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Review of disinfectant susceptibility of bacteria isolated in hospital to commonly used disinfectants

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Summary: The susceptibility of clinical isolates and indigenous bacteria to commonly used disinfectants was investigated during different time periods. Among the clinical isolates tested during Period I (August 1985–July 1986, 6 genera, 9 species, 353 strains) there were many resistant strains not killed within a short period of time by the recommended concentration of chlorhexidine gluconate (CHG) or benzalkonium chloride (BAC). During Period II (October 1987–May 1988, 6 genera, 9 species, 152 strains), however, a reduction in the number of strains resistant to these disinfectants was observed. The use of the broad spectrum disinfectant povidone-iodine (PVP-I) increased between those two time periods.

With regard to the susceptibility of indigenous bacteria, tests were carried out on bacteria isolated from sinks and physicians' hands in the gastroenterology division of the Departments of Internal Medicine and Surgery at the hospital. During Phase I (April–June 1987), strains of *Pseudomonas* and *Serratia* resistant to CHG and BAC were isolated from sinks, while the same strains of *Serratia* were also isolated from physicians' hands. During Phase II (March–May 1988), however, no resistant strains were isolated. A comparison of the consumption of disinfectants during the two phases revealed that a greater amount of CHG was consumed during Phase I, while a greater amount of PVP-I was consumed during Phase II. There was a strong indication, therefore, that bacteria resistant to CHG and BAC decrease with the increased use of PVP-I.

Introduction

There are an increasing number of reports of hospital infections in compromised patients caused by bacteria isolated in the hospital environment. Medical staff are particularly concerned to prevent such hospital infections and it is therefore necessary to employ appropriate measures for sterilization and disinfection. Because the strains of clinical isolates and the choice of drugs and disinfectants differ between hospitals, there may be differing disinfectant susceptibilities even amongst the same strain of bacteria. Each hospital therefore is required to determine the susceptibility of bacteria in that hospital in order to select an appropriate disinfectant.

Although a number of papers deal with disinfectant susceptibilities of bacteria isolated from clinical material,[1–3] there are few papers which examine this problem over a prolonged period and analyse the results in terms of the consumption of particular disinfectants. We therefore performed such investigations in our hospital using nine strains of bacteria from six genera isolated within our hospital and four types of disinfectant regularly used for handwashing: chlorhexidine gluconate (CHG), benzalkonium chloride (BAC), saponated cresol solution (SAC) and povidone-iodine solution (PVP-I). We also performed a study of the incidence and disinfectant susceptibility of indigenous hospital bacteria and again, over two phases, we compared the changes in susceptibility of the bacterial isolates with changes in disinfectant use.

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Clinical isolates

Disinfectant susceptibility

Bacterial susceptibility to disinfectants was determined by the two step method. In the first primary screening step, measurements of minimum inhibitory concentrations (MIC) were made. For strains with low susceptibility to the disinfectants (i.e. high MICs), measurements of minimum bactericidal concentrations (MBC) were made as the second step.

Table I Ratio of clinical isolate strains with low disinfectant susceptibility: Period I (August 1985–July 1986)

Clinical isolates tested	*No. of strains tested*	*No. of strains with lower susceptibility than standard strain (%)*			
		CHG	*BAC*	*SAC*	*PVP-I*
Staphylococcus aureus	76	6 (8)	25 (33)	0	0
Staphylococcus epidermidis	20	12 (60)	0	0	0
Escherichia coli	54	23 (43)	1 (2)	0	0
Klebsiella pneumoniae	53	2 (4)	0	0	0
Serratia marcescens	47	42 (89)	6 (13)	0	0
Pseudomonas aeruginosa	51	1 (2)	11 (22)	33 (65)	0
Pseudomonas cepacia	17	15 (88)	12 (70)	16 (94)	0
Proteus mirabilis	19	0	4 (21)	1 (5)	0
Proteus vulgaris	16	7 (44)	0	0	0

CHG: chlorhexidine digluconate, BAC: benzalkonium chloride, SAC: saponated cresol, PVP-I povidone-iodine.

Table II Secondary testing by the MBC method: Period I

Clinical isolates tested			*Time required for disinfection (sec)*			
			*CHG 0.1%**	*BAC 0.1%**	*SAC 2.0%**	*PVP-I 0.1%**
Serratia marcescens	ATCC 10031		30	30	30	30
	Clinical isolate	No. 1	180	60	30	30
		2	180	60	30	30
		3	180	180	30	30
Pseudomonas aeruginosa	IFO 3445		30	30	30	30
	Clinical isolate	No. 1	30	30	30	30
		2	30	30	30	30
		3	30	30	30	30
Psuedomonas cepacia	M-0257		> 180	30	30	30
	Clinical isolate	No. 1	> 180	180	30	30
		2	> 180	> 180	30	30
		3	> 180	> 180	30	30
Proteus mirabilis	IFO 3849		180	30	30	30
	Clinical isolate	No. 1	180	30	30	30
		2	> 180	30	30	30
		3	> 180	30	30	30
Proteus vulgaris	IFO 3988		30	30	30	30
	Clinical isolate	No. 1	180	30	30	30
		2	> 180	30	30	30
		3	180	30	30	30

*regular concentration (%).
Boxes highlight killing times > 60 sec

During the first study period (August 1985–July 1986, Period I) we screened 6 genera, 9 species, 353 strains of clinical isolates and found strains of *S. aureus, S. epidermidis, E. coli* and *K. pneumoniae* to be sensitive at the ordinary recommended concentrations of the disinfectants tested, even though their MICs were higher than respective standard strains (Table I). On the other hand strains of *Se. marcescens, Ps. aeruginosa, Ps. cepacia, Pr. mirabilis* and *Pr. vulgaris* had MICs almost corresponding to or higher than the recommended concentrations of the disinfectants. These strains were therefore expected to be resistant to the disinfectant during handwashing and three strains of each were subjected to the secondary test.

The results of the secondary test are shown in Table II. PVP-I and SAC at recommended concentrations (0.1% and 2% respectively) killed all strains within 30 seconds. *Se. marcescens* and *Ps. cepacia* were resistant to BAC at the recommended concentration (0.1%) and only the clinical isolates of *Ps. aeruginosa* were sensitive to CHG 0.1% with all 12 strains of *Se. marcescens, Ps. cepacia,*

Table III Ratio of clinical isolate strains with low disinfectant susceptibility: Period II (October 1987–May 1988)

Clinical isolates tested	*No. of strains tested*	*No. of strains with lower susceptibility than standard strain (%)*			
		CHG	*BAC*	*SAC*	*PVP-I*
Staphylococcus aureus	36	0	35 (97)	0	0
Staphylococcus epidermidis	16	14 (87)	4 (25)	0	0
Escherichia coli	20	11 (55)	0	0	0
Klebsiella pneumoniae	20	0	0	0	0
Serratia marcescens	21	20 (95)	3 (14)	0	0
Pseudomonas aeruginosa	15	0	0	15 (100)	0
Pseudomonas cepacia	2	0	0	2 (100)	0
Proteus mirabilis	18	1 (5)	14 (77)	0	0
Proteus vulgaris	4	4 (100)	0	0	0

Table IV Secondary testing by the MBC method: Period II

Clinical isolates tested			*Time required for disinfection (sec)*			
			*CHG 0.1%**	*BAC 0.1%**	*SAC 2.0%**	*PVP-I 0.1%**
Serratia marcescens	ATCC 10031		30	30	30	30
	Clinical isolate	No. 1	30	30	30	30
		2	30	30	30	30
		3	30	30	30	30
Pseudomonas aeruginosa	IFO 3445		30	30	30	30
	Clinical isolate	No. 1	30	30	30	30
		2	30	30	30	30
		3	30	30	30	30
Proteus mirabilis	IFO 3849		[180]	30	30	30
	Clinical isolate	No. 1	30	30	30	30
		2	[60]	30	30	30
		3	[60]	[60]	30	30
Proteus vulgaris	IFO 3988		30	30	30	30
	Clinical isolate	No. 1	[60]	[60]	30	30
		2	30	30	30	30
		3	30	[60]	30	30

*regular concentration (%).
Boxes highlight killing times > 60 sec

Pr. mirabilis and *Pr. vulgaris* being resistant. These results supported previous findings[7–9] that some isolates of *Pseudomonas, Serratia* and *Proteus* are not susceptible to CHG or BAC.

We repeated the study two years later (October 1987–May 1988, Period II). We screened 6 genera, 9 species and 152 strains of clinical isolates during this period and found strains of *Se. marcescens, Ps. aeruginosa, Pr. mirabilis* and *Pr. vulgaris* gave MICs corresponding to or higher than the recommended concentrations of the disinfectants. Three strains of each bacteria were therefore subjected to secondary testing (Table IV). Once again, all strains were sensitive to PVP-I 0.1% and SAC 2% but on this occasion only three of the 12 strains were resistant to BAC 0.1% and four strains resistant CHG 0.1%.

Influence of commonly used disinfectants on the disinfectant susceptibility

We compared the consumption of disinfectants between the two periods in which test strains were isolated. As shown in Figure 1, the amount of PVP-I used increased in Period II and there was a modest fall in the use of the other three disinfectants. We related this change to the clinical isolates detected during the two study periods, taking only that period from Period I which related directly in time to the shorter Period II.

The number of strains isolated and tested of *Ps. cepacia* and *Pr. vulgaris* in Period II was extremely small. This was probably related to a general decrease in these species noted by Watanabe *et al.*[11] and our own laboratory data.

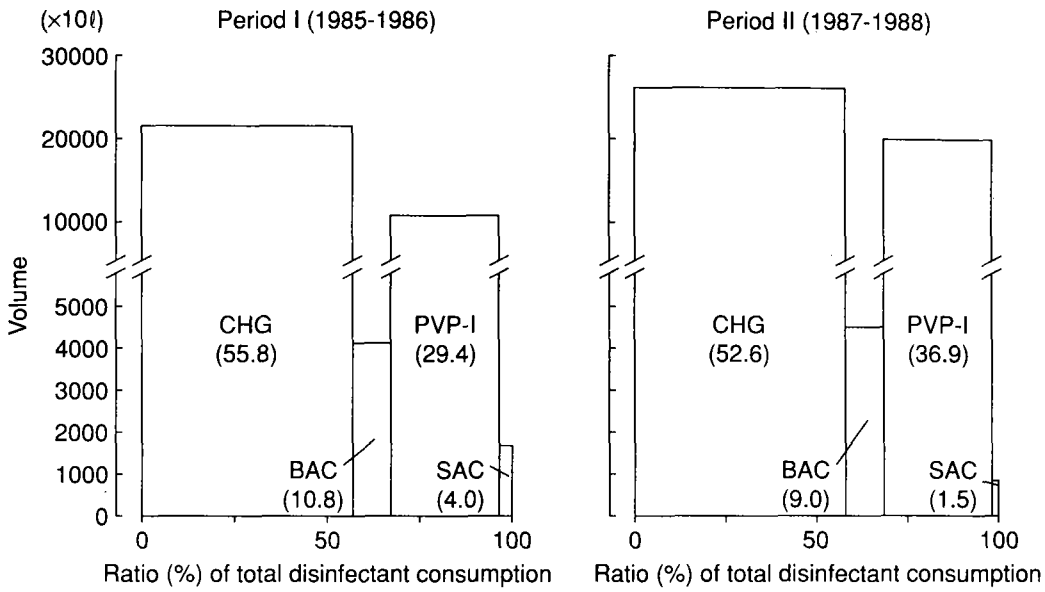

Figure 1 Consumption of disinfectants at Yamagata University Hospital during Period I and Period II (converted to regular concentrations).

The reason for this decline is not clear although the use of antibiotics has been proposed. In the present study a clear reduction in incidence and changes in disinfectant susceptibility for both species were observed. We consider that these changes were attributable to changes in the consumption of frequently used disinfectants.

During the eight months of Period I which corresponded to Period II, 7% of the isolated strains of *Se. marcescens* were resistant to CHG and 13% were resistant to BAC (15% of the total were resistant to both). Also resistant to both CHG and BAC were 70% of the isolates of *Ps. cepacia*. During Period II these strains were completely susceptible to CHG and BAC. All strains tested during both Period I and II were susceptible to PVP-I. We attribute the improvement in susceptibility to the increased use of PVP-I strongly affecting the overall disinfectant susceptibility.

It has been reported that the use of PVP-I decreases the isolation rate of strains of *Pseudomonas*.[10] The difference in susceptibility of clinical isolates between Periods I and II may be attributable more to change in the use of disinfectants rather than the use of antibiotics.

On the other hand, the fact that two species of *Proteus* which were not susceptible to disinfectants were still isolated in Period II indicate that the use of PVP-I did not influence the clinical materials from which these strains were isolated. Compared with the findings obtained in Period I, there was a tendency for a similar degree of disinfectant susceptibility among strains for all of the bacterial species isolated in that period and that the MIC or MBC became lower.[4,5]

Indigenous hospital bacteria

Disinfectant susceptibility

Although the indigenous bacterial flora vary between hospitals and it is impossible to eliminate these indigenous bacteria, the eradication of *Serratia* spp. and *Pseudomonas* spp. is felt to have a beneficial effect on the incidence of opportunistic infection. In the studies described above it was noted that there were strains of *Se. marcescens, Ps. cepacia, Pr. mirabilis* and *Pr. vulgaris* which were resistant to CHG and BAC. We examined the hospital environment in an attempt to isolate the source of these bacteria. We found that the sinks in almost all our hospital wards grew *Se. marcescens, Ps. cepacia* and *Ps. aeruginosa* and that *Se. marcescens* was isolated from physicians fingers.[12] We therefore undertook a definitive study in two phases to examine the incidence and susceptibility to disinfectants of bacteria obtained

Table V Indigenous bacteria isolated from sinks and physicians' hands: Phase I

Species isolated	*No. of strains isolated*	
	Div. of Gastroenterology Dept. of Surgery	*Div. of Gastroenterology Dept. of Internal Medicine*
Sinks		
Serratia marcescens	2	6
Pseudomonas aeruginosa	2	6
Pseudomonas cepacia	2	0
Hands		
Serratia marcescens	0	2
Pseudomonas aeruginosa	0	0
Pseudomonas cepacia	0	0

Table VI Disinfectant susceptibility of indigenous bacteria: Phase I

Strains tested		*MBC (%)*			
		CHG	*BAC*	*SAC*	*PVP-I*
Serratia marcescens					
Standard strain	IFO 3064	0.01	0.1	2.0	0.01
Isolated strains	No. 1	0.01	0.1	2.0	0.01
	2	[1.00]	[1.0]	2.0	0.0001
(Sinks)	3	[1.00]	1.0	2.0	0.001
	4	0.10	1.0	2.0	0.01
	5	[1.00]	[1.0]	2.0	0.01
	6	[1.00]	0.1	2.0	0.01
	7	[1.00]	[1.0]	2.0	0.001
	8	[1.00]	[1.0]	2.0	0.01
(Hands)	11	[1.00]	0.1	2.0	0.01
	12	[1.00]	[1.0]	2.0	0.01

Duration of contact: 30 sec.
Boxes indicate higher than ordinary recommended concentration

from the sinks of the Surgical and Medical divisions of the Department of Gastroenterology and also the fingers of physicians working at both divisions.

During Phase I (April–June 1987) *Se. marcescens* and *Ps. aeruginosa* were isolated from the sinks of both divisions and *Se. marcescens* was isolated from the fingers of physicians working on the medical ward (Table V). The results of disinfectant susceptibility testing of these isolates are shown in Tables VI to VIII. Six out of eight strains of *Se. marcescens* and both of the two strains of *Ps. cepacia* were not killed within 30 seconds by CHG at the ordinary recommended concentration (0.1%). Moreover many of the strains were not susceptible to BAC at its recommended concentration (0.1%). Two strains of *Se. marcescens*, isolated both from the sinks and from the fingers of physicians, were consistent in biochemical properties and it was therefore assumed that these two strains were present at both sites. It is possible therefore that strains of *Se. marcescens* resistant to CHG and BAC at their recommended concentrations were not eradicated by the disinfectant and could have caused hospital infections through ordinary clinical practices.

During Phase II (March–May 1989), strains of *Se. marcescens* and *Ps. cepacia* resistant to disinfectants were not isolated. All strains isolated during this period (Table IX) were susceptible to disinfectants.

Influence of commonly used disinfectants on disinfectant susceptibility

The amount of disinfectants used at the surgical and medical divisions was compared between the Phases I and II (Figure 2). In Phase I, in which strains with resistance to CHG and BAC were isolated, the sum of the amount of CHG and BAC used was similar to the combined consumption of SAC and PVP-I. In contrast, during Phase II when no resistant strains were isolated, the combined use of SAC and PVP-I was about 95% of total disinfectant consumption in the

Table VII Disinfectant susceptibility of indigenous bacteria: Phase I

		MBC (%)			
Strains tested		*CHG*	*BAC*	*SAC*	*PVP-I*
Pseudomonas aeruginosa					
Standard strain	ATCC 9027	0.01	0.01	2.0	0.001
Isolated strains	No. 1	0.01	0.01	2.0	0.01
(Sinks)	2	0.01	0.01	2.0	0.001
	3	0.10	0.01	2.0	0.01
	4	0.01	0.01	2.0	0.01
	5	0.01	0.01	2.0	0.01
	6	0.01	0.01	2.0	0.01
	7	0.01	0.01	2.0	0.01
	8	0.01	0.01	2.0	0.01

Duration of contact: 30 sec.

Table VIII Disinfectant susceptibility of indigenous bacteria: Phase I

		MBC (%)			
Strains tested		*CHG*	*BAC*	*SAC*	*PVP-I*
Pseudomonas cepacia					
Standard strain	M-0257	1.0	0.1	2.0	0.1
Isolated strains	No. 1	1.0	1.0	2.0	0.1
(Sinks)	2	1.0	0.1	2.0	0.1

Duration of contact: 30 sec.
Boxes indicate higher than ordinary recommended concentration.

surgical division and about 66% in the medical division. This study also demonstrated the fact that the most important reason for the non-isolation of bacteria resistant to CHG and BAC during Phase II was the increase in the use of PVP-I and SAC.

Discussion

There have been reports even for PVP-I that a loss of activity is seen in the presence of protein[15,16] and that resistant strains have appeared in some foreign countries.[17,18] Luckily at our hospital, despite the increase in use of PVP-I year by year over the last seven years (Figure 3), no strains with resistance to PVP-I have been isolated although we will continue to monitor the situation. Although not referred to in this review, SAC and PVP-I are highly effective against MRSA and are therefore considered to be very useful for the prevention of hospital infections caused by MRSA.

In clinical isolates we found strains of *Se. marcescens, Ps. cepacia* and *Proteus* which were not killed by CHG and BAC at their recommended concentrations in an appropriate time period. Fur-

Table IX Indigenous bactria isolated from sinks and physicians' hands: Phase II

	No. of strains isolated	
Species isolated	*Div. of Gastroenterology, Dept. of Surgery*	*Div. of Gastroenterology, Dept. of Internal Medicine*
Hands		
Acinetobacter calcoaceticus	4	4
Pseudomonas fluorescens	0	1
Sinks		
Acinetobacter calcoaceticus	4	6

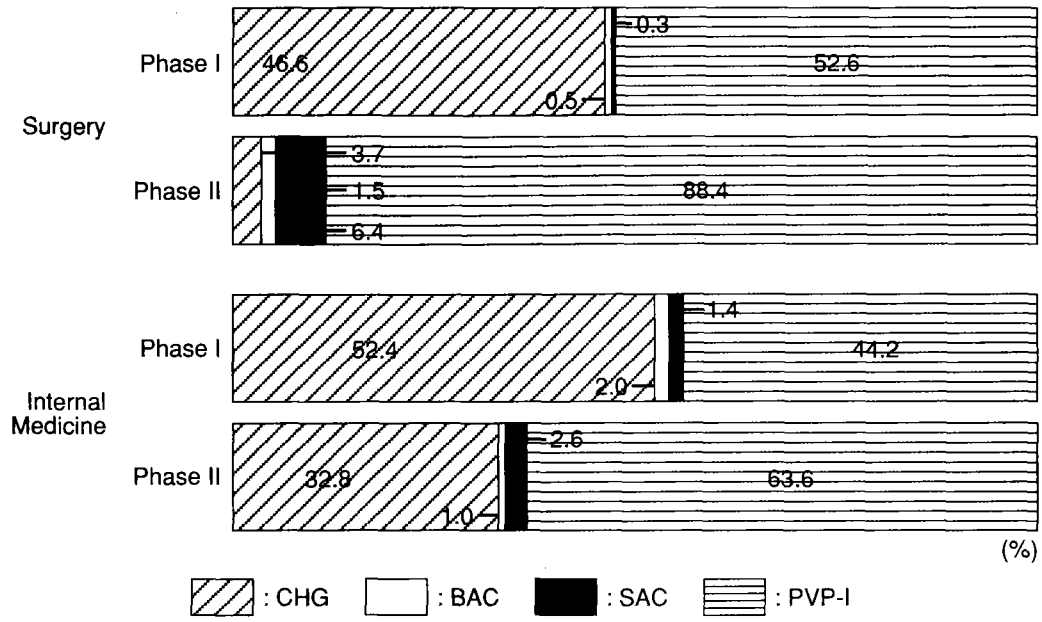

Figure 2 Ratios (%) of disinfectants consumed in the Gastroenterologic Divisions of the Departments of Surgery and Internal Medicine.

thermore, there were strains of *Se. marcescens* and *Ps. cepacia* in the ward sinks which were also resistant to CHG and BAC. Identical strains of *Se. marcescens* to those residing in the sinks were found on physicians' fingers and we believe that hospital infections may have been caused

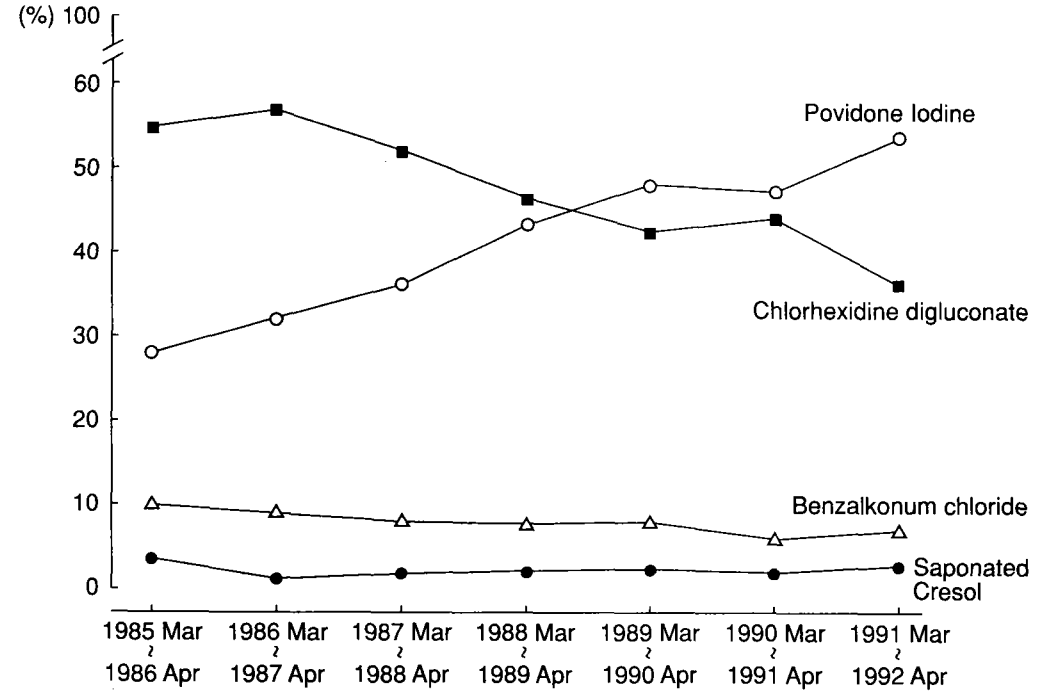

Figure 3 Changes in ratio of disinfectants consumed at Yamagata University Hospital in past 7 years.

by resistant *Se. marcescens* remaining after disinfection. On the other hand, taking into consideration that the decrease in the number of strains resistant to CHG and BAC were seen in association with an increase in the use of SAC and PVP-I, it appears that the use of these latter disinfectants with broad spectrum bactericidal activity[13,14] had great influence on the reduction in incidence of strains resistant to CHG and BAC.

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