

THE EFFICACY OF A NOVEL QUARTENARY AMMONIUM FOOT SPRAY (NQAFS) AGAINST FOOT ODOR CAUSING MICROORGANISMS

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Abstract

*A newly marketed foot spray, designated as NQAFS, developed to control foot odor was tested for its antimicrobial activity against bacterial and fungal isolates using the disk diffusion technique. Test microorganisms comprised of unidentified bacterial and fungal strains isolated directly from feet and bacterial isolates *Micrococcus luteus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Serratia marcescens*, and fungal isolates *Trichophyton mentagrophytes*, and *Trichophyton rubrum* purchased from PML Microbiologicals. Antimicrobial activity of the novel foot spray was compared to other commercially available foot treatment products containing an array of active ingredients. NQAFS was found to inhibit the growth of all microorganisms tested in this study. Although the degree of inhibition was not as great as the brand products containing clotrimazole or miconazole as the active ingredient, NQAFS can be considered an effective, overall, and affordable antimicrobial product. The inhibitory efficacy of two batches of NQAFS produced three years apart was also examined in this study. Not only did NQAFS prove to be a good broad spectrum antimicrobial, it demonstrated to be actively stable over a three year period.*

1 Introduction

Foot odor is caused by the metabolic activities of normal bacterial inhabitants of feet. The foot provides a warm, moist environment allowing bacteria such as *Micrococcus* sp., *Staphylococcus* sp. (Figure 1), and *Corynebacteria* to thrive. These particular bacteria use long chain fatty acids released by the sweat and oil glands as food sources. Metabolism of these long chain fatty acids results in the production of shorter volatile fatty acids that are responsible for odors associated with feet (Prescott et al., 2002). For many individuals this may be a small annoyance, for others, foot odor can be substantial. More seriously, increased growth of these bacteria can provide environmental conditions conducive to infections by dermatophytic fungi. Dermatophytic fungi have the capacity to invade skin cells of the feet causing a more serious condition commonly referred to as athlete's foot or tinea pedis (Weitzman and Summerbell, 1995). Etiological agents of dermatophytoses include members in the genera *Epidermophyton*, *Microsporum*, and *Trichophyton* (Figure 2). Dermatophytosis can be very difficult to treat and debilitating for immune compromised individuals infected with these microorganisms (Kuzucu et al., 2004).

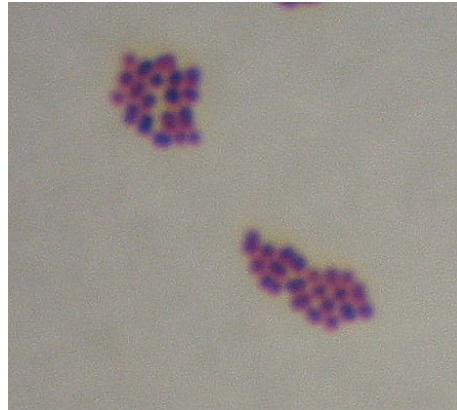


Figure 1: Microgram of a *Staphylococcus* sp

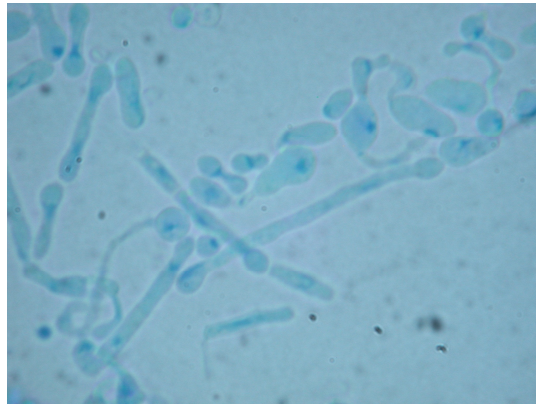


Figure 2: Microgram of *Trichophyton mentagrophytes*

The objective of this study was to determine if a novel shoe spray product NQAFS exhibited antimicrobial activity against representative microorganisms commonly associated with foot odor and foot disease and to compare its effectiveness with other commercially produced over the counter foot spray products. *Staphylococcus* sp. and *Micrococcus* sp. were chosen for this study as they are common bacterial inhabitants of the skin and are often isolated from the foot. These bacteria are most frequently identified as those responsible for causing foot odor. *Serratia* and *Pseudomonas* were chosen for this study as they represent ubiquitous microorganisms. These organisms can be isolated from a variety of environments that include soil, water as well as the human body. While these organisms are not thought to contribute to foot odor, they were chosen for this study as they represent opportunistic microorganisms that have potential to cause disease under appropriate conditions (Tortora et al., 2004).

Trichophyton rubrums and *Trichophyton mentagrophytes* are fungal isolates that may only contribute slightly to foot odor, but *T. rubrums*, *T. mentagrophytes*, and other members of this genus are known to cause a variety of associated skin diseases collectively known as ring worm. Tinea pedis, or athlete's foot, results from an imbalance between normal human microbiota and immune responses. Athlete's foot is characterized by dry and scaly lesions on or between the toes and in many cases is

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accompanied with intense itching. If left untreated, lesions caused by dermatophytic fungi can become colonized by bacteria. Colonization by bacteria may soon result in a serious secondary infection.

Unidentified bacterial and fungal cultures were isolated from feet and included in this study in part for comparison purposes. In many cases, repeated culturing of microorganisms can result in the loss of metabolic capabilities and degree of pathogenesis. This is often the case with commercially purchased cultures. It was of interest to us to ascertain if this novel foot spray product had the same antimicrobial effects on the isolated cultures compared to the commercially purchased cultures.

2 Methods

2.1 Test Isolates

Bacterial test isolates *Micrococcus luteus*, *Staphylococcus epidermidis*, *Serratia marcesens*, and *Pseudomonas aeruginosa* and fungal isolates *Trichophyton mentagrophytes* and *T. rubrums* were purchased from PML Microbiologicals (Wilsonville, OR, USA). Unidentified bacterial and fungal cultures were isolated from the feet of young volunteers. Sterile cotton-tipped applicators were soaked in sterile saline buffer (0.65 % NaCl w/v) prior to swabbing between the toes of the volunteers. These swabs were then used to inoculate prepared nutrient agar for the cultivation of bacterial isolates and potato dextrose agar for the cultivation of fungal isolates. After sufficient incubation at 37°C, bacterial and fungal colonies were randomly chosen from the appropriate agar and streaked for isolation using the streak plate technique. Two unidentified bacterial and two fungal isolates were used for this study.

2.2 Disc Diffusion Tests

Inhibitory effects of NQAFS on the growth of bacterial and fungal isolates were determined using the disc diffusion method as outlined in Tortora et al. (2004). Bacterial cultures were grown in 5 ml nutrient broth for 18-24 h. After the 18-24 h incubation period, 100µl aliquots of culture were aseptically spread over the surface of nutrient agar plates using the spread plate technique. After the bacterial lawn had been prepared, 5 mm filter paper discs saturated with commercial foot odor products were placed on the inoculated agar plates. Zone inhibition diameters resulting from exposure of bacterial isolates to foot odor products were measured using a metric ruler after 18-24 h incubation at 37°C (Figure 3).

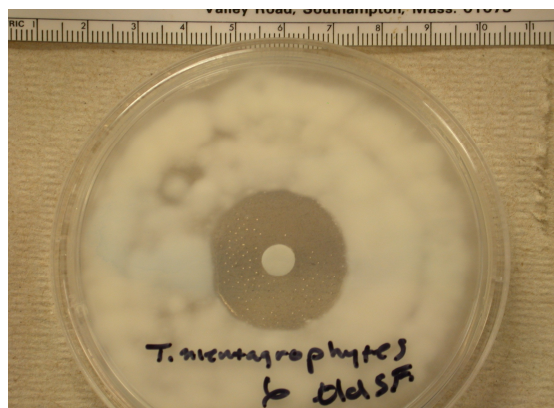


Figure 3: Disk Diffusion test showing zone inhibition

Fungal isolates were grown on potato dextrose agar contained in dilution bottles for approximately two weeks. After sufficient incubation, a fungal spore suspension was obtained by aseptically dispensing 10 ml of sterile saline (0.65 % NaCl) into the bottles and gently removing spores and hyphal fragments from the surface of the plate using a sterile glass rod. A fungal lawn was prepared by aseptically inoculating the surface of potato dextrose agar plates using 100 µl of fungal spore suspension using the spread plate technique. As previously described, filter paper discs saturated with foot spray product were placed on the prepared fungal lawns. Zone inhibition diameters were measured after fungal plates were incubated for 3-5 days at 37°C. All experiments were conducted in triplicate.

3 Results

3.1 Effect of NQAFS on Bacterial and Fungal Isolates

NQAFS was found to have inhibitory effects on all bacterial and fungal isolates tested (Table 1). *Staphylococcus epidermidis* appears to be the most sensitive of the bacterial isolates tested as indicated by the largest zone inhibition diameter (26.0 mm.), while *Serratia marcesens* appears to be the most resistant bacterium (zone inhibition diameter = 7.0 mm). Zone inhibition diameters were generally higher for fungal isolates indicating a slightly higher susceptibility to the novel product (Table 1). Little difference in degree of susceptibility was observed among the fungal isolates.

Table 1: Zone Inhibition Diameters (mm) for NQAFS against bacterial and fungal isolates

Bacterial Isolates	Zone Inhibition Diameter (mm)
<i>Micrococcus luteus</i>	19.3 ± 5
<i>Staphylococcus epidermidis</i>	26.0 ± 8
<i>Serratia marcesens</i>	7.0 ± 0
<i>Pseudomonas aeruginosa</i>	15.0 ± 1
Unidentified Isolate #1	16.0 ± 1
Unidentified Isolate #2	16.3 ± 2
Fungal Isolates	
<i>Trichophyton mentagrophytes</i>	23.5 ± 5
<i>Trichophyton rubrums</i>	25.0 ± 10
Unidentified Isolate #1	22.7 ± 3
Unidentified Isolate #2	9.3 ± 1

3.2 Comparison of Antimicrobial Activity of Different Commercial Foot Spray Products and the NQAFS

3.2.1 Activity Against Bacterial Isolates

Competing commercial foot spray products tested for comparison with NMSF contained different biologically active compounds. The competing foot spray product containing benzalkonium chloride is represented by Brand A, tolnaftate by Brands B, C, and D, alcohols by Brands E and F, clotrimazole by Brand G, miconazole by Brands H and I and an undisclosed active ingredient found in Brand J. Two forms of the commercial products were tested in this study: liquid spray (Brands A, B, E, F, G, H, J and NQAFS) and powder spray (Brands C, D, and I). Zone inhibition diameters for liquid antimicrobial control products are presented in Table 2. Data for powder forms are presented in Table 3.

Table 2: Zone inhibition diameters (mm) for different liquid antifungal control products against bacterial isolates

Antifungal Control Product	<i>M. luteus</i>	<i>S. epidermidis</i>	<i>S. marcesens</i>	<i>P. aeruginosa</i>	Isolate 1	Isolate 2
Brand A (Benzalkonium Chloride 0.13%)	19.6 ± 4	23.7 ± 1.5	4.6 ± 1	19.0 ± 3	18.7 ± 2	14.6 ± 1
Brand B (Tolnaftate, 1.0%)	15.7 ± 4	11.0 ± 0	1.3 ± 2	0.3 ± 1	0.7 ± 1	0.7 ± 1
Brand G (Clotrimazole, 1.0% w/v)	35.0 ± 5	33.3 ± 4.6	1.0 ± 0	25.7 ± 1	38.6 ± 3	26.6 ± 6
Brand H (Miconazole, 2.0%)	38.6 ± 2	35.0 ± 0	NE	23.0 ± 3	32.0 ± 3	10.0 ± 17
Brand F (Isobutanol, SD Alcohol)	NE	14.0 ± 0	10.0 ± 0	NE	7.3 ± 2	NE
Brand E (Methanol)	NE	NE	NE	NE	NE	NE
Brand J	56.6 ± 3	25.3 ± 5	NE	11.0 ± 5	10.0 ± 2	16.6 ± 3

NE = no effect

Table 3: Zone inhibition diameters (mm) for different spray powder products against bacterial isolates

Antifungal Control Product	<i>M. luteus</i>	<i>S. epidermidis</i>	<i>S. marcesens</i>	<i>P. aeruginosa</i>	Isolate 1	Isolate 2
Brand C (Tolnaftate, 1.0%)	NE	NE	NE	NE	NE	NE
Brand D (Tolnaftate, 1.0%)	NE	NE	NE	NE	NE	3.3 ± 6
Brand I (Miconazole nitrate, 2.0%)	21.7 ± 4	14.0 ± 2	NE	18.0 ± 9	21.0 ± 4	19.6 ± 18

NE = no effect

Commercial Brands A, B and G were the only other liquid products, in addition to NQAFS, that appeared to have antimicrobial activity against all bacterial test isolates (Table 2). Zone inhibition diameters for Brand B were generally much smaller when tested against *S. marcesens*, *P. aeruginosa*, Isolate 1, and Isolate 2 suggesting a greater resistance to this product by these organisms. While *Serratia marcesens* was resistant to Brand H, Brand D, and Brand G showed the greatest antibacterial activity against all other isolates as determined by the largest zone inhibition diameters (Table 2). Zone inhibition diameters observed for Brand J were similar to those observed for NQAFS with the exception

that no activity was observed with Brand J against *Serratia Marcesens*. In this study, Brand E had no inhibitory effect on any of the bacterial isolates tested.

Results of the disc diffusion technique testing inhibitory activity of powder foot sprays suggest that these products have less of an effect on the bacterial isolates tested (Table 3). None of the isolates were susceptible to Brand C while unidentified isolate #2 showed some susceptibility to Brand D. Brand I had the greatest inhibitory effect with zone inhibition diameters similar to those observed for NQAFS (Tables 1 and 3).

3.2.2 Activity Against Fungal Isolates

Commercial foot spray products showed generally greater inhibitory effects against the fungal isolates (Table 4 and Table 5) compared to bacterial isolates (Table 1, Table 2, and Table 3) as indicated by larger zones of inhibition. However, unlike NQAFS (Table 1), none of the commercially purchased products inhibited the growth of all fungal isolates tested (Tables 4 and 5).

Table 4: Zone inhibition diameters (mm) for different liquid antifungal control products against fungal isolates

Antifungal Control Product	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	Isolate 1	Isolate 2
Brand A (Benzalkonium Chloride 0.13%)	NE	30.7 ± 6	27.7 ± 3	14.6 ± 1
Brand B (Tolnaftate, 1.0%)	57.3 ± 3	NE	32.7 ± 9	ND
Brand G (Clotrimazole, 1.0% w/v)	NE	41.6 ± 6	51.7 ± 8	ND
Brand H (Miconazole, 2.0%)	45.0 ± 0	NE	25.6 ± 6	40.0 ± 0
Brand F (Isobutanol, SD Alcohol)	NE	NE	NE	ND
Brand E (Methanol)	3.6 ± 6	NE	NE	15.0 ± 13
Brand J	NE	21.7 ± 3	NE	NE

NE = no effect ND = not determined

Table 5: Zone inhibition diameters (mm) for different spray powder products fungal isolates

Antifungal Control Product	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	Isolate 1	Isolate 2
Brand C (Tolnaftate, 1.0%)	15.0 ± 3	45.0 ± 0	13.3 ± 3	NE
Brand D (Tolnaftate, 1.0%)	41.7 ± 8	48.3 ± 10	5.6 ± 5	NE
Brand I (Miconazole nitrate, 2.0%)	40.0 ± 1	NE	15.0 ± 0	31.7 ± 3

NE = no effect

Unlike bacterial isolates, fungal isolates tested appeared to be more sensitive to spray powder products with the growth of at least three of the four fungal isolates tested inhibited by each product (Table 5).

3.3 Comparison of the Antimicrobial Activity of Two NQAFS Manufactured at Different Times

In addition to comparing the antimicrobial activity of NQAFS with that of other commercial products, a study was conducted to assess if age of NQAFS greatly affected inhibitory activities. Zones inhibition diameters were determined for the same bacterial and fungal isolates resulting from the activity of two batches of NQAFS, one produced three years ago and one produced within the year of this study (Table 6). Zone inhibition diameters are similar for both batches of NQAFS suggesting that NQAFS has an extended shelf life of at least three years.

Table 6: Comparison of the zone inhibition diameters (mm) for two different batches of No More Stinky Feet against bacterial and fungal isolates

	<i>M. luteus</i>	<i>S. epidermidis</i>	<i>S. marcesens</i>	<i>P. aeruginosa</i>	Isolate 1	Isolate 2
New NQAFS	37.5 ± 6	32.5 ± 6	10.0 ± 0	31.8 ± 7	33.0 ± 13	32.5 ± 6
Old NQAFS	31.3 ± 9	26.0 ± 8	7.0 ± 0	29.5 ± 11	25.5 ± 7	37.5 ± 3
	<i>T. mentagrophytes</i>		<i>T. rubrum</i>	Isolate 1	Isolate 2	
New NQAFS	23.3 ± 6		26.7 ± 3	18.7 ± 2	10.3 ± 1	
Old NQAFS	28.3 ± 3		28.3 ± 3	26.7 ± 3	9.3 ± 1	

4 Discussion and Conclusions

This study compares the antimicrobial activity of a novel quaternary ammonium foot spray product (NQAFS) with antimicrobial activities of commercially available foot products advertised to control foot odor (Brands D, E, F, and J) and those advertised as treatments for tinea pedis (Brands A, B, C, G, H, and I). These products contain an array of chemical compounds listed as their active ingredient. Active ingredients include alcohols, benzalkonium chloride, tolnaftate, clotrimazole, and miconazole. NQAFS is the only product in this study claiming a quaternary ammonium compound as the active ingredient.

Generally, liquid spray products had the greatest antimicrobial activity compared to powder spray products. This was not necessarily a surprising result as the efficacy of the disc diffusion test used to determine antimicrobial activity is dependent on the ability of product to diffuse into the agar. Regardless of the type of product, those with alcohol listed as the active ingredient were found to have very little or no antimicrobial activity. Of those products, Brand E was the weakest performer. One of the most widely used treatments for tinea pedis is tolnaftate (Weitzman and Summerbell, 1995). It is found to be an effective treatment for fungal agents responsible for tinea pedis, but in this study products containing tolnaftate performed only marginally better than the alcohol based products when tested against bacterial isolates. Of all the products tested, those exhibiting the greatest antimicrobial activity as indicated by the large zone inhibition diameters were those containing clotrimazole or miconazole as their active ingredient. These products were effective against both bacterial and fungal isolates with the greatest activity observed against fungal isolates. Brand J is a main competitor of NQAFS. The active ingredient is not known for this product. Generally, this product performed similarly to NQAFS when tested against bacterial isolates but had limited activity against the fungal isolates.

NQAFS was the only product that exhibited activity against all bacterial and fungal isolates tested with activity remaining high over a three-year period. Presently, there is very little data available on the inhibitory effects of quaternary ammonium compounds (Kampf and Kramer, 2004), but this study shows that NQAFS had the broadest range of antimicrobial activity showing potential for an effective foot odor and foot disease control product. While the degree of inhibition was not as great as for those brand products containing either clotrimazole or miconazole, according to this study, NQAFS can be considered an effective, overall, and affordable antimicrobial product.

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