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Efficacy of the antimicrobial agent triclosan in topical deodorant products: Recent developments *in vivo*

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Synopsis

In vivo studies have been conducted to determine the effects of application of deodorant and antiperspirant products on aerobic axillary microbial populations and to quantify the influence of the antimicrobial agent triclosan on product efficacy.

Antibacterial effects of alcohol and antiperspirant ingredients were augmented by inclusion of triclosan in deodorant compositions and, in the case of a deospray composition containing 0.15% triclosan, improvement in deodorancy was established by olfactory studies.

The axillary microflora, predominently Gram-positive micrococci and coryneform bacteria, showed a sustained reduction during six months' usage of triclosan-containing deodorants, and Gram-negative bacteria, carried in low numbers by 50% of test subjects, were, in general, quickly eliminated. Resistant populations, though, were not established, and no replacement or "overgrowth" with opportunist transients was evident. On discontinuation of deodorant application, bacterial numbers in the axillae returned to pre-usage levels within four to seven days.

INTRODUCTION

Although a variety of materials have been suggested to reduce perception of body and underarm malodor (1), the majority of deodorants (aerosols, pumps, sticks, roll-ons, creams, and soaps) currently marketed incorporate an antimicrobial agent as active ingredient. Such compounds are included to inhibit growth of the microbial populations responsible for sweat degradation and malodor generation. The compound most widely used in this context has been triclosan. Marketed since 1967,[1] triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether) exhibits broad-spectrum antimicrobial activity, including efficacy against bacteria of hygiene and clinical importance (2,3).

Two main approaches are used to study *in vivo* efficacy of topical deodorants: determination of effects on skin microbial populations and olfactory evaluation of skin odors. Both approaches have proved useful in our experience for determination of triclosan efficacy following both short-term (3 days) and long-term (6 months) application of aerosol deodorants. In addition, studies have been conducted to examine the influence of triclosan inclusion on sustained antibacterial efficacy of antiperspirant roll-ons and sticks.

[1] Irgasan® DP 300, Ciba-Geigy.

MATERIALS AND METHODS

DETERMINATION OF EFFECT ON SKIN MICROFLORA

Aerobic bacteria were quantitatively recovered from the middle axillae region of subjects, utilizing the "Thran" pressurized spray gun as previously described by ourselves (4) and other workers (5) and enumerated, via serial dilution or where appropriate, by membrane filtration of sampling fluid, in suitable agar recovery medium, following 2–4 days incubation at 37°C. The axillary microflora were differentiated, where indicated, by use of selective growth media in conjunction with Gram-staining and diagnostic testing of isolated major colony types (API Systems 20E and STAPH).

Total aerobic bacterial counts were determined either on Brain-Heart-Infusion agar (Difco 0418) or Trypticase soy agar (Oxoid CM 131), supplemented with 0.3% lecithin and 1.0% Tween 80 to neutralize residual triclosan antimicrobial activity and also to facilitate growth of lipophilic coryneform bacteria. Coryneforms were selected on the same agar supplemented with 50 mg/l furoxone to suppress micrococci (6,7), Gram-negative bacteria on MacConkey agar (Difco 0075019), and *Pseudomonas* sp. on Pseudosel agar (BBL 11554) containing 300 mg/l cetrimide. Axillae isolates were cultured in Brain-Heart-Infusion broth (Oxoid CM 225), supplemented when necessary with 1% Tween 80 for lipophilic types. All microbiological studies were conducted on male subjects with unshaved axillae.

Axillae population following aerosol spray treatment.

- Aerosol sprays were manufactured containing either 0, 0.05%, or 0.2% triclosan. The sprays, based on ethanol and containing 0.75% isopropyl-myristate as emollient, utilized a 50:50 mix of Freon 11/12 as propellant. After one week's preconditioning period with placebo soap, both axillae of six subjects were washed for 1 min with placebo soap, dried, and deodorant applied by spraying at a distance of 10 cm from the skin for a period of 3 sec. Total aerobic bacterial counts in the axillae were determined before and 1, 4, 7, and 9 h following treatment. Application and sampling were repeated for an additional two days. Following additional conditioning periods with placebo soap, the treatment and sampling regime was repeated with the other two aerosol formulations.

- In a subsequent study the influence of six months *ad lib* usage of triclosan-containing aerosol sprays on the number, composition, and sensitivity of bacteria present in the axillae was determined. Two groups, each of eight subjects, applied a marketed deodorant spray (alcohol-based, containing 0.15% triclosan) or antiperspirant deodorant spray (aluminum chlorhydrate-based, containing 0.25% triclosan) at least once daily to both axillae, with bacteria sampling 4 h after treatment, during a one-week preconditioning period, i.e., using placebo soap only and following 1 day, 2 days, 5 days, 2 weeks, 4 weeks, 12 weeks, and 6 months usage of the deodorants. Sensitivity of axillae isolates to triclosan was examined by the agar incorporation method: Prepared plates of Brain-Heart-Infusion agar incorporating triclosan levels in the range 0.001 mg/l to 200 mg/l were surface-inoculated with one drop of a 48-hour-old broth culture of the isolate, diluted to approx. 10^7 cells/ml in sterile physiological saline. Following incubation of the plates for 2–4 days at 37°C, the minimum inhibitory concentration (MIC) of triclosan for each axillae isolate was determined.

Axillae population following application of antiperspirant deodorants.

- o/w emulsion roll-ons were produced containing 15% active aluminium chlorhydrate, with and without 0.2% triclosan included. After one week's preconditioning period with placebo soap, the axillae of ten subjects were washed, dried, and the roll-on with and without 0.2% triclosan applied to left and right axilla, respectively, for 15 sec per axilla. Total aerobic bacteria counts in the axillae were determined before and 1, 4, 6, and 8 h following treatment. Application and sampling were repeated for a further four days. Following a second conditioning period with placebo soap alone, roll-on application was repeated, again over five days, but with the test products reversed, to eliminate any left-right bias.

- Antiperspirant sticks containing as active ingredient an aluminium-zirconium tetrachlorohydrex-glycine complex, with and without 0.3% triclosan included, were evaluated on twenty male subjects. A seven-day "washout" period using placebo soap alone was followed by a four-day test week. During the test week panelists' axillae were washed according to a controlled regimen, and test sticks with and without 0.3% triclosan were applied once daily to opposite axilla at the rate of 0.4 g/axilla/application. Total aerobic bacteria counts in the axillae were determined before, and 4 h and 24 h after test product application on day 1 and 4 of the test week.

OLFACTORY EVALUATION OF SKIN ODORS

General guidelines for olfactory studies on deodorant efficacy have been defined (8), and such procedures were utilized to evaluate two aerosol spray formulations (Spray A: 0.15% triclosan, 0.75% isopropyl-myristate, 39.10% ethanol, 60.0% Freon 11/12. Spray B: 0.75% isopropyl-myristate, 39.25% ethanol, 60.0% Freon 11/12).

Following a one-week preconditioning period using unperfumed placebo soap, thirty-two subjects, male and female (from an initial panel of fifty volunteers), were selected

Table I
Effect of Deodorant Sprays on Axillae Bacterial Populations

		Mean $\log_{10}$ bacteria/cm^2 skin ± SD		
Day	Hour	Ethanol	Plus 0.05% triclosan	Plus 0.2% triclosan
1	1	2.82 ± 0.54	2.62 ± 0.78	1.83 ± 0.68
	4	3.36 ± 0.22	3.09 ± 0.50	2.07 ± 0.83
	7	3.58 ± 0.26	3.46 ± 0.31	2.40 ± 0.56
	9	4.09 ± 0.08	3.64 ± 0.46	2.55 ± 0.66
2	1	2.77 ± 0.37	2.40 ± 0.36	1.61 ± 0.50
	4	2.98 ± 0.23	2.85 ± 0.53	1.94 ± 0.66
	7	3.59 ± 0.22	3.25 ± 0.39	2.56 ± 0.65
	9	4.09 ± 0.20	3.65 ± 0.25	2.82 ± 0.68
3	1	3.28 ± 0.65	2.90 ± 0.23	1.96 ± 0.29
	4	3.57 ± 0.69	3.26 ± 0.36	2.40 ± 0.46
	7	4.05 ± 0.32	3.53 ± 0.40	2.93 ± 0.56
	9	4.13 ± 0.48	3.83 ± 0.41	3.31 ± 0.57

for the double-blind study on the basis of high axillary odor. The subjects were divided into two subgroups with equivalent odor distribution, and the selected spray applied to the selected axilla, following a standard soap wash, once daily for three days. Odor assessments were carried out independently by four judges, 12 h and 24 h after treatment, using a 10-point scoring system (0 = no objectionable odor, 5 = moderate malodor, 10 = strong disagreeable odor).

RESULTS AND DISCUSSION

Previous studies have demonstrated that the unwashed axilla maintains a fairly constant microbial population over three days, varying from 4.9×10^4 to 5.9×10^5 bacteria/cm^2 skin. When the axillae are washed with soap, the microbial count is initially reduced, but the intermediate counts each day show a build-up, with the axillary population increasing up to the unwashed level by the end of the day (4). The same tendency is evident following three days' application of an ethanol-based aerosol spray, although the initial reduction in microbial count is much greater, presumably due to the intrinsic antibacterial action of the alcohol itself. With 0.2% triclosan included, though, microbial counts are maintained at a reduced level even 9 h after product application (Table I). In olfactory studies, both sprays with and without triclosan gave odor score reductions, but spray A containing 0.15% triclosan was clearly superior, in particular when malodor was assessed 12 h after application (Table II).

In the study of six months' duration and prior to use of the test aerosol sprays, the total aerobic bacteria count was found to be on average $5.2 \times 10^5/cm^2$ skin. Of the sixteen test subjects, 50% normally carried Gram-negative bacteria in the axillae ($5.2 \times 10^1 - 1.0 \times 10^3/cm^2$ skin), in particular *Proteus mirabilis, Enterobacter cloacae,* and *Klebsiella spp.* None of the Gram-negative bacteria isolated throughout the study were oxidase-positive, and no bacteria were recovered on Pseudosel agar. Carriage of *Pseudomonas* spp. in the axillae is therefore not indicated.

From 430 primary isolates characterized during the course of the study, 88% could be identified further into three predominent genera, *Staphylococcus, Micrococcus,* and *Corynebacterium* spp. The majority of *Micrococcus* spp. and *Corynebacterium* spp. isolated were lipophilic, with optimal growth on nutrient media supplemented with Tween 80.

During six months' application of deodorant spray with 0.15% triclosan (average usage 580 ml/subject) or antiperspirant deodorant spray with 0.25% triclosan (average usage

Table II
Olfactory Evaluation of Deodorant Sprays: Axillae Malodor Levels 12 h and 24 h Following Application

Time	Application number	Spray A	Spray B	% Diff.	Sig. level
12 h	1	3.34	3.67	9.1 ± 9.4	0.056
	2	2.72	3.46	21.4 ± 9.6	<0.001
	3	2.72	3.23	15.7 ± 10.3	<0.001
24 h	1	3.47	3.60	3.7 ± 8.3	0.338
	2	3.31	3.66	9.6 ± 7.6	0.021
	3	3.20	3.59	10.9 ± 8.9	0.028

Table III
Effect of Six Months' Deodorant Usage on Axillae Bacteria Recoveries

	Mean bacteria recoveries/cm^2 skin	
Application period	0.15% triclosan deodorant spray	0.25% triclosan antiperspirant/ deodorant spray
Day 5 (pre-)	4.1×10^5	6.3×10^5
Day 1	6.76×10^2	4.75×10^2
Day 2	1.31×10^3	3.43×10^2
Day 5	3.32×10^2	2.93×10^2
Day 15	1.44×10^3	3.01×10^2
Day 29	9.54×10^2	2.94×10^2
Day 85	1.32×10^3	4.29×10^2
Day 180	1.24×10^3	4.81×10^2
$\bar{X}$	1.04×10^3	3.74×10^2
Day 4 (post-)	3.7×10^5	4.6×10^5
Day 7 (post-)	6.4×10^5	8.9×10^5

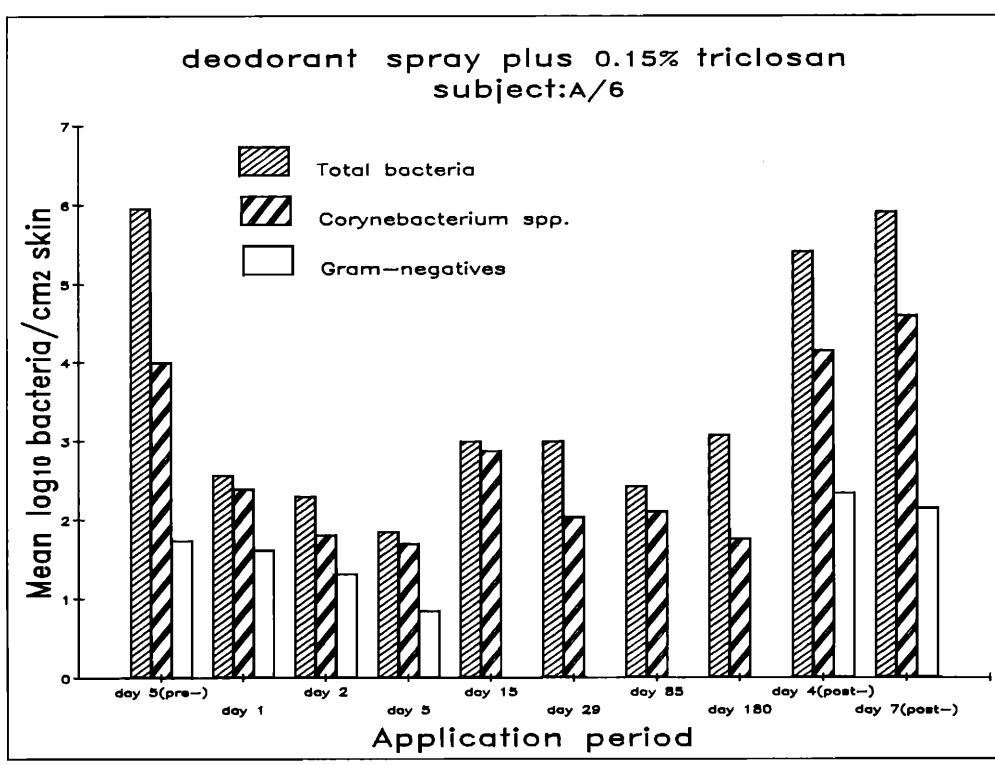

Figure 1. Effect of six months' usage of a triclosan-containing deodorant spray on axillary microflora composition.

Table IV
Sensitivity of Axillae Bacterial Isolates to Triclosan: Minimum Inhibitory Concentrations in mg/l

	Before application		During six months' usage		After discontinuation	
Axillae isolate	(a)	(b)	(a)	(b)	(a)	(b)
Staphylococcus sp.	0.5	0.05–5.0	0.1–1.0	0.1–5.0	0.5–5.0	0.1–1.0
Staph. epidermidis	0.05–5.0	0.05–5.0	0.05–5.0	0.02–5.0	0.1–5.0	0.05–5.0
Corynebacterium sp.	0.05–5.0	2.0–5.0	2.0–5.0	0.1–5.0	5.0	1.0–5.0
Micrococcus sp.	0.1–5.0	0.1–5.0	2.0–5.0	0.1–5.0	1.0–5.0	2.0
Klebsiella sp.	0.5	1.0	0.5	0.5	0.5	0.5
Enterobacter sp.	0.5	—	0.5	—	—	—
Proteus mirabilis	0.5	—	0.2–0.5	—	0.2–0.5	—
Acinetobacter calc.	0.5	—	0.5	—	—	—
Unidentified (Gram-ve)	0.2–0.5	0.05–0.5	—	—	—	—

(a) 0.15% triclosan deodorant spray.
(b) 0.25% triclosan antiperspirant deodorant spray.

630 ml/subject), the aerobic bacterial population of the axillae, assessed four hours after product application, exhibited a sustained reduction, to on average 1.04×10^3 bacteria/cm^2 skin and 3.74×10^2 bacteria/cm^2 skin, respectively. With both test deodorants, when usage was discontinued after six months' application, the total microbial count recovered to its original level within four to seven days (Table III). Findings for a typical subject are illustrated in Figure 1.

With the exception of one subject with persistent carriage of *Proteus mirabilis,* Gram-negative bacteria were quickly eliminated following deodorant application, and in the case of three subjects the Gram-negative population was not reestablished even after discontinuing deodorant application, suggesting that in these cases Gram-negative carriage was only of a transient nature. With six of the sixteen subjects, a simplification of the Gram-positive microflora was evident with deodorant usage, *Micrococcus* spp. and *Corynebacterium* spp. predominating. In no instance, however, were the normal resident bacteria replaced or "overgrown" by opportunist transients. Previous studies on long-term usage of antibacterial soaps confirm that under normal conditions Gram-negative "overgrowth" of the application site does not occur (9,10).

The premise that prolonged use of an antibacterial agent at sublethal concentrations may potentially give rise to a resistant population was investigated during the course of the study by determining *in vitro* sensitivity of axillae isolates to triclosan. Findings for all sixteen subjects and involving 380 bacteria isolates are summarized in Table IV. None of the isolates were found to be resistant to triclosan, with MIC values ranging between 0.02 and 5.0 mg/l.

Under the same testing conditions, laboratory strains of *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 11'299) gave triclosan MIC values of 0.05 and 0.05–1.0 mg/l, respectively.

Both the antiperspirant roll-on and stick compositions exerted a similar and pronounced antibacterial action compared to soap washing alone, reducing the total aerobic bacteria

Table V
Effect of Antiperspirant Deodorant Roll-Ons on Axillae Bacterial Populations

Day	Hour	Mean $\log_{10}$ bacteria/cm^2 skin ± SD		Significance of difference (8 h)
		Aluminum chlorhydrate	Plus 0.2% triclosan	
1	1	2.96 ± 0.49	2.85 ± 0.45	
	4	3.42 ± 0.70	3.04 ± 0.54	
	6	3.49 ± 0.56	3.52 ± 0.63	
	8	3.68 ± 0.57	3.64 ± 0.48	$2\alpha < 0.05$
2	1	2.60 ± 0.50	2.44 ± 0.48	
	4	3.68 ± 0.61	2.94 ± 0.63	
	6	3.52 ± 0.59	3.10 ± 0.72	
	8	4.00 ± 0.52	3.50 ± 0.54	$2\alpha < 0.01$
3	1	2.89 ± 0.59	2.57 ± 0.62	
	4	3.31 ± 0.61	3.34 ± 0.69	
	6	3.66 ± 0.52	3.34 ± 0.64	
	8	4.06 ± 0.55	3.58 ± 0.51	$2\alpha < 0.01$
4	1	3.07 ± 0.70	2.65 ± 0.65	
	4	3.55 ± 0.70	2.93 ± 0.73	
	6	3.76 ± 0.56	3.45 ± 0.56	
	8	4.31 ± 0.58	3.71 ± 0.71	$2\alpha < 0.01$
5	1	2.95 ± 0.61	2.45 ± 0.59	
	4	3.51 ± 0.77	2.92 ± 0.80	
	6	3.87 ± 0.53	3.23 ± 0.49	
	8	4.26 ± 0.69	3.65 ± 0.59	$2\alpha < 0.01$

in the axillae 4 h after application to, on average, $2.57 \times 10^3/\text{cm}^2$ skin and $4.90 \times 10^3/\text{cm}^2$ skin, respectively. Antibacterial efficacy of both the roll-on and stick was significantly enhanced by inclusion of triclosan, particularly following repeated application. In the case of the stick composition, an antibacterial action due to the active antiperspirant ingredient was supported in a parallel study where the base formulation (no aluminum-zirconium tetrachlorohydrex-glycine complex or triclosan included) had

Table VI
Effect of Antiperspirant Deodorant Sticks on Axillae Bacterial Populations

Day	Hour	Mean $\log_{10}$ bacteria/cm^2 skin ± SD			Significance of difference ZAG ≠ ZAG + triclosan
		Base formulation	Plus ZAG[1]	Plus ZAG & 0.3% triclosan	
1	4	5.49 ± 0.52	3.83 ± 0.59	3.18 ± 0.67	$2\alpha < 0.01$
	24	—	4.42 ± 0.60	4.02 ± 0.84	$2\alpha < 0.01$
4	4	5.36 ± 0.32	3.49 ± 0.49	2.75 ± 0.70	$2\alpha < 0.01$
	24	—	3.97 ± 0.55	3.05 ± 0.72	$2\alpha < 0.01$

[1] Aluminum-zirconium tetrachlorohydrex-glycine complex.

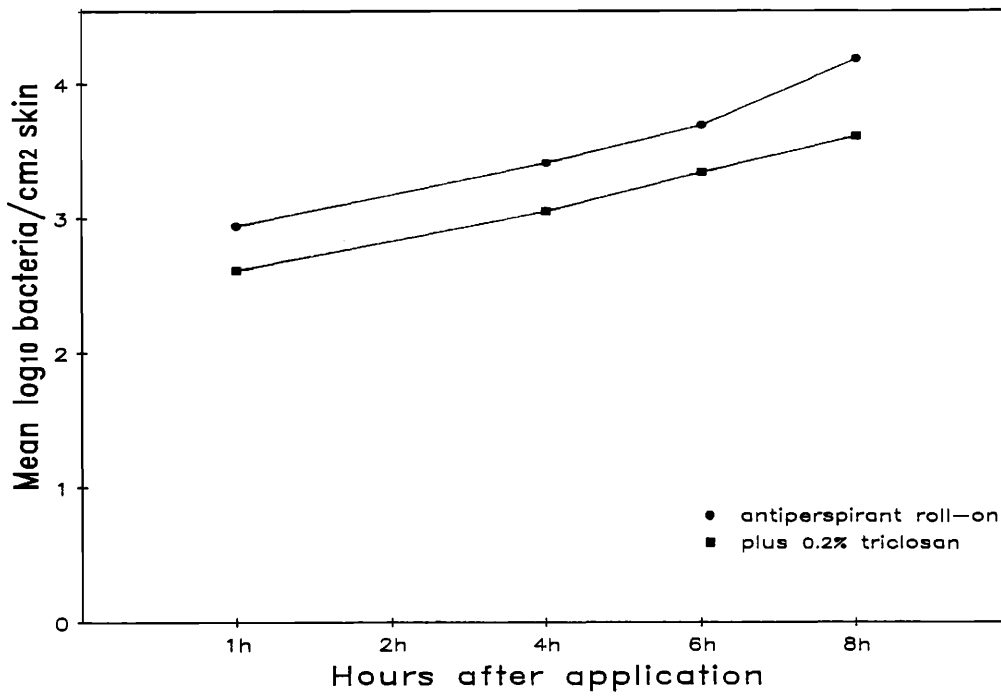

Figure 2. Sustained antibacterial effect of antiperspirant deodorant roll-ons. Mean recoveries of axillae bacteria over 8 h.

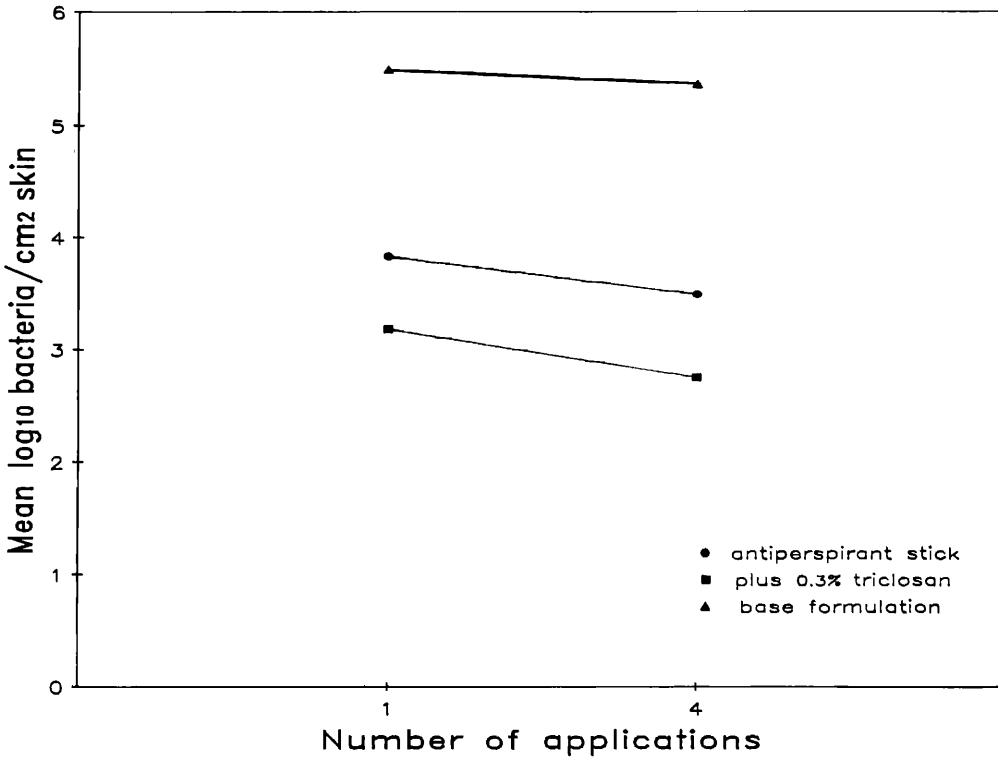

Figure 3. Reduction in axillae bacteria 4 h after application of antiperspirant deodorant sticks.

little or no influence on the axillary microflora. Findings are summarized in Tables V and VI and Figures 2 and 3.

In conclusion, a number of studies conducted in our laboratories have confirmed the *in vivo* antibacterial efficacy of triclosan incorporated in underarm deodorant and antiperspirant deodorant compositions (sprays, roll-ons, and sticks). A subsequent improvement in deodorancy has also been demonstrated from inclusion of triclosan in a deodorant spray composition; further olfactory studies, though, would be desirable, in order to establish similar benefits from usage of triclosan in combination with antiperspirant ingredients.

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