Bacteriological Study on the Contamination of Flower Vase Water

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Abstract

Water from flower vases at various locations in the hospital was cultured for isolation of bacteria, together with investigating the effects of disinfectants on flower vase water (FVW) contamination. Immediately after putting the flowers, viable bacteria counts were below the detection limit ($<10^3$ cfu/ml). With the lapse of time, these counts increased to 10^5 cfu/ml on the second to third days regardless of the location. A wide range of bacterial species including multidrug-resistant nonfermentative gram-negative rods such as *Flavobacterium, Pseudomonas,* and *Alcaligenes* sp. were isolated. These isolates were considered to originate not only from the flower stalks but also from the hospital environment. Out of five disinfectants, 1.25% sodium hypochlorite (Milton) and 1.0% benzethonium chloride (Hyamine) were proved effective for preventing contamination of FVW without affecting the flower's vitality. The use of a disinfectant in the FVW or at least a daily change of water is recommended in the ward of compromised patients.

Key words

contamination of flower vase water, hospital acquired infection, compromised patients, disinfectants

Introduction

In the latter half of the 1980's, hospital-acqiured infection (HAI) due to methicillin-resistant *Staphylococcus aureus* (MRSA) occurred explosively throughout Japan[1, 2)]. Since then, much attention has been paid to the hospital environment as a pathogen source and vehicle for their transmission. In fact, HAIs from the hydrotherapy pool, ventilator condensate, and holy water have been reported in other countries[3-6)]. Concerning bacteria in flower vase water (FVW) or plants, several reports[7-13)] were successively published mostly in the 1970's. However, most of them were reported in

short communication style, and full paper reports with detailed data were very few. Furthermore, some investigators[9, 11, 13] described that bacteria in FVW or plants would be a risk factor for HAI, but others[8, 12] denied such a possibility. There is no doubt that fresh flowers give psychological comfort to people, and it is therefore not possible to remove them from the hospital. We considered that it was important to investigate this situation in more detail through bacteriological studies. Thus, this study was initiated to clarify the following points: when FVW is contaminated, what species of bacteria are involved, where do they come from, what is their antibiotic sensitivity, and how do we prevent this situation.

Materials and Methods

Location of flower vases

Pairs of carnations, which were obtained from a florist in our university hospital, were put into nonsterile, conventional glass flower vases each holding one liter of tap-water. The vases were put at certain locations in the hospital including patient and stuff rooms, and a day room. In some studies, the vases were treated with disinfectant for 2 h at room temperature before use, or vases with water containing disinfectant were employed.

Bacteriological study

After putting the vases at the prescribed locations in the hospital, 10 ml of FVW was collected from each location into a sterile plastic conical tube at zero hour (=immediate after putting), 24 h, 48 h, and 72 h. Immediately after water collection, 1 ml of water with or without appropriate dilutions was processed for isolation of the bacteria on both Drigalski and PEA agar media (Eiken, Tokyo, Japan) to distinguish fermentative/nonfermentative gram-negative rods (FGNRs/NFGNRs) and gram-positive cocci, respectively. For isolation of bacteria from the flower stalks, they were cut into small pieces and dipped into 10 ml of sterile saline in a sterile plastic conical tube, followed by vigorous shaking for 10 min at room temperature. For isolation of bacteria from hand wash basins, tables, and floors in the patients' rooms, their surface (10 x 10 cm) was wiped with a sterile applicator, and then the applicator was dipped in saline. After removal of the flower stalks or applicator from saline, it was centrifuged to concentrate the bacteria and then processed to isolation as above. In the case of tap water, 100 ml was collected from the patients' rooms and concentrated by centrifugation before isolation. After incubation at 37℃ under an aerobic condition, colony numbers were counted by the bacteria categories described above and expressed as cfu/ml. The incubation period was usually overnight except in the case of tap water which was incubated for 3 days. Three plates were used for each sample to calculate the mean.

Identification and antibiotic sensitivity test of isolates were carried out by the standard procedures using Auto-scan W/A (Baxter Diagnostic Inc., West Sacramento, CA, USA) based on National Committee for Clinical Laboratory Standards method[14]. Antibiotics tested were Piperacillin (PIPC), Cefazolin (CEZ), Cefotiam (CTM), Ceftizoxime (CZX), Gentamycin (GM), Minocycline (M INO), and Imipenem/Cilastatin (IPM/CS). As for the disinfectants, sodium hypochloride (Milton), benzethonium chloride (Hyamine), glutaraldehyde (Sterihyde), alkylpolyamino ethylglycine (Tego 51), and chlorhexidine gluconate (Stericlon W) were used at the indicated concentrations as shown in the text.

Results

Time-related contamination of FVW

Each vase with a pair of carnations was located in 3 patient rooms, 3 stuff rooms, and a day room. On day zero, total viable counts were below

the detection limit (10^3 cfu/ml) (data not shown). With the lapse of time, these values increased but their patterns differed according to their bacterial categories (Fig. 1). In NFGNRs, considerably large numbers of colonies were detected on the first day. On the second day, these numbers reached their peaks (approximately 10^5 cfu/ml), and then decreased on the third day regardless of the location. In contrast, gram-positive cocci reached recognizable levels on the second day, and successively increased by the third day in all locations tested except for the patient rooms in which viable counts were very low. On the other hand, FGNRs were the minor population which could be detected marginally beyond the detection limit on the third day in all locations tested. On the whole, contamination was recognized in all specimens regardless of the vases' location. Since NFGNRs were the major population and seemed to act as definitive bacteria on the time-related pattern of contamination, we focused our attention on the NFGNRs in the following studies.

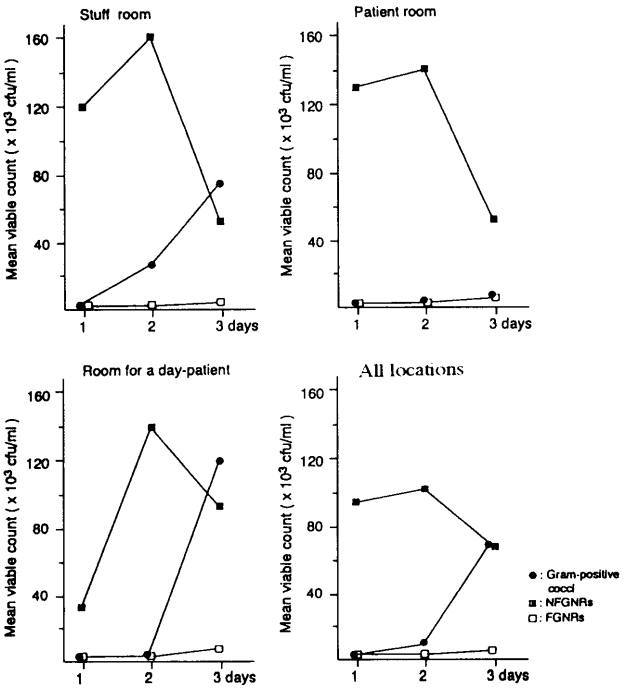

Figure 1. Time-related and site-specific isolation from the vase water. Vases with a pair of carnations were located in 3 patient rooms, 3 stuff rooms and a day room. The mean for all locations is shown in the right-lower panel.

Species identification of NFGNRs in the contamination

Identification of species names of NFGNRs was carried out randomly on 5 selected specimens collected at 48 h in the time-related study described above. In Table 1, 7 species in NFGNRs and 3 species in *Enterobacteriaceae* are listed in order of viable counts. Among NFGNRs isolated, *Flavobacterium* (6.6 x 10^5 cfu/ml), *Pseudomonas* (3.4 x 10^5 cfu/ml), and *Alcaligenes* sp. (2.0 x 10^5 cfu/ml) were the major species which occupied 80% of the isolates. Viable counts of *Acinetobacter* (4.8 x 10^4 cfu/ml), *Stenotrophomonas* (2.9 x 10^4 cfu/ml), and *Agrobacterium* sp. (1.2 x 10^4 cfu/ml) were approximately one-log less than those of the major three species. Non-identified NFGNRs were also isolated with a total of 7.0 x 10^4 cfu/ml. In the *Enterobacteriaceae* family, only 3 species of Genus *Enterobacter* (*Ent. agglomerans, intermedium* and *cloacae*) were isolated. Concerning the isolation rates from 5 specimens, *Pseudomonas, Flavobacterium* and *Ent. agglomerans* were isolated from 3-4 specimens, and other species showed relatively lower rates (1-2 specimens). Taken together these data, *Flavobacterium* and

Table 1. Bacteria species in gram-negative rods isolated from the vase water

Species	Mean viable count (x 10^3 cfu/ml)	Isolation rate
NFGNRs		
Flavobacterium sp.	661	3/5
Pseudomonas sp.	307	4/5
Alcaligenes sp.	200	1/5
Acinetobacter sp.	48	1/5
Comamonas sp.	30	1/5
Stenotrophomonas maltophilia	29	1/5
Agrobacterium sp.	10	2/5
Others	70	
Enterobacteriaceae		
Ent. agglomerans	38	3/5
intermedium	12	2/5
cloacae	5	1/5

Table 2. Susceptibility profile of the representative isolates from the vase water

Species	Susceptibility profile[1]						
	PIPC	CEZ	CTM	CZX	G M	MINO	IPM/CS
Flavobacterium sp.	S	R	R	S	R	S	S
Pseudomonas sp.	R	R	R	R	S	R	R
Alcaligenes sp.	R	R	R	S	S	S	S
Acinetobacter sp.	R	R	R	R	S	S	S
Stenotrophomonas maltophilia	S	R	R	S	S	S	S
Ent. agglomerans	R	S	S	S	S	S	S

Abbreviations for each antibiotic indicate in the text.
[1] S and R indicate sensitive and resistant, respectively.

Pseudomonas sp. may be ranked as the most common species in the contamination of FVW.

Sensitivity profiles of the representative isolates

Sensitivity profiles of the representative 6 species, *Flavobacterium, Pseudomonas, Alcaligenes, Acinetobacter,* and *Stenotrophomonas sp.*, and *Ent. agglomerans*, were examined (Table 2). Although 3-5 strains of the same species isolated from different locations were used for this test, sensitivity profiles were equal within the same species (data not shown). Among the tested drugs, GM was not effective only for *Flavobacterium sp.*, MINO for *Pseudomonas sp.* and IPM/CS for *Alcaligenes sp.* On the other hand, most of the isolates showed resistance to PIPC and cephems (CEZ, CTM, and CZX) which are frequently used throughout Japan including our own hospital. Overviewing the sensitivity profile in terms of species, *Stenotrophomonas* and *Ent. agglomerans* were ranked as the most sensitive species showing sensitivity to 5-6 antibi otics. *Flavobacterium, Alcaligenes*, and *Acinetobacter* sp. were ranked as the intermediate ones showing sensitivity to 3-4 antibiotics, whereas *Pseudomonas* was the most multidrug-resistant showing resistance to 6 antibiotics except GM.

Comparison of isolated species among flower stalks, FVW, and environmental materials

To define where the contaminating bacteria originated from, isolation of the bacteria was attempted from the flower stalks and environmental materials collected from the patient rooms (Table 3). Viable counts from the tap water itself were below the detection limit (data not shown). Although the level of colonies from the flower stalks at the point of purchase was extremely low (1-3 colonies from each flower stalk), more than 4 species of NFGNRs (*Flavobacterium, Pseudomonas, Alcaligenes,* and non-identified NFGNRs) and 4 species of Genus *Enterobacter* (*Ent. agglomerans, cloacae, sakazaki,* and *aerogenes*) were actually isolated from a total of 10 flower stalks in 5 vases. All species isolated from the flower stalks were also recovered from either FVW in 5 patient rooms. For example, *Pseudomonas* and *Enterobacter* were isolated from the FVW in 4 rooms, and *Flavobacterium* from 3 rooms. In contrast, *Acinetobacter, Stenotrophomonas,* and *Agrobacterium sp.* were not isolated from the flower stalks, but from FVW. In addition to these three species, certain species were also isolated from both FVW and environment, but not from the flower stalks. These data indicate that FVW may act as concentrator of environment-born bacteria as well as flower stalk-born bacteria.

Preventive efficacy of disinfectants on the FVW contamination

Whether water containing disinfectant shows preventive efficacy for FVW contamination without affecting the flower's vitality was examined

Table 3. Comparison of isolates among flower stalk, vase water and environment

Origin		Representative isolates[1] by patient room				
		W-2	W-3	W-6	E-6	E-7
Fiower stalk	A,B,C,H,O[2]					
Vase water		A, B, C, H,	A, H,	B, H,	A, B, D, O[1]	B, E, F H
Handwash basin		C, D, O[2]	D, E, F, G, H	B, G,	D, G,	A, B, D
Table		n. d.[2]	A, D	A, B, E	E	n. d.
Floor		D	n. d.	D, H, K	A, B, D, E	n. d.

[1] isolates with more than 5 colonies are listed.
In the case of flower stalk, isolates with 1 to 3 colonies are listed.
Underline indicates the same species as those isolated from the flower stalk.
[2] not detected (less than 4 colonies).
Abbreviation used for species as follows : A, *Flavobacterium* ; B, *Pseudomonas* ; C, *Alcaligenes* ; D, *Acinetobacter* ; E, *Stenotrophomonas* ; F, *Agrobacteirum* ; G, *Achromobacter* ; H, *Enterobacter* ; K, *Klebsiella pneumoniae* ; O, others (O[1], NFGNRs ; O[2], both NFGNRs and FGNRs).

Table 4. Comparison of protective efficacy of disinfectants on the contamination of vase water

Disinfectant	Concentration (%)	Mean viable count (x 10^3 cfu/ml)				State of fiowers
		0	1	2	3 day	
Water	-	0	61	92	1,100	healthy
Stericlon W	0.02	0	210	800	600	healthy
Tego 51	0.05	0	1	400	1,000	healthy
Sterihyde	0.4	0	0	0	0	wilted
Hyamine	1.0	0	0	0	0	healthy
Milton	1.25	0	0	0	0	healthy
	0.125	0	0	0	48	healthy
	0.0125	0	0	100	520	healthy

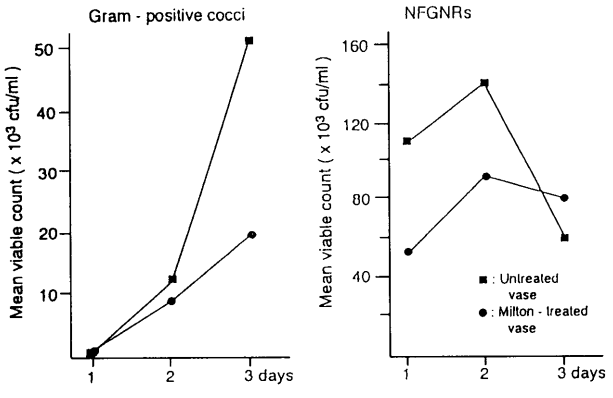

Figure 2. Preventive efficacy of Milton-treated vase. The vase was treated with 1.25% Milton for 2 h at room temperature before a pair of carnations were put in it. Thereafter, the vase water was collected daily to monitor the degree of contamination.

using 5 disinfectants at a conventional dose. As shown in Table 4, both Milton (1.25%) and Hyamine (1.0%) were effective for contamination and did not affect the flower's vitality, whereas Sterihyde caused wilting. The preventive effect of the remaining 2 disinfectants was negligible. When the efficacy of Milton was further tested at a lower concentration range by 10- and 100-fold dilutions, 48 colonies were isolated on the third day at 0.125%, and 100 colonies on the second day at 0.0125%, indicating that 1.25% Milton was suitable.

Furthermore, the preventive efficacy of a Milton-treated vase was examined (Fig. 2). The two vases were pretreated with 1.25% Milton for

2 h at room temperature, and then flowers were put into tap water without Milton to monitor the contamination. Compared with the two untreated control vases, viable bacteria counts were lower in Milton-pretreated vases by one log on the first day, but both counts reached a comparable level on the second day. These data suggest that the preventive efficacy of pretreatment of a vase with Milton is limited unless the disinfectant is present in the water.

Discussion

It is well known that FVW becomes stagnant within a few days unless water is renewed. However, this situation has not yet been studied in detail from the aspects of hospital sanitation and HAI. We have shown in this study that FVW may become a potential bacterial reservoir. At the time of purchase of the flowers, level of colonization on the flower stalks was extremely low, but with the lapse of time, this increased to a considerable level of contamination in FVW. At the same time, the contamination was enhanced by the occasionally incoming environmental flora, resulting in stagnant water with a mass of mixed bacterial growth as mush as 10^5 cfu/ml. Conversely, increased contamination may spread from FVW into the environment. Namely, flower stalk-born bacteria as well as environment-born bacteria could be cited as possible sources of contamination. A similar situation has recently been pointed out in holy water used by certain patients in the hospital[6].

In this study, the representative isolates were NFGNRs such as *Flavobacterium, Pseudomonas,* and *Alcaligenes* sp. which are distributed widely in nature[13] and are acquiring recognition as important pathogens for HAI[16]. Unlike the reports by Taplin and Mertz[9], or Bartzokas et al[12], *Escherichia coli* and *Klebsiella* sp. were not major organisms in our study. Bartzokas et al[12] reported that *Proteus* and *Pseudomonas* sp. were not isolated from FVW. As described previously[17, 18], these isolates were shown to be multidrug-resistant, especially resistant to cefem antibiotics which have been frequently used in Japan, although the excess use of these antibiotics has been controlled since unusually high isolation rates of MRSA have been found in Japan. At the present time, MRSA and *Ps. aeruginosa* are major pathogens in our university hospital[1]. However, MRSA was not isolated from the FVW or environmental materials.

Concerning the preventive efficacy of certain disinfectants, Hyamine (1.0%) and Milton (1.25 %) were effective for FVW decontamination without affecting the flower's vitality. A similar effect of chlorhexidine (Hibitane)[7, 10] or hydrogen peroxide[8,12] have been reported. Taplin and Mertz[9] reported that the removal of flowers from a burn unit and the provision of new dry mops for daily floor cleaning was followed by a dramatic decrease in wound colonization and infections by gram-negative bacteria. But, it is difficult to remove flowers from the patient rooms, because patients feel relaxed to see them. Fortunately, in our hospital we have not yet experienced any outbreak of HAIs in which involvement of FVW-born pathogens was considered. However, we must consider the possibility that FVW is a potential risk factor for HAI as a concentrator and reservoir of pathogens, so the use of disinfectant-containing FVW or at least daily change of water is recommended in the ward of debilitated patients such as those with AIDS, severe burns, trauma, post-operative wounds, or intravenous lines.

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Contamination of flower vase water

花器水汚染の細菌学的検討

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要　旨

病院内の色々な場所に設置した花器水の汚染度を細菌学的に検討した，同時に汚染予防として消毒剤の効果も調べた．花器に生花を生けた直後の生菌数は検出限界以下（$<10^3$ cfu/ml）であったが，花器の設置場所に拘らず，経日的に増加し，汚染度は2～3日目では10^5 cfu/mlに達した．汚染菌種は多様で，その中には多剤耐性ブドウ糖非発酵菌（フラボバクテリウム属，緑膿菌属やアルカリゲネス属）が含まれていた．これらの汚染菌の由来は，生花の茎ばかりでなく院内環境もあると推定された．5種の消毒剤のうち，1.25% sodium hypochloride（ミルトン）と1.0% benzathonium chloride（ハイアミン）が生花の状態に影響を与えずに汚染を予防することが明らかになった．これらのことから，易感染者のいる病室や病棟においては，これらの消毒剤の使用あるいは少なくとも1日1回の花器水の交換が推奨された．

キーワード

花器水汚染，院内感染，易感染者，消毒剤