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Survival of *Serratia marcescens* in Benzalkonium Chloride and in Multiple-Dose Medication Vials: Relationship to Epidemic Septic Arthritis

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In an epidemic of septic arthritis due to *Serratia marcescens*, the intra-articular injection of contaminated methylprednisolone may have played a key role. The epidemic strain was found in used multiple-dose vials of methylprednisolone and in a canister of cotton balls soaked in benzalkonium chloride. The cotton balls had been used for antisepsis and disinfection. Growth characteristics of the epidemic strain of *S. marcescens* were compared with those of control strains of *S. marcescens* which had been obtained from unrelated nosomial outbreaks. The epidemic strain was able to survive in 1:100 dilutions of benzalkonium chloride and was able to grow to $>10^5$ CFU/ml in multiple-dose vials of methylprednisoline; control strains could not be recovered after 24 h in the same solutions. The preservative in methylprednisolone is γ-myristyl picolinium chloride, a compound chemically related to benzalkonium chloride. We speculate that the epidemic strain of *S. marcescens*, which was resistant to benzalkonium chloride, had cross-resistance to γ-myristyl picolinium chloride. If the cotton balls were used to disinfect the tops of the multiple-dose vials of methylprednisolone, small numbers of organisms subsequently introduced into the solution could have grown to high concentrations.

Multiple-dose parenteral medication vials have been thought to be potential sources of infection in hospitals and other clinical settings. However, few reports have documented actual infections resulting from their use. We investigated an epidemic of 10 cases of *Serratia marcescens* septic arthritis in an office practice. In this practice, the use of a multiple-dose steroid medication, methylprednisolone, for joint injections may have played a key role (8). All case patients had received joint injections with both methylprednisolone and lidocaine. However, only multiple-dose vials of methylprednisolone used for joint injections and a canister of cotton balls soaked in benzalkonium chloride used for antisepsis and disinfection contained *S. marcescens*; multiple-dose vials of lidocaine were culture negative. To further characterize the epidemic strain of *S. marcescens* and to clarify the roles of benzalkonium chloride, methylprednisolone, and lidocaine in the epidemic, we designed a laboratory study, the purpose of which was twofold: (i) to determine the MBC of benzalkonium chloride for the epidemic strain and control strains of *S. marcescens*; and (ii) to compare the growth kinetics of the epidemic strain and six control strains of *S. marcescens* in multiple-dose vials of methylprednisolone and lidocaine.

MATERIALS AND METHODS

Bacterial isolates. *S. marcescens* strains selected for testing included several isolates of the epidemic strain and six control strains obtained from different, unrelated nosocomial outbreaks. All strains had been identified by standard methods.

Growth conditions. Since growth conditions may have been critical to its resistance properties, we studied the epidemic strain under different growth conditions. For *S. marcescens* 1a, a sterile glass canister, filled with 100% cotton fiber balls saturated with full-strength benzalkonium chloride (Zephiran chloride [aqueous solution, 1:750]; Winthrop Laboratories) was allowed to stand for 48 h. This canister was inoculated with benzalkonium chloride squeezed from cotton balls recovered in the office practice and known to be contaminated with the epidemic strain of *S. marcescens*. The canister was allowed to stand at room temperature for several weeks. It served as the environmental reservoir of *S. marcescens* 1a to be used as inocula throughout our studies. For test purposes, an inoculum of *S. marcescens* 1a was prepared by aseptically removing a single cotton ball from the canister and placing it in a sterile garlic press. Approximately 6 ml of contaminated fluid, containing about 10^6 CFU/ml, was expressed from each cotton ball. For *S. marcescens* 1b, 0.3 ml of *S. marcescens* 1a was added to 5 ml of Trypticase soy broth (BBL Microbiology Systems), and the mixture was incubated for 18 h at 25°C. The cell suspension was then concentrated by centrifugation (1,000 × *g* for 20 min), washed twice, and suspended in 0.85% saline to eliminate trace nutrients and to yield a concentration of approximately 10^6 CFU of *S. marcescens* 1b per ml. For *S. marcescens* 1c and 1d, which were two isolates of the epidemic strain from the synovial fluid of two different patients and which had been stored on motility media, we inoculated a single colony of each isolate into 5 ml of Trypticase soy broth and incubated it for 18 h at 25°C. The broth-cell suspension was centrifuged (1,000 × *g* for 20 min), washed twice with 0.85% saline, and suspended in 5 ml of 0.85% saline to yield a final concentration of approximately 10^6 CFU/ml.

For the control strains, six strains, *S. marcescens* 2 to 7, had been obtained during past nosocomial outbreaks and had been stored on motility media. They were prepared by the same procedures as for *S. marcescens* 1c and 1d.

Determination of benzalkonium chloride sensitivity. The following dilutions of stock benzalkonium chloride were prepared: undiluted, 1:10, 1:20, and 1:100. An adsorbed

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TABLE 1. Survival of *S. marcescens* in benzalkonium chloride (1:750)

Isolate	Benzalkonium chloride dilution	Expected bacterium concn[a] at $t=0$ min	Measured concn[a] at time (min):							
			0	5	10	60	360	1,440	2,880	4,320
Epidemic strain										
1a	Undiluted	6.3	4.2	2.7	2.2	–[b]	–	–	ND[c]	ND
	1:10	5.9	5.1	4.8	4.8	4.6	2.0	–	–	ND
	1:20	5.9	4.8	4.7	4.8	4.7	4.6	4.0	2.2	–
	1:100	6.2	5.6	4.7	4.9	4.8	4.8	4.7	4.8	4.6
1b	Undiluted	6.5	4.3	3.0	2.0	1.7	–	–	–	ND
	1:100	6.6	5.4	5.4	4.8	5.1	5.2	5.2	4.9	5.2
Control strain										
2	Undiluted	6.0	–	–	–	–	–	–	–	ND
	1:10	5.8	2.4	–	–	–	–	–	–	ND
	1:20	6.0	3.9	–	–	–	–	–	–	ND
	1:100	6.0	4.7	2.9	–	–	–	–	–	–

[a] Expressed as $\log_{10}$ CFU/ml; values represent the average of two determinations.
[b] –, No growth detected.
[c] ND, Not done.

solution of benzalkonium chloride was also prepared by adding stock solution to sterile cotton balls and storing them for 48 h at 25°C. After storage, these cotton balls were aseptically squeezed to yield adsorbed benzalkonium chloride solution.

Tubes, each containing 2.7 ml of undiluted, 1:10-, 1:20-, and 1:100-diluted benzalkonium chloride, were inoculated with 0.3-ml cell suspensions of *S. marcescens* 1a and 2. *S. marcescens* 1a was also inoculated into adsorbed benzalkonium chloride, and *S. marcescens* 1b was inoculated into undiluted and 1:100-diluted benzalkonium chloride. Inoculated tubes were incubated at 25°C, and 0.1-ml aliquots were taken for subculture at the following time intervals: 0, 5, 10, 60 min (1 h), 360 min (6 h), 1,440 min (24 h), 2,880 min (48 h), and 4,320 min (72 h). Each 0.1-ml aliquot was inoculated into 0.9 ml of brain heart infusion broth. Serial dilutions to 10^{-3} were made for each brain heart infusion broth tube. The surfaces of sheep blood agar plates were inoculated with 0.1 ml of each dilution. The brain heart infusion broth dilution tubes and plates were incubated at 35°C. Plate counts were performed at 24 h; tubes were saved for 3 days and discarded if there was no growth. Each of the above experiments was done in duplicate, and the reported results are the average of duplicate runs.

TABLE 2. Survival of *S. marcescens* in methylprednisolone

Isolate	Expected bacterial concn[a] at $t=0$ h	Measured concn[a] at time (h):				
		0	24	48	72	148
Epidemic strain						
1a	2.1	2.2	3.4	5.9	5.8	6.4
1b	2.7	2.5	3.6	6.7	7.1	6.5
1c	3.3	3.5	3.9	6.3	7.0	5.9
1d	3.4	3.7	4.0	6.4	7.0	6.1
Control strains						
2	2.9	–[b]	–	ND[c]	ND	–
3	3.4	1.5	–	–	ND	–
4	3.1	1.0	–	–	ND	–
5	3.2	–	–	–	ND	–
6	3.2	–	–	–	ND	–
7	3.0	1.7	–	–	ND	–

[a] Expressed as $\log_{10}$ CFU/ml; values represent the average of two determinations.
[b] –, No growth detected.
[c] ND, Not done.

Growth in medications. The two medications to be tested, methylprednisolone (Depo-Medrol; The Upjohn Co., Kalamazoo, Mich.) and lidocaine (Xylocaine 1%; Astra Pharmaceutical Products, Inc.), were obtained as sterile solutions in 10- and 50-ml multiple-dose vials, respectively, and handled as specified by the manufacturers. Using microliter syringes, we inoculated between 10^2 and 10^3 CFU of *S. marcescens* 1a to 1d and the six control strains per ml into vials of methylprednisolone. Similarly, *S. marcescens* la and lb and the six control strains were inoculated into vials of lidocaine. Inoculated multiple-dose vials were covered with sterile aluminum foil and incubated for 168 h (7 days) at 25°C. Aliquots of 0.1 ml were aseptically removed for subculture at the following intervals: 0, 1, 2, 3, and 7 days. Aliquots were handled as previously described for benzalkonium chloride experiments. The medication experiments were done in duplicate, and the reported results are the average of duplicate runs.

RESULTS

The epidemic strain, *S. marcescens* 1a, survived in 1:100 dilutions of benzalkonium chloride after 72 h, but showed gradual die-off at all other dilutions tested (Table 1). *S. marcescens* 1b also survived in 1:100 dilution of benzalkonium chloride. One control strain, *S. marcescens* 2, showed rapid die-off in less than 1 h for all dilutions of benzalkonium chloride (Table 1). At all dilutions, die-off of *S. marcescens* 2 was more rapid than that of *S. marcescens* 1a and 1b. Die-off of the epidemic strain was slower at higher dilutions. Absorbed benzalkonium chloride solution caused die-off of *S. marcescens* 1a at a rate between those in undiluted and 1:10 dilutions of benzalkonium chloride (data not shown). All *S. marcescens* strains tested showed complete die-off in multiple-dose vials of lidocaine in less than 24 h. *S. marcescens* 1a to 1d were not only able to survive, but proliferated in methylprednisolone to between 10^5 and 10^6 CFU/ml (Table 2). In contrast, the six control strains were unable to survive in methylprednisolone and had completed die-off in 24 h (Table 2).

DISCUSSION

The epidemic strain of *S. marcescens* was resistant to low concentrations of benzalkonium chloride under a variety of

antecedent growth conditions. Many previous outbreaks caused by gram-negative organisms with similar resistance to these types of aqueous quaternary ammonium compounds have led to recommendations that aqueous quaternary ammonium compounds should not be used as disinfectants or antiseptics in areas of patient care (2, 3, 7). Such resistance may result from the adaptation of an environmental strain after prolonged exposure to a weak solution of benzalkonium chloride, e.g., in a canister of the benzalkonium chloride-soaked cotton balls. Previous reports have shown that cotton and other materials readily adsorb benzalkonium chloride and lower the active concentration of the antiseptic (6).

The preservative contained in methylprednisolone is γ-myristyl picolinium chloride, a compound chemically related to benzalkonium chloride, i.e., a quaternary ammonium compound. The preservatives in lidocaine are methylparaben and sodium metabisulfite, which are both unrelated to benzalkonium chloride. Since the epidemic strain of *S. marcescens* was able to grow in the methylprednisolone, we speculate that there was cross-resistance of this particular strain of *S. marcescens* to both benzalkonium chloride and γ-myristyl picolinium chloride. Many multiple-dose medications contain such preservatives to retard microbial growth. Previous studies have suggested that these preservatives or the medications themselves may be only partially effective in preventing infections. Therefore, since the risk of contamination by bacteria is unpredictable, several authors recommend that unit-dose vials of medication be used whenever possible (1, 4, 5, 9–11).

On the basis of our results, the epidemic could possibly have been prevented in at least two ways: (i) the use of an antiseptic-disinfectant other than aqueous quaternary ammonium compounds, and (ii) the use of unit-dose medications for joint injections.

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