-	-	-	-
H: Cafeteria	2.3	-	-	-	2.0	-	-	-	-	-	<1.0	-	-	-	<1.0	-	2.5	-	-
I: Cafeteria	-	-	4.1	<1.0	3.0	<1.0	3.9	4.0	3.6	<1.0	<1.0	<1.0	<1.0	<1.0	4.6	<1.0	-	-	-
J: Cafeteria	-	-	4.1	<1.0	3.4	<1.0	3.5	<1.0	2.8	<1.0	3.8	<1.0	3.0	<1.0	2.7	<1.0	-	-	-
K: Hospital	-	-	-	-	<1.0	-	-	-	-	-	-	-	-	-	<1.0	-	<1.0	-	-
L: Hotel	-	-	-	-	<1.0	-	-	-	-	-	<1.0	-	-	-	-	-	-	-	-
M: Hotel	-	-	5.7	<1.0	3.6	<1.0	5.5	<1.0	3.9	<1.0	<1.0	<1.0	2.0	<1.0	3.4	<1.0	-	-	-
N: Hotel	4.0	-	-	-	4.1	-	3.5	-	-	-	-	-	-	-	4.3	-	4.0	-	-

The number of viable bacteria present over an area of 100 cm² on each section of the door-type and conveyor-type dishwashers are presented as Log₁₀ (CFU/100 cm²) where CFU represents colony forming units at 35 and 60°C. (*): The same model of dishwasher. Different models of dishwashers except as otherwise noticed. (-): not tested. The sections of door-type dishwasher include 1: Washing tank (inaccessible to detergent), 2: Washing tank (exposed to detergent), 3: Wall (inaccessible to detergent), 4: Wall (exposed to detergent), 5: Washing nozzle, 6: Rinsing nozzle. The sections of conveyor-type dishwasher include 1: Washing tank (inaccessible to detergent), 2: Washing tank (exposed to detergent), 3: Wall of washing room, 4: Washing nozzle, 5: Rinsing tank, 6: Wall of rinsing room, 7: Rinsing nozzle, 8: Rack rail, 9: Curtain.

Table 2. Gram-positive and -negative bacteria isolated from door-type dishwashers.

	Section 1		Section 2		Section 3		Section 4		Section 5		Section 6	
	35°C	60°C	35°C	60°C	35°C	60°C	35°C	60°C	35°C	60°C	35°C	60°C
Gram-positive bacteria												
<i>Anoxybacillus</i> * ^a	-	D	B	B,D	-	B(2)	-	-	A	-	-	B(2)
<i>Bacillus</i> *	A	-	C	-	B,F(2)	-	-	B	-	C	-	-
<i>Geobacillus</i> * ^a	-	-	-	C	-	C	-	C	-	-	-	-
<i>Aerococcus</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Barrientosiimonas</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Exiguobacterium</i>	A	-	-	-	-	-	-	-	-	-	-	-
<i>Gordonia</i>	-	-	-	-	C(2)	-	-	-	-	-	-	-
<i>Isoptricola</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Kocuria</i>	-	-	-	-	-	-	-	A	-	-	-	-
<i>Micrococcus</i>	A	-	A	-	C	-	-	-	-	-	-	-
<i>Microbacterium</i>	-	-	-	-	-	-	-	-	-	-	B	-
<i>Pseudoxanthomonas</i> ^a	-	-	D(2)	D	-	-	-	-	-	-	-	-
<i>Rhodococcus</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Staphylococcus</i>	-	-	B	-	-	-	-	-	-	-	-	-
Gram-negative bacteria												
<i>Acinetobacter</i>	C(2)	-	A	-	-	-	-	-	C	-	-	-
<i>Brevundimonas</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Caulobacter</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Meiothermus</i> ^a	-	-	-	-	-	B(3)	-	-	-	-	-	-
<i>Moraxella</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Paracoccus</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Petrobacter</i>	-	-	D	-	-	-	-	-	-	-	-	-
<i>Pseudomonas</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Psychrobacter</i>	-	-	-	-	-	-	-	-	-	-	-	-
Unidentified	-	-	-	-	A,C(2)	-	-	-	-	-	-	-

(*): Spore-forming bacteria. (^a): Thermophilic bacteria grown at 60°C. (-): not detected. The alphabet represents the dishwasher shown in Table 1. The number in parentheses shows the number of isolated bacteria that were assigned to the same genus, grown at the same temperature, and isolated from the same section of the same dishwasher. No strains were satisfactorily identified from the dishwasher of E: Hotel. The sections of door-type dishwasher include 1: Washing tank (inaccessible to detergent), 2: Washing tank (exposed to detergent), 3: Wall (inaccessible to detergent), 4: Wall (exposed to detergent), 5: Washing nozzle, 6: Rinsing nozzle.

Table 3. Gram-positive and -negative bacteria isolated from conveyor-type dishwashers.

	Section 1		Section 2		Section 3		Section 4		Section 5		Section 6		Section 7		Section 8		Section 9	
	35°C	60°C	35°C	60°C	35°C	60°C	35°C	60°C	35°C	60°C	35°C	60°C	35°C	60°C	35°C	60°C	35°C	60°C
Gram-positive bacteria																		
<i>Anoxybacillus</i> * ^a	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-
<i>Bacillus</i> *	H	-	I	-	H,J	-	I,N	-	G,I	-	G,J	-	M	-	I,J	-	-	-
<i>Geobacillus</i> * ^a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Aerococcus</i>	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-
<i>Barrientosii</i> monas	-	-	-	-	-	-	-	-	M	-	-	-	-	-	-	-	-	-
<i>Exiguobacterium</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Gordonia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Isoptricola</i>	H	-	-	-	G,N	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Kocuria</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	-
<i>Micrococcus</i>	-	-	M(2)	-	-	-	I	-	I	-	-	-	-	-	-	-	-	-
<i>Microbacterium</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M	-	-	-
<i>Pseudoxanthomonas</i> ^a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Rhodococcus</i>	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Staphylococcus</i>	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-
Gram-negative bacteria																		
<i>Acinetobacter</i>	-	-	-	-	-	-	-	-	M	-	-	-	-	-	-	-	N	-
<i>Brevundimonas</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	-
<i>Caulobacter</i>	-	-	-	-	-	-	M	-	-	-	-	-	-	-	-	-	-	-
<i>Meiothermus</i> ^a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Moraxella</i>	-	-	-	-	-	-	-	-	M	-	-	-	-	-	-	-	H	-
<i>Paracoccus</i>	G	-	-	-	M	-	I,M	-	M	-	-	-	-	-	M(3)	-	-	-
<i>Petrobacter</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	N	-
<i>Psychrobacter</i>	-	-	-	-	-	-	N	-	I	-	-	-	-	-	-	-	-	-
Unidentified	-	-	M	-	I,M(3)	-	M	-	-	-	-	-	-	-	M	-	-	-

(*): Spore-forming bacteria. (a): Thermophilic bacteria grown at 60°C. (-): not detected. The alphabet represents the dishwasher shown in Table 1. The number in parentheses shows the number of isolated bacteria that were assigned to the same genus, grown at the same temperature, and isolated from the same section of the same dishwasher. No strains were satisfactorily identified from the dishwashers of K: Hospital and L: Hotel. The sections of conveyor-type dishwasher include 1: Washing tank (inaccessible to detergent), 2: Washing tank (exposed to detergent), 3: Wall of washing room, 4: Washing nozzle, 5: Rinsing tank, 6: Wall of rinsing room, 7: Rinsing nozzle, 8: Rack rail, 9: Curtain.

Geobacillus spp.) were isolated from four door-type dishwashers (Table 2). Both strains also form biofilm on various abiotic surfaces [14,15,16]. Further studies for effective biofilm and spore removal should be needed to improve hygiene of dishwashers.

Of the strains isolated from samples taken from door-type dishwashers, only 3 genera were classified as Gram-negative strains (Table 2). In contrast, the conveyor-type dishwashers hosted 7 distinct Gram-negative species (Table 3). It is interesting to note that no Gram-negative bacteria were isolated from rinsing nozzles of door- (Table 2 Section 6) and conveyor types (Table 3 Section 7) of dishwashers, though Gram-positive bacteria were isolated from the rinsing nozzles. These results suggest that the rinsing step, and not the washing step, is critical for the reduction in the number of Gram-negative bacteria. The cell walls of Gram-negative bacteria are weaker than those of Gram-positive bacteria. Gram-negative bacteria are more susceptible to disintegration by mechanical stress [17], though Gram-negative bacteria are more resistant to antibiotics because of the outer membrane, which contributes to the reduced cell permeability [18]. It is possible that the rinsing step at 80°C is more detrimental to Gram-negative bacteria compared to Gram-positive bacteria. It is also important to note that mesophilic Gram-negative bacteria were isolated from the rinsing tank, the rack rail and the curtain (Table 3 Section 5, 8, and 9 respectively) which are rarely exposed to high temperatures in the conveyor-type dishwashers. These sections are not present in the door-type dishwasher. All the bacteria that were isolated in this study were grown under aerobic conditions. It is important to note that the use of different culture conditions, for example growth under anaerobic conditions and in selective medium, should elucidate different microflora that form biofilms within dishwashers using in the food service industry.

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