Kang et al | Article 8385
J Pure Appl Microbiol. 2023;17(2):780-787. doi: 10.22207/JPAM.17.2.03
Received: 27 December 2022 | Accepted: 07 March 2023
Published Online: 13 April 2023

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Pretreatments for Microbial Analysis and Evaluation of Hygiene of Wet Towels and Wet Wipes

Joohyun Kang[1], Miseon Sung[1], Jung Hoan Kim[3] and Yohan Yoon[1,2]*

[1]Department of Food and Nutrition, Sookmyung Women's University, Seoul 04310, Korea.
[2]Risk Analysis Research Center, Sookmyung Women's University, Seoul 04310, Korea.
[3]Food Technology and Service, Eulji University, Gyeonggi 13135, Korea.

Abstract

The demand for hygiene products has increased worldwide since the outbreak of global COVID-19. As the hygiene products market is expanding, it is necessary to manage microbial contamination in wet towels and wet wipes. This study evaluated pretreatment methods for microbial recovery from wet towels and wipes and microbial contamination levels in wet towels and wipes with the pretreatment method. *Escherichia coli* (NCCP14038 and NCCP14039), *Staphylococcus aureus* (ATCC25923 and ATCC29213), and *Pseudomonas aeruginosa* (NCCP10250 and NCCP11229) were inoculated on five fabric materials of wet towels and wet wipes. The recovery rates of the bacteria from wet towels and wet wipes using three pretreatment methods (pummeling, hand shaking, and portion cutting method) were investigated. Using the selected pretreatment method, the contamination levels of *E. coli*, *S. aureus*, and *P. aeruginosa* were evaluated for 238 wet towels and 244 wet wipes, which were collected in April to August, 2019. The presence of *tox*A and antibiotic resistance of *P. aeruginosa* isolated from wet towels were evaluated. The overall recovery rates of the pummeling method and hand shaking method were higher than the portion cutting method. Considering the convenience, the pummeling method was used to investigate the microbial contamination in the wet towels and wet wipes. *P. aeruginosa* was detected in two wet towels at an average of 9.9×10^2 CFU/towel. *E. coli* and *S. aureus* were not detected in both wet towels and wipes. *P. aeruginosa* isolates showed no resistances to piperacillin, piperacillin-tazobactam, aztreonam, and gentamicin, but had *toxA*. The results indicate that the pummeling method is the most appropriate pretreatment method for the recovery of microorganisms, and microbial analysis showed that this method could be useful in monitoring microbial contamination in wet towels and wet wipes.

Keywords: Wet Towels, Wet Wipes, Hygiene Products, Pretreatment Method, Microbial Contamination

***Correspondence:** yyoon@sookmyung.ac.kr

Citation: Kang J, Sung M, Kim JH, Yoon Y. Pretreatments for Microbial Analysis and Evaluation of Hygiene of Wet Towels and Wet Wipes. *J Pure Appl Microbiol.* 2023;17(2):780-787. doi: 10.22207/JPAM.17.2.03

INTRODUCTION

As the level of awareness about food safety and the amount of eating-out increases, the consumption of hygiene products also increases.[1] In addition, consumption of hygiene products such as wet towels and wet wipes has increased since the outbreak and spread of COVID-19.[2-4] *Escherichia coli* and *Staphylococcus aureus* are often isolated from human hands.[5,6] *E. coli* is Gram-negative bacteria, and contamination is spread through direct contact between hands and surfaces.[7,8] *S. aureus* can cause contamination through lesions on the hands or arms of workers or through coughing.[9] Because a few *S. aureus* isolates produce enterotoxins, the growth and proliferation of *S. aureus* present a potential risk to consumer health.[10] As *Pseudomonas aeruginosa* is ubiquitous in nature, the frequency of contact with humans is relatively high.[11] It is resistant to many antibiotics, and thus, the infection is very difficult to treat.[12] Exotoxin A produced by *P. aeruginosa,* can cause diseases in humans through the inhibition of protein synthesis, cellular degeneration, and interference with the immune function of host cells.[13] As such, there is a possibility of being exposed to diseases due to the use of wet towels and wet wipes. In addition, wet wipes consist of several fabrics, including natural and synthetic fibers.[14] Because Korea does not suggest pretreatment methods for the microbial test of wet towels and wet wipes in detail, the pretreatment methods to increase the recovery rates of microorganisms contaminated with samples of various fabrics should be prepared. Therefore, the objective of this study was to evaluate pretreatment methods and evaluate microbial contaminations in wet towels and wet wipes with the selected pretreatment method.

MATERIALS AND METHODS

Comparison of pretreatment methods

Selection pretreatment methods

The materials used in wet towels and wet wipes were investigated in the literature, applicable experimental pretreatment methods were selected, and bacterial recovery rates according to the pretreatment method were compared for each material. For the pummeling method, entire sheets of wet towel (15-25 g) or wet wipes (10-25 g) were placed in sample bags (3M, Maplewood, MN, USA), and they were 10-fold diluted with 0.1% buffered peptone water (BPW; BD Difco, Sparks, MD, USA) of the sample weight and homogenized by pummeler (BagMixer, Interscience, St. Nom, France) for 3 min. For the hand shaking method, 0.1% BPW was added to a sample bag (3M, USA) at a 10-fold dilution of the sample weight, and the sample bag was shaken by hand for 3 min. For portion cutting method, 2 g of hygiene products made of textile materials and 100 mL of distilled water were placed in a sample bag (3M, USA), and the sample bag was shaken using a rocker (RF300, ForBioKorea Co., Ltd., Gyeonggi, Korea) for 2 h.

Preparation of inocula and inoculation

Isolated colonies of *E. coli* strains NCCP14038 and NCCP14039 on MacConkey agar (BD Difco), or isolated colonies of *S. aureus* strains ATCC25923 and ATCC29213 on Baird-Parker agar (BPA; Oxoid Ltd., Basingstoke, Hants, UK) were inoculated in 10 mL of tryptic soy broth (TSB; BD Difco). They were cultured at 35°C for 24 h. Isolated colonies of *P. aeruginosa* strains NCCP10250 and NCCP11229 on cetrimide agar (BD Difco) were inoculated in Luria-Bertani broth (LB broth; BD Difco). It was cultured at 35°C for 24 h. Aliquots (0.1 mL) of *E. coli* or *S. aureus* cultures were transferred into 10 mL TSB, and an aliquot (0.1 mL) of *P. aeruginosa* culture was transferred into 10 mL LB broth. The cells were then subcultured at 35°C for 24 h. After the incubation, the subcultures of each bacterial strain were mixed and centrifuged at 1,912×g and 4°C for 15 min, and the cell pellets were washed twice with phosphate buffered saline (PBS, pH 7.4; NaCl 8.0 g, $NaHPO_4$ 1.5 g, KH_2PO_4 0.2 g, KCl 0.2 g in 1 L distilled water). The cell suspensions were then diluted with PBS at 5-6 log CFU/mL and used for the inocula.

Inoculation and evaluation for bacterial cell recovery

For wet towels, cotton (24 cm × 24 cm, 15 g) without antimicrobials used were examined because only cotton is used for the wet towel. For wet wipes, four materials [rayon (20 cm × 15 cm, 2 g), pulp (20 cm × 15 cm, 3 g), lyocell (20 cm × 15 cm, 3 g), and spunlace nonwoven (20 cm × 15

cm, 3 g)] were examined. These materials were sterilized at 121°C for 15 min. The aliquots (1 mL) of *E. coli, S. aureus*, or *P. aeruginosa* inocula were inoculated on the wet towels and wet wipes samples in filter bags. The samples were rubbed to spread the bacterial cells out with hands for 3 min. The samples were then treated with the pummeling, hand shaking, and portion cutting methods. The homogenates from the three methods were serially diluted with 0.1% BPW, and 0.1-mL aliquots of the diluents were spread-plated on MacConkey agar (BD Difco) for *E. coli*, BPA (Oxoid Ltd.) for *S. aureus*, and cetrimide agar (BD Difco) for *P. aeruginosa*. The plates were then incubated at 35°C for 24 h. After incubation, the typical colonies were counted manually. The recovery rate was calculated as follows:

$$\text{Recovery Rate (\%)} = \frac{\text{No. of Bacteria after Pretreatment(CFU/g)}}{\text{No. of Inoculated Bacteria (CFU/g)}} \text{ X100}$$

Investigation of microbial contamination level

Sample preparation

A total of 238 wet towels (24 cm × 28 cm, 15-25 g) and 244 wet wipes (20 cm × 15 cm, 5-15 g) were collected from restaurants or online shops from April to August in 2019. The collected samples were placed in an ice cooler and then transported to a laboratory within 4 h. According to the sample preparation procedure for hygiene products, a whole sheet of wet towel and wet wipe was aseptically placed into a filter bag and 10-fold diluted with 0.1% BPW (BD Difco) of the sample weight. The samples were homogenized with the pretreatment method test. The homogenized samples were then subjected to microbial analysis.

E. coli

Aliquots (1 mL) of the homogenates for wet towels and wet wipes were placed in EC Petrifilm (3M) and spread out for *E. coli* enumeration. The petrifilms were incubated at 35°C for 24 h, and red colonies with air bubbles were counted manually for detection *E. coli*. Also, 1-mL aliquotes of the homogenates were cultured in 10 mL of EC broth (BD Difco) containing Durham fermentation tubes at 35°C for 24 h. One loopful of the culture medium of the gas-generation positive sample was streaked on eosin methylene blue agar (EMB agar; BD Biosciences). After incubating the plate at 35°C for 24 h, the presence of greenish metallic sheen in light, a typical colony of *E. coli*, was confirmed.

S. aureus

For qualitative analysis, aliquots (1-mL) of the homogenates were inoculated into 9 mL of TSB plus 10% NaCl and enriched at 35°C for 24 h. A loopful of the enriched cultures was streaked on BPA (Oxoid Ltd.). The plates were incubated at 35°C for 24 h. Glossy black colonies surrounded by clear zones were identified as *S. aureus*. For quantification, aliquots (1 mL) of the homogenates were spread evenly in BPA (Oxoid Ltd.) for complete absorption and then incubated at 35°C for 24 h. Afterward, typical colonies were manually counted.

P. aeruginosa

Aliquots (1 mL) of the homogenates were inoculated in 9 mL of TSB (BD Difco) and incubated at 35°C for 24 h for enrichment. The enriched media were streaked on cetrimide agar (BD Difco), and the plates were incubated at 35°C for 24 h. Four colonies with green fluorescence in the plates were selected, and 16s rRNA was analyzed for identification. The proportion of positive colonies was multiplied by the number of colonies in the plates for *P. aeruginosa* cell counts. For DNA extraction, a colony was taken from cetrimide agar plates and suspended in a 1.5-mL microcentrifuge tube (Axygen Inc., New York, USA) containing 100 µL sterile distilled water. It was heated in a heat block at 99°C for 10 min, centrifuged at 15,814×g for 3 min, and the supernatant was used as template DNA. The universal bacterial 16s rRNA primers 27F (5'–AGA GTT TGA TCM TGG CTC AG–3') and 1492R (5'–CGG TTA CCT TGT TAC GAC TT–3')[15] were used for identification with 16s rRNA sequencing. The PCR product mixture was amplified with modified cycling conditions [95°C for 4 min (initial denaturation), 30 cycles of 95°C for 30 sec (denaturation), 55°C for 40 sec (annealing), and 72°C for 1 min (extension)] using Rotor-Gene Q (Qiagen).[15] Fluorescence-labeled ddNTP was added to the PCR product using a BigDye Terminator v3.1 matrix standard kit (ThermoFisher Scientific). Sequencing was

Table 1. Primer sequences for *toxA* gene amplification of *Pseudomonas aeruginosa*

Gene		Sequence (5' to 3')	Size (bp)	Reference
toxA	Forward Reverse	GGTAACCAGCTCAGCCACAT TGATGTCCAGGTCATGCTTC	352	Khattab et al., 2015[16]

performed with Applied Biosystems 3730 DNA Analyzer (Thermo Fisher Scientific). The homology of the isolates was compared by the BLAST search program of NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). For quantitative analysis, aliquots (1 mL) of homogenates were spread-plated evenly on cetrimide agar (BD Difco). The plates were incubated at 35°C for 24 h. Colonies with green fluorescence on the plates were counted manually.

Characterization of isolated *P. aeruginosa*

Identification of pathogenic genes

PCR was used to detect the toxin-generating gene (*tox*A) in *P. aeruginosa* colonies, which were determined to be positive in the qualitative test. For DNA extraction, a colony was taken from cetrimide agar plates and suspended in a 1.5-mL microcentrifuge tube containing 100 µL of sterile distilled water. It was heated in a heat block at 99°C for 10 min, centrifuged at 15,814×g for 3 min, and the supernatant was used as template DNA. DNA was extracted from two cultured *P. aeruginosa* isolates and used as template DNA, and the primers presented in Table 1 were used to amplify the *tox*A gene. The initial denaturation at 94°C for 5 min, denaturation at 94°C for 1 min, annealing at 62.4°C for 1 min, and extension at 72°C for 1 min, was repeated 30 times, followed by a final extension at 72°C for 10 min,[16] after which the PCR reaction was terminated. The PCR product was electrophoresed on a 1.5% agarose gel, and the bands were visualized by a UV-transilluminator (Vilber Lourmat, Collégien, France).

Examination of antibiotic resistance

To examine the antibiotic resistance of the *P. aeruginosa* isolates having *tox*A gene, the antibiotic diffusion method was used according to the 'performance standards for antimicrobial susceptibility testing' of the Clinical and Laboratory Standards Institute (CLSI).[17] *E. coli* ATCC43887 was used as a control according to CLSI, and one isolate with *tox*A per sample was selected, and *P. aeruginosa* isolates were spread evenly on Mueller-Hinton agar (MHA; BD Difco, USA) with sterile cotton and dried for 5 min. Antibiotic discs (piperacillin 100 µg, piperacillin-tazobactam 100/10 µg, aztreonam 30 µg, and gentamicin 10 µg) were then placed on the plates and incubated at 35°C for 24 h. Diameters of the inhibition zones around the antibiotic discs were measured after incubation, and the antibiotic resistance was determined according to the performance standards for antimicrobial susceptibility testing.[17]

Statistical analysis

Experimental data were analyzed using a pairwise *t*-test with the general linear procedure of SAS® version 9.4 (SAS Institute Inc., Cary, NC, USA) to determine significant differences ($p<0.05$) in the mean values among the different pretreatment methods.

RESULTS AND DISCUSSION

Selection of pretreatment method

In general, the portion cutting method had a lower ($p<0.05$) cell recovery rate than the pummeling and hand shaking methods (Table 2). In cotton, rayon, and lyocell materials, *E. coli* and *S. aureus* had higher ($p<0.05$) cell recovery rates by the hand shaking method than the portion cutting method, and *P. aeruginosa* had a higher ($p<0.05$) cell recovery rate using the pummeling method than the portion cutting method. In pulp materials, more *E. coli* was recovered by the pummeling method than hand shaking method, but the difference was not significant. In addition, the cell recovery rates of *S. aureus* and *P. aeruginosa* using the hand shaking method were higher than those using the pummeling method, but the difference was not significant. In spunlace

Table 2. Bacterial cell recoveries from various material used for wet towels and wet wipes after pretreatments

Bacteria	Material	Pretreatment method	No. of inoculated bacteria (CFU/g)	No. of bacteria after pretreatment (CFU/g)	Recovery rate (%)
E. coli	Cotton	Pummeling	9.1×10^3	5.3×10^3	48.5^{c}
		Hand shaking		6.7×10^3	59.3^{bc}
		Portion cutting		2.5×10^3	23.0^{e}
	Rayon	Pummeling	3.9×10^4	3.8×10^4	82.2^{a}
		Hand shaking		3.9×10^4	87.0^{a}
		Portion cutting		2.3×10^4	48.0^{c}
	Lyocell	Pummeling	7.3×10^4	6.8×10^4	82.2^{a}
		Hand shaking		7.1×10^4	83.5^{a}
		Portion cutting		3.5×10^4	43.6^{cd}
	Pulp	Pummeling	6.0×10^4	5.3×10^4	79.9^{ab}
		Hand shaking		5.2×10^4	77.3^{ab}
		Portion cutting		3.3×10^4	49.8^{c}
	Spunlace nonwoven	Pummeling	4.3×10^4	4.2×10^4	86.6^{a}
		Hand shaking		3.5×10^4	75.3^{ab}
		Portion cutting		1.8×10^4	44.3^{cd}
S. aureus	Cotton	Pummeling	2.5×10^3	1.4×10^3	57.4^{bc}
		Hand shaking		1.5×10^3	61.0^{bc}
		Portion cutting		8.0×10^2	28.8^{de}
	Rayon	Pummeling	9.7×10^3	7.6×10^3	80.0^{ab}
		Hand shaking		8.1×10^3	83.1^{a}
		Portion cutting		3.1×10^3	34.0^{de}
	Lyocell	Pummeling	1.6×10^4	1.3×10^4	84.7^{a}
		Hand shaking		1.4×10^4	85.7^{a}
		Portion cutting		6.0×10^3	35.5^{cd}
	Pulp	Pummeling	1.5×10^4	1.1×10^4	73.6^{ab}
		Hand shaking		1.2×10^4	79.5^{ab}
		Portion cutting		7.6×10^3	49.4^{c}
	Spunlace nonwoven	Pummeling	9.1×10^3	6.7×10^3	76.9^{ab}
		Hand shaking		7.6×10^3	82.9^{a}
		Portion cutting		4.6×10^3	45.9^{cd}
P. aeruginosa	Cotton	Pummeling	5.5×10^3	2.5×10^3	48.0^{c}
		Hand shaking		2.7×10^3	44.3^{cd}
		Portion cutting		1.4×10^3	24.7^{de}
	Rayon	Pummeling	2.4×10^4	1.9×10^4	78.4^{ab}
		Hand shaking		1.6×10^4	69.0^{b}
		Portion cutting		1.2×10^4	44.6^{cd}
	Lyocell	Pummeling	3.1×10^4	2.4×10^4	80.4^{a}
		Hand shaking		2.3×10^4	78.5^{ab}
		Portion cutting		1.2×10^4	34.3^{de}
	Pulp	Pummeling	3.6×10^4	2.5×10^4	67.8^{b}
		Hand shaking		2.5×10^4	69.5^{b}
		Portion cutting		2.1×10^4	57.2^{bc}
	Spunlace nonwoven	Pummeling	1.9×10^4	1.6×10^4	85.5^{a}
		Hand shaking		1.4×10^4	75.8^{ab}
		Portion cutting		5.4×10^3	31.1^{de}

a-e Values are significant different in different pretreatment method ($p<0.05$)

Table 3. Clear zone sizes (mean ± standard deviation; mm) of *Pseudomonas aeruginosa* isolated from wet towels against antibiotics

Classification	Penicillins	β-lactams	Monobactams	Aminoglycoside
	Piperacillin (100 μg)	Piperacillin-tazobactam (100/10 μg)	Aztreonam (30 μg)	Gentamicin (10 μg)
Resistance limit	≤14.0	≤14.0	≤15.0	≤12.0
Control (*E. coli* ATCC43887)	34.5±0.7	33.5±0.7	40.0±0.0	23.0±0.0
P. aeruginosa SMFM201908-WT1	34.7±0.6	34.3±2.1	26.7±2.5	24.7±1.2
P. aeruginosa SMFM201908-WT49	31.7±1.2	32.3±1.2	27.7±1.2	21.2±1.5

nonwoven materials, *E. coli* and *P. aeruginosa* had higher recovery rates by the pummeling method than the hand shaking method, and *S. aureus* had high recovery rates by the hand shaking method than the pummeling method, but the difference was not significant. Regardless of the material types, the pummeling and hand shaking methods had higher recovery rates than the portion cutting method, and both methods had similar recovery rates. The hand shaking method may induce variation in recovery rates by individuals. Thus, the pummeling method was selected for further analyses.

Application of selected pretreatment method for monitoring

Of 238 wet towels, *P. aeruginosa* was identified in two samples (0.8%), and the average detection level was 9.9×10^2 CFU/towel. However, *E. coli* and *S. aureus* were not detected in any sample. Even though *E. coli* and *S. aureus* were not detected, if microorganisms may remain owing to insufficient hygiene management by workers or insufficient washing in the wet towel production stage. Microorganisms may multiply during storage at room temperature in a factory after wet towels are packaged, or during delivery in a vehicle. In addition, if individual package of wet towels is removed and stored in restaurant, cross-contamination may occur among wet towels. Therefore, the wet towel should be stored at cold temperatures and the delivery vehicle should have refrigeration system. In addition, in restaurants, the individual package should be kept from the consumer use. *E. coli*, *S. aureus*, and *P. aeruginosa* were not detected in 244 wet wipes.

Pathogenic gene and antibiotic resistance of *P. aeruginosa isolates*

Exotoxin A is a virulence factor of *P. aeruginosa* in clinical infections that can cause tissue and organ damage.[18] The *tox*A gene regulates exotoxin A synthesis.[19] In two wet towel samples, *P. aeruginosa* was detected at an average level of 9.9×10^2 CFU/towel. The colonies were analyzed to detect *tox*A gene by PCR analysis, and the isolates had *tox*A gene. It indicates that the *P. aeruginosa* isolates could be pathogenic.

P. aeruginosa is generally resistant to penicillin, β-lactams, monobactams, and aminoglycan antibiotics.[20,21] Thus, in the present study, antibiotic resistance tests were conducted for four antibiotics most effective on *P. aeruginosa*. Two *P. aeruginosa* isolates did not resist the antibiotics (Table 3). *P. aeruginosa* is a non-fermenting, gram-negative bacillus that occurs widely in the natural environment, and most strains are opportunistic pathogens that rarely cause diseases in humans.[22] Because *P. aeruginosa* is a common flora on human skin,[23] *P. aeruginosa* contamination might occur due to poor personal hygiene during the wet towel packaging. There were also cases of microbial contamination by bare hands during the manufacturing and packaging of wet towels.[24] *P. aeruginosa* can cause skin diseases, leading to keratitis and peritonitis.[23,25] Although *P. aeruginosa* isolates do not have antibiotic resistance, if the *P. aeruginosa* isolates possessing *tox*A gene infect the immune-compromised, it might cause pathogenicity.

CONCLUSION

Among the pretreatment methods used in this study, there was no noticeable difference between the pummeling method and the hand shaking method, and the portion cutting method was inefficient in recovering bacteria. With the pummeling method, only *P. aeruginosa* was detected in two wet towels, and *P. aeruginosa*, *S. aureus* and *E. coli* were not detected in wet wipes. In addition to the existing pretreatment test methods, it may be necessary to develop a new pretreatment method that can increase the recovery rates of microorganisms from wet towels and wet wipes in the future study.

ACKNOWLEDGMENTS

The authors would like to thank Ministry of Food and Drug Safety for their support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

FUNDING

This study was funded by the Ministry of Food and Drug Safety under Grant (19162MFDS017).

DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

Not applicable.

REFERENCES

1. Kolanowski W, Karaman AD, Akgul FY, Lugowska K, Trafialek J. Food safety when eating out – perspectives of young adult consumers in Poland and Turkey – a pilot study. *Int J Environ Res Public Health*. 2021;18(4):1884. https://doi.org/10.3390/ijerph18041884
2. Chong MS. Bacterial contamination in disposable wet wipes from general restaurants. *Korean J Clin Lab Sci*. 2016;48(3):237-241. https://doi.org/10.15324/kjcls.2016.48.3.237
3. Koksoy Vayisoglu S, Oncu E. The use of cleaning products and its relationship with the increasing health risks during the COVID-19 pandemic. *Int J Clin Pract.* 2021;75(10):e14534. https://doi.org/10.1111/ijcp.14534
4. Hu T, Shen M, Tang W. Wet wipes and disposable surgical masks are becoming new sources of fiber microplastic pollution during global COVID-19. *Environ Sci Pollut Res*. 2022;29: 284–292. https://doi.org/10.1007/s11356-021-17408-3
5. Abd-Elaleem R, Bakr WMK, Hazzah WA, Nasreldin O. Assessment of the personal hygiene and the bacteriological quality of butchers' hands in some abattoirs in Alexandria, Egypt. *Food Control*. 2014;41:147-150. doi: 10.1016/j.foodcont.2014.01.016 https://doi.org/10.1016/j.foodcont.2014.01.016
6. Tan SL, Lee HY, Mahyudin NA. Antimicrobial resistance of Escherichia coli and Staphylococcus aureus isolated from food handler's hands. *Food Control*. 2014;44:203-207. doi: 10.1016/j.foodcont.2014.04.008
7. King MF, López-García M, Atedoghu KP, et al. Bacterial transfer to fingertips during sequential surface contacts with and without gloves. *Indoor Air.* 2020;30:993-1004. doi: 10.1111/ina.12682
8. Rusin P, Maxwell S, Gerba C. Comparative surface-to-hand fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria, and phage. *J Appl Microbiol.* 2002;93(4):585-592. doi: 10.1046/j.1365-2672.2002.01734.x
9. Kluytmans JAJW, Wertheim HFL. Nasal carriage of Staphylococcus aureus and prevention of nosocomial infections. *Infection.* 33:3-8. doi: 10.1007/s15010-005-4012-9
10. Mahros MA, Abd-Elghany SM, Salla KI. Multidrug-, methicillin-, and vancomycin-resistant Staphylococcus aureus isolated from ready-to-eat meat sandwiches: An ongoing food and public health concern. *Int J Food Microbiol.* 2021;346:109165. doi: 10.1016/j.ijfoodmicro.2021.109165
11. Ramsey MM, Whiteley M. Pseudomonas aeruginosa attachment and biofilm development in dynamic environments. *Mol Microbiol.* 2004;53(4):1075-1087. https://doi.org/10.1111/j.1365-2958.2004.04181.x
12. Bonomo RA, Szabo D. Mechanisms of multidrug resistance in Acinetobacter species and Pseudomonas aeruginosa. *Clin Infect Dis.* 2006;43(Suppl. 2):S49-S56. https://doi.org/10.1086/504477
13. Pollack M. The role of exotoxin A in pseudomonas disease and immunity. *Rev Infect Dis.* 1983;5(Suppl. 5):S979-S984. https://doi.org/10.1093/clinids/5.Supplement_5.S979
14. Köklü R, Ateş A, Deveci EÜ, Sivri N. Generic foresight model in changing hygiene habits with the pandemic: use of wet wipes in next generations. *J Mater Cycles Waste Manag.* 2023;25(1):74-85. https://doi.org/10.1007/s10163-022-01515-5
15. Raja CE, Pandeeswari R, Ramesh U. Characterization of high fluoride resistant Pseudomonas aeruginosa species isolated from water samples. *Environ Res Tec.* 2022;5(4):325-339.

16. Khattab MA, Nour MS, ElSheshtawy NM. Genetic identification of Pseudomonas aeruginosa virulence genes among different isolates. *J Microb Biochem Technol.* 2015;7(5):274-277.
17. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 26th ed. CLSI supplement M100S. Clinical and Laboratory Standards Institute, Pennsylvania. 2016.
18. Jenkins CE, Swiatoniowski A, Issekutz AC, Lin TJ. Pseudomonas aeruginosa exotoxin A induces human mast cell apoptosis by a caspase-8 and -3-dependent mechanism. *J Biol Chem.* 2004;279(35):37201-37207. https://doi.org/10.1074/jbc.M405594200
19. Dong D, Zou D, Liu H, et al. Rapid detection of Pseudomonas aeruginosa targeting the toxA gene in intensive care unit patients from Beijing, China. *Front Microbiol.* 2015;6:1100. https://doi.org/10.3389/fmicb.2015.01100
20. Pang Z, Raudonis R, Glick BR, Kin TJ, Cheng Z. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. *Biotechnol Adv.* 2019;37(1):177-192. https://doi.org/10.1016/j.biotechadv.2018.11.013
21. Glen KA, Lamont IL. β-lactam resistance in Pseudomonas aeruginosa: current status, future prospects. *Pathogens.* 2021;10(12):1638. https://doi.org/10.3390/pathogens10121638
22. Diggle SP, Whiteley M. Microbe profile: Pseudomonas aeruginosa: opportunistic pathogen and lab rat. *Microbiol.* 2020;166(1):30-33. https://doi.org/10.1099/mic.0.000860
23. Spernovasilis N, Psichogiou M, Poulakou G. Skin manifestations of *Pseudomonas aeruginosa* infections. *Curr Opin Infect Dis.* 2021;34(2):72-79. https://doi.org/10.1097/QCO.0000000000000717
24. Ministry of Food and Drug Administration. Investigation of contamination status among hygiene products. 2021. https://scienceon.kisti.re.kr/commons/util/originalView.do. Accessed 25 Oct 2021.
25. Cole N, Krockenberger M, Stapleton F, Khan S, Hume E, Husband AJ, Willcox M. Experimental Pseudomonas aeruginosa keratitis in interleukin-10 gene knockout mice. *Infect Immun*. 2003;71(3):1328-1336. https://doi.org/10.1128/IAI.71.3.1328-1336.2003