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INVESTIGATION OF ANTIBIOTIC AND ANTIBACTERIAL SUSCEPTIBILITY AND RESISTANCE IN *STAPHYLOCOCCUS* FROM THE SKIN OF USERS AND NON-USERS OF ANTIBACTERIAL WASH PRODUCTS IN HOME ENVIRONMENTS

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Abstract- Background: Amidst continued calls for more research investigating the relationship between the use of antibacterial wash products and antibiotic and antibacterial resistance in human-source bacteria, this study aimed to describe susceptibilities in staphylococcal skin isolates from community users of antibacterial wash products, compared to isolates from non-users.

Methods: Qualified and randomly selected participants (n=210) comprised three equal groups: 1) those that frequently used wash products containing triclosan; 2) those that frequently used products containing triclocarban; 3) control group that used no antibacterial wash products. A forearm swab sample was collected from each participant and processed for coagulase-negative *Staphylococcus* species and *S. aureus* (SA). Standard antibiotic and antibacterial minimal inhibitory concentration (MIC) testing was performed on all isolates (n=317).

Results: There was no statistically significant difference in antibiotic resistance in *Staphylococcus* isolates from regular antibacterial wash product users compared with non-users. None of the isolates were resistant to vancomycin, and the rate of methicillin resistant *S. aureus* (MRSA) detected was appreciably less than that reported in the literature for both hospital inpatient and outpatient isolates of SA. There was also a definitive lack of antibiotic/antibacterial cross-resistance.

Conclusion: An extensive community study of resident skin *Staphylococcus* showed no increased antibiotic resistance in participant groups regularly using wash products containing triclocarban (TCC) or triclosan (TCS), as compared with participants using wash products containing no TCC or TCS. This adds to and confirms previous yet limited community data showing lack of evidence that the use of antibacterial wash products facilitates antibiotic resistance and antibiotic/antibacterial cross-resistance.

Keywords: Antibiotic, antibacterial, susceptibility, resistance, *Staphylococcus*, skin, products, home

Introduction

As improved personal hygiene practices in the community continue to be advocated in light of infectious disease outbreaks of great concern, antibacterial wash products continue to come under scrutiny as potential contributors to increasing antibiotic resistance in pathogenic bacteria affecting humans. Published data have indicated that triclosan, one of the most widely used topical antibacterial agents, functions intracellularly as a site-directed enzyme inhibitor much like an antibiotic [1]. Although there are similarities in the mechanisms of antibacterial wash product actives and antibiotics, there has been very limited evidence that there is a correlation between antibiotic and antibacterial agent resistance in community bacteria associated with humans [2-4]. In this regard, there has been a call for more research [2, 5, 6]. Thus, the purpose of this study was to generate additional community-based interpretive data by investigation of: 1) a potential relationship between antibiotic resistance in normal human skin flora and the regular use of antibacterial wash products containing the antibacterial agents, triclosan (TCS) and triclocarban (TCC); and 2) antibiotic/antibacterial cross-resistance - i.e. whether highly anitibiotic-resistant isolates also exhibited decreased susceptibility to one or both of the antibacterial agents, and vice-versa.

Methods

Study design

The investigation was designed as a controlled trial where qualified and randomly selected participants in each of three study groups underwent swabbing of forearm skin for the collection and subsequent isolation, identification, and antibiotic and antibacterial susceptibility testing of target *Staphylococcus* species. Comparison of susceptibility profiles across study groups would then characterize the relationship between the use of antibacterial wash products and antibiotic and antibacterial agent resistance.

Target organisms

Staphylococcus aureus (SA) and coagulase-negative *Staphylococcus* (CNS) species were selected as the target indicator bacteria because of their recognition as common bioburden component of normal skin flora, and their potential to develop antibiotic resistance and act as human pathogens.

Participants

There were a total of 210 randomly selected, qualified adult subjects (male and female) comprising three study groups:

- Participants (n=70) that frequently used liquid bath and/or shower products containing triclosan (TCS);
- Participants (n=70) that frequently used bar soaps containing trichlorocarbanalide (TCC);
- Participants (n=70) that did not use any antibacterial bath and/or shower products or bar soaps, and served as the control group.

Up to two participants per home were utilized, with composite sampling for skin bacteria on both forearms from each participant, utilizing a pre-validated method. Antibacterial "users" were defined as those who used products (either TCC or TCS based) on a regular basis during the last thirty days or longer for body washing, including the forearm.

The study protocol and its methods were reviewed and approved as consistent with all relevant federal guidelines and institutional policies by an independent Institutional Review Board (IRB), and consisted of all details pertaining to: 1) participant recruitment, enrollment (to include solicitation materials) informed consent, and confidentiality; 2) the home assessment survey and product inventory; 3) clinical sample collection; 4) sample processing and isolation and confirmatory identification of CNS and SA isolates; 5) standard antibiotic susceptibility testing; 6) standard antibacterial agent susceptibility testing; and 7) data reporting.

Potential participants were determined to be qualified for the study based on the following exclusion criteria:

- Antibiotic therapy within the last 90 days
- The use of topical skin medications, medicated shampoos, anti-acne products
- Employment in a health care, day-care, or animal care facility
- Frequent swimmer or hot tub user
- Have pets other than fish
- Have routine exposure to solvents

Qualified participants were sorted into "pools" based on their grouping (Non-User, TCC-User, and TCS-User). From a pool of over 450 pre-qualified individuals, 70 participants from each group were randomly selected for home visits and sample collection.

Study personnel visited participants to administer a home assessment survey and collect bacterial samples from skin. The visit confirmed study group qualification and assignment of a participant to a user or non-user group through a brief questionnaire and inventory and examination of all personal hygiene and environmental cleaning, sanitizing, and disinfecting products. Following qualification confirmation and informed consent, swab samples from the designated skin areas were collected.

Skin sampling and sample processing

Using a previously verified method, one composite sample was collected from each participant in each study group using a sterile 4 x 16 cm (64 cm^2) template and a Stuart's modified medium-filled plastic transport tube containing a single rayon swab (CultureSwab® Transport System). The template was placed on the forearm of the participant and eight wipes with the swab were made up and back along the length of the forearm, along with 32 wipes back and forth along the width of the forearm. An individual sample was comprised of the combined sampled areas from both forearms.

All samples were processed within 24 to 48 hours. Each sample was eluted in 1.0 ml sterile FTAb (phosphate buffered saline with 0.1% Tween 80), vortex-mixed for 60 seconds, followed by 10-fold serial dilution. A 0.1 ml aliquot was inoculated onto duplicate plates of trypticase soy agar with 5% sheep blood, spread for isolation until dry, and incubated for 18-24 hours at 37°C. Plates were then examined for isolated colonies, and target bacteria presumptively identified according to standard criteria of morphology, pigmentation, texture, hemolysis, and other distinguishing characteristics. One representative of each colony type from each sample was selected for identification and confirmation as a target organism. In addition to colony morphology, pigmentation, texture, and hemolysis, preliminary identification of organisms was based on gram stain reaction, catalase, and coagulase results. Additional identification confirmations were subsequently provided by LabCorp (Laboratory Corporation of America, Burlington, NC) as part of their standardized antibiotic susceptibility testing.

Antibiotic susceptibility testing

Antibiotic susceptibility testing using standard panels was conducted on all 317 *Staphylococcus* isolates by LabCorp. Isolates were tested using the MicroScan automated procedure (Dade MicroScan, Inc., West Sacramento, CA) following the testing and quality assurance practices outlined in the M7-A4 NCCLS document [7]. Test panels consisted of Ampicillin, Ciprofloxacin, Clindamycin, Erythromycin, Nitrofurantoin, Oxacillin, Penicillin G, Tetracycline, Trimethoprim-Sulfamethoxazole, and Vancomycin, which included those antibiotics currently recognized as drugs of choice (primary and secondary) by the medical community for treatment of *Staphylococcus* infections. Interpretation of results was based on the M100-S9 NCCLS document [8].

Antibacterial susceptibility testing

Antibacterial MIC testing of all 317 isolates from user and non-user groups, was conducted on both antibiotic susceptible and resistant strains, against two of the most common antibacterial agents found in bar soaps and body washes: 2,4,4'-trichloro-2'-hydroxy-diphenyl-ether (triclosan) and 3,4,4'-Trichlorocarbanilide (triclocarban). Testing was conducted using industrial preparations of TCC and TCS, and a standard broth micro-dilution method [9], and derived from the NCCLS Method M7-A4 [7]. Prior to testing the isolates, the micro-dilution method was validated using selected isolates. Control organisms with confirmed triclosan MIC value (i.e., *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 11229) were included in the test profile to verify that appropriate dilutions were utilized, as triclosan has an MIC of less than 1 PPM against those two species. Testing also included a fully TCS-susceptible strain of CN *Staphylococus* species. Initial concentrations of the active ingredients were formulated such that a typical use-dilution concentration was achieved near the middle of the dilution scheme. Positive and negative controls were run simultaneously with all test isolates.

Data analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) software for personal computers release 14.0. Conventional frequency tables, t-tests, and chi-square distributions were employed to analyze. The level of significance was set at 0.05.

Results

Antibiotic resistance to standard test panels

Of critical significance in regard to antibiotic susceptibility is the fact that none of the 317 isolates from the user and non-user groups exhibited full or intermediate resistance to vancomycin; and also that rates of resistance to oxacillin, as a measure of methicillin resistance across all isolates, was shown to be appreciably less than rates reported in the literature for both hospital-acquired and community-acquired staphylococcal infections [10-12].

Test results generated by MIC testing showed a rate for methicillin resistant *Staphylococcus aureus* (MRSA) of 12.5% (2/16) for all study isolates. This compares to a rate of 22.6% for MRSA isolates from clinical outpatient samples of all types from 23 US hospitals for the 1998-1999 period [11]; a rate of 20.2% for MRSA isolates from 50,759 blood samples from hospitals in Europe from 1999-2002 [12]; and a rate of 50% for MRSA isolates from all clinical inpatient and outpatient samples from 1999-2004 from a large university medical center [10]. In the present study, the non-user group showed an MRSA rate of 25% (1/4), while the pooled TCC/TCS groups showed an 8.3% (1/12) rate. It must be kept in mind however, that the number of SA isolates (n = 16) is too small for meaningful statistical analysis.

For CNS, the methicillin resistance rate was 20.6% (62/301) across all isolates, as compared to 43.6% for all outpatient isolates from 23 US hospitals from 1998-1999 [10], and 73.3% from all clinical inpatient and outpatient samples of all types from 1999-2004 from a large university medical center [10]. Across participant groups, CNS methicillin resistance rates were 17.9% (19/106) for the Non-Users (NU), 23.5% (24/102) for the TCC users, and 20.4% (19/93) for the TCS users. As shown in Tables 1 and 2, these differences were not significant.

Table 1 presents the distribution of resistance across all 10 drugs for CNS isolates from the NU group (n = 106) versus the TCC group (n = 102) and the TCS group (n = 93), and shows comparable resistance rates for the NU group versus the TCC and TCS groups. And when both TCC and TCS group resistance data for CNS are pooled (n = 195) and compared with the NU group (n = 106), as shown in Table 2, there are no statistically significant differences across the drugs tested, with the exception of tetracycline, which showed a greater resistance in the NU group (17.0%) than the pooled TCC and TCS groups (9.7%).

Comparative distribution of resistance across all 10 drugs for SA isolates from the NU group (n = 4) versus the pooled TCC (n = 7) and TCS (n = 5) groups, as shown in Table 3, showed no significant differences across the drugs tested, with the exception of ampicillin, which showed greater resistance in the NU group (100%) than the pooled user groups (33.3%).

Antibiotic resistance to preferred drugs

Of the 10 antibiotics included in the standard test panel, 6 are considered preferred treatment drugs for *Staphylococcus* infections – ciprofloxacin (CIP), clindamycin (CLD), oxacillin (OX), tetracycline (TET), trimethoprin/sulfamethoxasole (TMP/SMX), and vancomycin (VAN). Table 4 shows comparable antibiotic resistance among the non-user and user groups for CNS for both resistance to one or more preferred drugs and two or more preferred drugs. No significant resistance was shown to one or more preferred treatment drugs for 4 SA isolates from the TCC and TCS groups over those in the NU group – recognizing again that the number of SA isolates is very small. Results were similar for 2 SA isolates resistant to 2 or more of the preferred drugs – again recognizing that 2 SA isolates remain too few for meaningful interpretation.

Antibacterial resistance

All *Staphylococcus* isolates from all participant groups (n = 317) were tested for their resistance to triclocarban and triclosan using a standardized micro-broth dilution method. Results are presented in Table 5 as ranges of antibacterial MIC values from lowest (least resistance) to highest (most resistance) for each participant group and each antibacterial agent. CNS isolates (n = 301) for all three participant groups showed comparable MIC values when tested against TCC; while the NU group showed a narrower range of resistance (i.e. less susceptibility) than exhibited by the TCC or TCS groups when tested against TCS. For all SA isolates (n = 16) antibacterial MIC ranges of all participant groups were comparable when tested against TCC and TCS, with the exception that TCS isolates had a slightly narrower range of resistance (i.e. less susceptibility) when tested against TCS.

Cross-resistance

The data were evaluated for cross-resistance – i.e. whether highly antibiotic-resistant isolates also exhibited decreased susceptibility to one or both of the antibacterial agents, and vice-versa. The antibacterial MIC values for 9 CNS isolates (NU = 3; TCC = 2; TCS = 4) most resistant to preferred treatment drugs (4-5), when tested against TCC were comparable among the 3 participant groups, and none exhibited the highest MIC values, as did other less antibiotic-resistant isolates. Likewise, antibacterial MIC values for isolates tested against TCS were comparable among the participant groups, and with one exception, none exhibited the highest MIC value that other less antibiotic-resistant isolates did.

Conversely, we looked at isolates of CNS and SA with the highest antibacterial MIC values (most resistant) and their resistance to numbers of preferred treatment drugs. Results showed that resistance to number of preferred treatment drugs for 7 CNS isolates (NU = 1; TCC = 4; TCS = 2) with the highest TCC MIC value (0.75) for the 3 participant groups was comparable, with resistance to 0, 1, or 2 preferred drugs, as opposed to resistance to 4-5 drugs exhibited by less TCC resistant isolates. Similarly, resistance to number of preferred treatment drugs for 60 CNS isolates with the highest TCS MIC value (2.02) for the 3 participant groups were comparable, as NU isolates (n = 22) were resistant to 0-3 preferred drugs, TCC isolates (n = 19) were resistant to 0-2 drugs, and TCS isolates (n = 19), with one exception, resistant to 0-3 drugs. The exception was one isolate resistant to 4 preferred drugs, which is not significant.

For SA, there were no isolates demonstrating the highest possible MIC value for TCC. There were however, 3 isolates exhibiting the highest MIC value for TCS, and none of them were shown to be resistant to any of the preferred treatment drugs.

Discussion

This community study of resident skin *Staphylococcus* showed no statistically significant increase in antibiotic resistance in participant groups regularly using wash products containing triclocarban (TCC) or triclosan (TCS), as compared with participants using wash products containing no TCC or TCS. Additionally, none of the 317 study isolates were found to be resistant to vancomycin, and the rate of methicillin resistant *S. aureus* (MRSA) detected was appreciably less than that reported in the literature for both hospital inpatient and outpatient isolates of SA. Also, the study data showed a definitive lack of antibiotic/antibacterial cross-resistance when the most resistant staphylococci in each category were comparatively assessed across the three participant groups. These study results confirm similar findings from previous assessments of antibiotic and antibacterial resistance in home environments [2, 3] and further discount claims that the use of antibacterial wash products have contributed to the selection and propagation of drug-resistant bacteria on human skin in the community to this time.

Conclusion

Based on current knowledge and a continued public health emphasis on reducing the risk of transmission of communicable disease agents, the use of topical antimicrobial wash products in combination with recommended personal hygiene practices appears to present a low risk for the development of antibiotic and/or antibacterial resistance in human skin flora.

Abbreviations

CIP Ciprofloxacin
CLD Clindamycin
CNS Coagulase-negative *Staphylococcus*
FTab Phosphate buffered saline with 0.1% Tween 80
IRB Institutional Review Board
MIC Minimal inhibitory concentration testing
MRSA Methicillin resistant *S. aureus*
NCCLS National Committee for Clinical Laboratory Standards
NU Non-user group; those who use neither trichlorocarban nor triclosan
OX Oxacilln
SA *Staphylococcus aureus* or *S. aureus*
SPSS Statistical Package for Social Sciences software
TCC Triclocarban
TCS Triclosan
TET Tetracycline
TMP/SMX Trimethoprim/sulfamethoxazole
VAN Vancomycin

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Table 1- Comparative distribution of antibiotic resistance data for CNS isolates from non-user, TCC users, and TCS user groups

Drug	Non-users (n=106)	TCC-users (n=102)	TCS-users (n=93)
AMP	30.2%	35.3%	36.6%
CIP	5.7%	3.9%	8.6%
CLD	5.7%	3.9%	8.6%
ERY	43.4%	39.2%	35.5%
NF	2.8%	1.0%	1.1%
OX	17.9%	23.5%	20.4%
PEN	50.9%	53.9%	53.8%
TET	17.0%	10.8%	8.6%
TMP/SMX	3.8%	7.8%	8.6%
VAN	0.0%	0.0%	0.0%
1+Preferred[a]	53.8%	49.5%	50.0%
≥2Preferred[b]	25.5%	18.3%	24.5%

[a]1+Preferred means resistant to one or more preferred drugs
[b]≥2Preferred means resistant to two or more preferred drugs

Table 2- Comparative distribution of antibiotic susceptibility/resistance data for CNS isolates from non-user and pooled TCC and TCS user groups

Drug	Non-users (n=106)		Users (n=195)		p-value[a]
	Susceptible	Resistant	Susceptible	Resistant	
AMP	69.8%	30.2%	64.1%	35.9%	0.192
CIP	94.3%[b]	5.7%	93.8%	6.2%	0.541
CLD	94.3%[b]	5.7%	93.8%[b]	6.2%	0.541
ERY	56.6%[b]	43.4%	62.6%[b]	37.4%	0.187
NF	97.2%	2.8%	99.0%[b]	1.0%	0.237
OX	82.1%	17.9%	77.9%	22.1%	0.245
PEN	49.1%	50.9%	46.2%	53.8%	0.359
TET	83.0%	17.0%	90.3%[b]	9.7%	0.052
TMP/SMX	96.2%	3.8%	91.8%	8.2%	0.106
VAN	100.0%	0.0%	100.0%	0.0%	
[c]1+Preferred	46.2%	53.8%	50.3%	49.7%	0.292
[d]≥2Preferred	74.5%	25.5%	78.5%	21.5%	0.262

[a]Based on Chi-square; [b]Contains 1-5 isolates with intermediate resistance
[c]1+Preferred means resistant to one or more preferred drugs; [d]≥2Preferred means resistant to two or more preferred drugs

Table 3- Comparative distribution of antibiotic susceptibility/resistance data for SA isolates from non-user and pooled TCC and TCS user groups

Drug	Non-users (n=4)		Users (n=12)		p-value[a]
	Susceptible	Resistant	Susceptible	Resistant	
AMP	0.0%	100.0%	66.7%	33.3%	0.038
CIP	75.0%	25.0%	100.0%	0.0%	0.250
CLD	100.0%	0.0%	83.3%[b]	16.7%	0.550
ERY	25.0%	75.0%	83.3%	16.7%	0.063
NF	100.0%	0.0%	91.7%	8.3%	0.750
OX	75.0%	25.0%	91.7%	8.3%	0.450
PEN	0.0%	100.0%	58.3%	41.7%	0.069
TET	100.0%	0.0%	100.0%	0.0%	
TMP/SMX	100.0%	0.0%	91.7%	8.3%	0.750
VAN	100.0%	0.0%	100.0%	0.0%	
[c]1+Preferred	75.0%	25.0%	75.0%	25.0%	0.728
[d]≥2Preferred	75.0%	25.0%	91.7%	8.3%	0.450

[a]Based on Chi-square; [b]Contains one isolate with intermediate resistance
[c]+Preferred means resistant to one or more preferred drugs
[d]≥2Preferred means resistant to two or more preferred drugs

Table 4- Distribution of all isolates according to resistance to 1 or more preferred drugs, 2 or more drugs, and usage of antibacterials

Category	**Resistant to 1 or more drugs**	**p-value***	**Resistant to 2 or more drugs**	**p-value[a]**
CNS Isolates				
All CNS (n=301)	51.2%	0.687	22.9%	<.0001
Non-users (n=106)	53.8%	0.437	25.5%	<.0001
TCC users (n=102)	50.0%	1.000	24.5%	<.0001
TCS users (n=93)	49.5%	0.917	18.3%	<.0001
SA Isolates				
All SA (n=16)	25.0%	0.046	12.5%	0.003
Non-users (n=4)	25.0%	0.317	25.0%	0.317
TCC users (n=7)	14.3%	0.059	0.0%	
TCS users (n=5)	40.0%	0.655	20.0%	0.180

[a]Based on Chi-square distribution of resistant and susceptible isolates

Table 5- Antibacterial MIC ranges for all isolates tested for susceptibility to TCC and TCS

User Group	**TCC**	**TCS**
CNS		
Non-users (n=106)	0.0117-0.750	0.120-2.020
TCC-users (n=102)	0.0234-0.750	0.004-2.020
TCS-users (n=98)	0.0117-0.750	0.008-2.020
SA		
Non-users (n=4)	0.0469-0.1875	0.510-2.020
TCC-users (n=7)	0.0029-0.1875	0.120-1.010
TCS-users (n=5)	0.0469-0.1875	1.010-2.020