Investigation of Antibiotic and Antibacterial Resistance in *Staphylococcus* from the Skin of Users and Non-Users of Antibacterial Wash Products in Home Environments*

Cole EC[1], Addison RM[2], Dulaney PD[3], Leese KE[4]

[1]Brigham Young University, Provo, UT; [2]Duke University Medical Center, Durham, NC; [3]Applied Environmental, Inc., Cary NC; [4]Restoration Sciences, Cary, NC

ABSTRACT

Background. Antibacterial wash products have come under scrutiny as potential contributors to the problem of antibiotic resistance. This study investigated the extent of, and relationship between, antibiotic and antibacterial resistance in human skin bacteria isolated from individuals in the home environment, relative to their routine use or non-use of antibacterial hand and bath soaps, and other antibacterial body wash products.

Methods. Qualified study participants (n=210) were randomly selected from qualified applicant pools and comprised three groups of 70 each: 1) those that routinely used wash products containing triclosan (TCS); 2) those that frequently used products containing triclocarban (TCC); and 3) a control group that used no antibacterial wash products. A 64 cm^2 composite swab sample was collected from each participant's forearm skin and processed for coagulase-negative *Staphylococcus* (CNS) species and *S. aureus* (SA). Standard antibiotic and antibacterial minimal inhibitory concentration (MIC) testing was performed on all 317 isolates (301 CNS; 16 SA).

Results. There was no increased antibiotic resistance in *Staphylococcus* isolates from 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 resistant to vancomycin, and the rate of methicillin resistant *S. aureus* (MRSA) detected in the TCS/TCC groups was less than that in the non-user group, and appreciably less than that reported in the literature for both hospital inpatient and outpatient isolates of SA. Additionally, the 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.

Conclusion. This randomized community study of resident skin *Staphylococcus* has shown 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. These study results confirm similar findings from recent assessments of antibiotic and antibacterial resistance in home environments (Aiello et al, 2005; Cole et al, 2003), and further discount the speculative claim that the use of antibacterial wash products contribute to the selection and propagation of drug-resistant bacteria on human skin.

BACKGROUND

Antibacterial wash products have come under increased scrutiny as potential contributors to increasing antibiotic resistance in pathogenic bacteria affecting humans. Recently published data have indicated that triclosan (one of the most widely used topical antibacterial agents) functions intracellularly as a site-directed enzyme inhibitor (Levy et al, 1999) much like an antibiotic. Although there are similarities in the mechanisms of antibacterial wash product actives and antibiotics, there has not been strong evidence that there is a correlation between antibiotic and environmental antibacterial resistance in bacteria associated with humans (Cole et al, 2003; Rutala et al, 1997).

OBJECTIVE

The objective of this study was to investigate the extent of, and relationship between, antibiotic and antibacterial resistance in human skin bacteria isolated from individuals in the home environment, relative to their routine use or non-use of antibacterial hand and bath soaps, and other antibacterial body wash products. Specifically, we sought to investigate:

1. A potential relationship between antibiotic resistance in normal human skin flora and the long-term use of wash products containing the most common antibacterial agents, triclosan (TCS) and triclocarban (TCC);
2. Antibiotic/antibacterial cross-resistance - i.e. whether highly anitibiotic-resistant isolates also exhibited increased resistance to one or both of the antibacterial agents, and vice-versa.

APPROACH

The investigation was designed as a randomized, controlled trial where qualified 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 specified target indicator organisms. Comparison of resistance 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 components of normal skin flora, and their potential to act as human pathogens.

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

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

Potential participants were excluded if any of the following applied:

- 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 routine exposure to solvents

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 (30) days for body washing, including the forearm.

METHODS and MATERIALS

Participant Bacterial Skin Sampling

Using the aforementioned, verified sampling 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 study personnel made 8 wipes with swab up and back along the length of the forearm and 32 wipes back and forth along the width of the forearm. An individual sample was comprised of the combined sampled areas from both forearms.

Once collected, samples were placed into individual, sterile, labeled transport tubes and stored in insulated containers for protection from the elements and extremes in temperatures during transport to the laboratory for processing. Collection from all participants resulted in 210 samples, each to be processed for target bacterial isolates. Isolates were not recovered from 8 TCC Users, 12 TCS Users, and 9 Non-Users. There were 317 confirmed target bacterial isolates for antibiotic and antibacterial susceptibility testing.

Sample Processing

All collected 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 SBA (trypticase soy agar with 5% sheep blood), spread for isolation until dry, and incubated for 18-24 hours at 37°C. After appropriate incubation, plates were examined for isolated colonies, and target bacteria presumptively identified according to standard criteria of morphology, pigmentation, texture, hemolysis, and other distinguishing characteristics. The primary target organisms were coagulase-negative *Staphylococcus* sp., with occasional isolates of *S. aureus* expected. 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. Following identification confirmation, isolates were prepared in duplicate, i.e. one for quantitative antibiotic and antibacterial susceptibility/resistance testing by standard Minimum Inhibitor Concentration (MIC) methods, and one for archiving at -70°C for future reference.

Antibiotic Susceptibility/Resistance Testing

Antibiotic susceptibility testing using standard panels was conducted on all 317 *Staphylococcus* isolates by LabCorp (Laboratory Corporation of America, Burlington, NC). 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 (NCCLS 1999). 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 (NCCLS, 1999).

Antibacterial Susceptibility/Resistance 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 by Applied Environmental using a standard broth micro-dilution method (Barry et al, 1999), and derived from the NCCLS Method M7-A4 (NCCLS 1997. 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 these two species. Testing also included a fully TCS-susceptible strain of CN *Staphylococcus* 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 – TCS concentrations (0.004 - 2.02 ppm). TCC concentrations (0.0015 - 0.75 ppm). Positive and negative controls were run simultaneously with all test isolates.

DATA ANALYSIS

Comparative analysis of susceptibility/resistance test results for the bacterial isolates from the three groups (non-user, TCS-user, TCC-user) was performed using descriptive statistics (percentages, ranges), as well as Chi-square analysis for statistical significance.

RESULTS

Antibiotic Susceptibility

Resistance to standard test panels. Of critical significance in regard to antibiotic susceptibility is the fact that none of the 317 isolates 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.

Table 1 presents the comparative distribution of resistance across all 10 drugs for CNS isolates from the NU group (n = 106) versus the TCC group (n = 102), and shows no significant differences between the groups for all 10 drugs tested. Likewise, Table 2 shows comparable CNS resistance rates for the NU group (n = 106) versus the TCS group (n = 93). 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 3, 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%).

Table 4 presents the comparative distribution of resistance across all 10 drugs for SA isolates from the NU group (n = 4) versus the TCC group (n = 7), and shows no significant differences across the drugs tested, with the exception of penicillin, which showed greater resistance in the NU group (100%) than the TCC group (28.6%). Likewise, Table 5 shows comparable SA resistance rates for the NU group (n = 4) versus the TCS group (n = 5); as does Table 6 when the NU group (n = 4) is compared with the TCC and TCS pooled data (n = 12), with the exception of increased resistance to ampicillin in the NU group.

Resistance to preferred drugs. Of the 10 antibiotics included in the standard test panel, 6 are considered preferred treatment drugs – ciprofloxacin (CIP), clindamycin (CLD), oxacillin (OX), tetracycline (TET), trimethoprin/sulfamethoxasole (TMP/SMX), and vancomycin (VAN). Results relative to these preferred antibiotics, as shown in Table 7 indicates no significant resistance 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. Similarly, with 154 CNS isolates, the rates of resistance to one or more than one preferred drug for each of the three groups were comparable: NU = 53.8% (57/106); TCC = 50.0% (51/102); and TCS = 49.5% (46/93).

Table 8 presents the distribution data for 69 CNS isolates and 2 SA isolates resistant to 2 or more of the preferred drugs. Again, rates of resistance were comparable among the participant groups for 69 CNS isolates: NU = 25.5%; TCC = 24.5%, and TCS = 18.3%; while 2 SA isolates remain too few for meaningful interpretation.

Methicillin-resistant S. aureus (MRSA) and CNS

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 (Fridkin et al, 2002); a rate of 20.2% for MRSA isolates from 50,759 blood samples from hospitals in Europe from 1999-2002 (Tiemersma et al, 2004); and a rate of 50% for MRSA isolates from all clinical inpatient and outpatient samples from 1999-2004 from a large university medical center (DUMC, 2005). 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 in this study, 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 (Fridkin et al, 2002), and 73.3% from all clinical inpatient and outpatient samples of all types from 1999-2004 from a large university medical center (DUMC, 2005). Across participant groups, CNS methicillin resistance rates were 17.9% (19/106) for the Non-Users, 23.5% (24/102) for the TCC users, and 20.4% (19/93) for the TCS users. And these differences were not significant.

Antibacterial Susceptibility

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 9 as ranges of MIC values from lowest (least resistance) to highest (most resistance) for each participant group and each antibacterial agent. CNS isolates 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 group when tested against TCS. For all SA isolates (n = 16) 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 when tested against TCS.

Cross-Resistance

The data were evaluated for cross-resistance – i.e. whether highly anitibiotic-resistant isolates also exhibited increased resistance to one or both of the antibacterial agents, and vice-versa. Table 10 presents the antibacterial MIC values for 9 CNS isolates most resistant to preferred treatment drugs (4-5). MIC values for isolates tested against TCC were comparable among the 3 participant groups, and none exhibited the highest MIC values, as other less antibiotic-resistant isolates did. Likewise, 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. Table 11 shows resistance to number of preferred treatment drugs for 7 CNS isolates with the highest MIC values for TCC. Isolates from the participant groups were comparable, with resistance to 0, 1, or 2 preferred drugs, as opposed to resistance to 4-5 drugs exhibited by less TCC resistant isolates, as presented previously. Similarly, Table 12 shows resistance to number of preferred treatment drugs for 60 CNS isolates with the highest antibacterial MIC values for TCS. Results show comparable results for the participant groups, as NU isolates were resistant to 0-3 preferred drugs, TCC isolates resistant to 0-2 drugs, and TCS isolates, with one exception, resistant to 0-3 drugs. The exception was one isolate out of 19 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, as shown in Table 13, three isolates exhibiting the highest antibacterial MIC values for TCS. And none of those isolates were resistant to any of the preferred treatment drugs.

CONCLUSION

This randomized community study of resident skin *Staphylococcus* has shown 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. Additionally, none of the 317 study 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. Also, the study data show a definitive lack of antibiotic/antibacterial cross-resistance when the most resistant staphylococci in each category are comparatively assessed across the three participant groups. These study results confirm similar findings from recent assessments of antibiotic and antibacterial resistance in home environments (Aiello et al, 2005; Cole et al, 2003), and further discount the speculative claim that the use of antibacterial wash products contribute to the selection and propagation of drug-resistant bacteria on human skin.

REFERENCES

Aiello AE, Marshall B, Levy SB, Della-Latta P, Lin SX, and Larson E (2005). Antibacterial Cleaning Products and Drug Resistance, *Emerging Infectious Diseases*, 11(10):1565-1570.

Barry AL, Fuchs PC, and Brown SD (1999). Lack of Effect of Antibiotic Resistance on Susceptibility of Microorganisms to Chlorhexidine Gluconate or Povidone Iodine, *Eur J Clin Microbiol Infect Dis* 18 (12):920-1.

Cole EC, Addison R, Rubino J, Leese K, Dulaney P, Newell M, Wilkins J, Gaber D, Wineinger T, and Criger D (2003). Investigation of Antibiotic and Antibacterial Agent Cross-Resistance in Target Bacteria from Homes of Antibacterial Product Users and Non-Users, *Journal of Applied Microbiology* 95:664-676.

DUMC (2005). htpp://pathology.mc.duke.edu/microbiology/susceptibility.htm, Summary of Antimicrobial Susceptibility Test Results 1999-2004, Duke University Medical Center, Durham, NC.

Fridkin SF, Hill HA, Volkova NV, Edwards JR, Lawton RM, Gaynes RP, McGowan Jr JE (2002). Temporal changes in prevalence of antimicrobial resistance in 23 U.S. hospitals, *Emerging Infectious Diseases*, (8)7:697-701.

Levy CW, Roujeinikova A, Sedelnikova S, Baker PJ, Stuitje AR, Slabas AR, Rice DW, and Rafferty JB (1999). Molecular Basis of Triclosan Activity. *Nature* 398(6726):383-4.

NCCLS (1997). Performance Standards for Antimicrobial Disk Susceptibility Tests, 6th edition, Approved Standard, Document M7-A4, NCCLS, Wayne, PA.

NCCLS (1999). Performance Standards for Antimicrobial Susceptibility Testing, 9th Informational Supplement. Document M100-S9, NCCLS, Wayne, PA.

Tiemersma EW, Bronzwaer SL, Lyytikainen O, Degener JE, Schrijnemakers P, Bruinsma N, Monen J, Witte W, Grundmann H (2004). Methicillin-resistant Staphylococcus aureus in Europe, 1999-2002, *Emerging Infectious Diseases*, 10(9):1627-1634.

Rutala WA, Steigel MM, Sarubbi FA, and Weber DJ (1997). Susceptibility of Antibiotic-Susceptible and Antibiotic-Resistant Hospital Bacteria to Disinfectants, *Infection Control & Hospital Epidemiology* 18:417-421.

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Table 1. Comparative distribution of antibiotic susceptibility/resistance data for CNS isolates from non-user and TCC user groups.

Drug	Non-users (n=106)		TCC-users (n=102)		p-value*
	Susceptible	Resistant	Susceptible	Resistant	
AMP	69.8%	30.2%	64.7%	35.3%	0.262
CIP	94.3%	5.7%	96.1%	3.9%	0.398
CLD	94.3%[§]	5.7%	96.1%[§]	3.9%	0.398
ERY	56.6%[§]	43.4%	60.8%[§]	39.2%	0.319
NF	97.2%	2.8%	99.0%	1.0%	0.325
OX	82.1%	17.9%	76.5%	23.5%	0.204
PEN	49.1%	50.9%	46.1%	53.9%	0.386
TET	83.0%	17.0%	89.2%	10.8%	0.138
TMP/SMX	96.2%	3.8%	92.2%	7.8%	0.169
VAN	100.0%	0.0%	100.0%	0.0%	
1+Preferred	46.2%	53.8%	50.0%	50.0%	0.343
>1Preferred	74.5%	25.5%	75.5%	24.5%	0.500

* Based on Chi-square
§ Contains 1-4 isolates with intermediate resistance
1+Preferred means resistant to one or more preferred drugs
>1Preferred means resistant to two or more preferred drugs

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

Drug	Non-users (n=106)		TCS-users (n=93)		p-value*
	Susceptible	Resistant	Susceptible	Resistant	
AMP	69.8%	30.2%	63.4%	36.6%	0.211
CIP	94.3%	5.7%	91.4%	8.6%	0.297
CLD	94.3%[§]	5.7%	91.4%[§]	8.6%	0.297
ERY	56.6%	43.4%	64.5%	35.5%	0.160
NF	97.2%	2.8%	98.9%[§]	1.1%	0.361
OX	82.1%	17.9%	79.6%	20.4%	0.394
PEN	49.1%	50.9%	46.2%	53.8%	0.399
TET	83.0%	17.0%	91.4%[§]	8.6%	0.061
TMP/SMX	96.2%	3.8%	91.4%	8.6%	0.129
VAN	100.0%	0.0%	100.0%	0.0%	
1+Preferred	46.2%	53.8%	50.5%	49.5%	0.321
>1Preferred	74.5%	25.5%	81.7%	18.3%	0.147

* Based on Chi-square
§ Contains 1-2 isolates with intermediate resistance
1+Preferred means resistant to one or more preferred drugs
>1Preferred means resistant to two or more preferred drugs

Table 3. 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*
	Susceptible	Resistant	Susceptible	Resistant	
AMP	69.8%	30.2%	64.1%	35.9%	0.192
CIP	94.3%[§]	5.7%	93.8%	6.2%	0.541
CLD	94.3%[§]	5.7%	93.8%[§]	6.2%	0.541
ERY	56.6%[§]	43.4%	62.6%[§]	37.4%	0.187
NF	97.2%	2.8%	99.0%[§]	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%[§]	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%	
1+Preferred	46.2%	53.8%	50.3%	49.7%	0.292
>1Preferred	74.5%	25.5%	78.5%	21.5%	0.262

* Based on Chi-square
§ Contains 1-5 isolates with intermediate resistance
1+Preferred means resistant to one or more preferred drugs
>1Preferred means resistant to two or more preferred drugs

Table 4. Comparative distribution of antibiotic susceptibility/resistance data for SA isolates from non-user and TCC user groups.

Drug	Non-users (n=4)		TCC-users (n=7)		p-value*
	Susceptible	Resistant	Susceptible	Resistant	
AMP	0.0%	100.0%	71.4%	28.6%	0.045
CIP	75.0%	25.0%	100.0%	0.0%	0.364
CLD	100.0%	0.0%	85.7%	14.3%	0.636
ERY	25.0%	75.0%	85.7%	14.3%	0.088
NF	100.0%	0.0%	100.0%	0.0%	
OX	75.0%	25.0%	100.0%	0.0%	0.364
PEN	0.0%	100.0%	71.4%	28.6%	0.045
TET	100.0%	0.0%	100.0	0.0%	
TMP/SMX	100.0%	0.0%	100.0%	0.0%	
VAN	100.0%	0.0%	100.0%	0.0%	
1+Preferred	75.0%	25.0%	85.7%	14.3%	0.618
>1Preferred	75.0%	25.0%	100.0%	0.0%	0.364

* Based on Chi-square
1+Preferred means resistant to one or more preferred drugs
>1Preferred means resistant to two or more preferred drugs

Table 5. Comparative distribution of antibiotic susceptibility/resistance data for SA isolates from non-user and TCS user groups.

Drug	Non-users (n=4)		TCS-users (n=5)		p-value*
	Susceptible	Resistant	Susceptible	Resistant	
AMP	0.0%	100.0%	60.0%	40.0%	0.119
CIP	75.0%	25.0%	100.0%	0.0%	0.444
CLD	100.0%	0.0%	80.0%[§]	20.0%	0.556
ERY	25.0%	75.0%	80.0%	20.0%	0.167
NF	100.0%	0.0%	80.0%	20.0%	0.556
OX	75.0%	25.0%	80.0%	20.0%	0.722
PEN	0.0%	100.0%	40.0%	60.0%	0.278
TET	100.0%	0.0%	100.0	0.0%	
TMP/SMX	100.0%	0.0%	80.0%	20.0%	.556
VAN	100.0%	0.0%	100.0%	0.0%	
1+Preferred	75.0%	25.0%	60.0%	40.0%	0.595
>1Preferred	75.0%	25.0%	80.0%	20.0%	0.722

* Based on Chi-square
§ Contains one isolate with intermediate resistance
1+Preferred means resistant to one or more preferred drugs
>1Preferred means resistant to two or more preferred drugs

Table 6. 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*
	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%[§]	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%	
1+Preferred	75.0%	25.0%	75.0%	25.0%	0.728
>1Preferred	75.0%	25.0%	91.7%	8.3%	0.450

* Based on Chi-square
§ Contains one isolate with intermediate resistance
1+Preferred means resistant to one or more preferred drugs
>1Preferred means resistant to two or more preferred drugs

Table 7. Distribution of all isolates according to resistance to 1 or more preferred drugs and usage of antibacterials.

Category	Resistant		Susceptible		p-value*
	Number	Percentage	Number	Percentage	
CNS Isolates					
All CNS	154	51.2	147[§]	48.8	0.687
Non-users	57	53.8	49[§]	46.2	0.437
TCC users	51	50.0	51[§]	50.0	1.000
TCS users	46	49.5	47[§]	50.5	0.917
SA Isolates					
All SA	4	25.0	12[§]	75.0	0.046
Non-users	1	25.0	3	75.0	0.317
TCC users	1	14.3	6	85.7	0.059
TCS users	2	40.0	3[§]	60.0	0.655

* Based on Chi-square
§ Contains 1-4 isolates with intermediate resistances

Table 8. Distribution of all isolates according to resistance to 2 or more preferred drugs and usage of antibacterials.

Category	Resistant		Susceptible		p-value*
	Number	Percentage	Number	Percentage	
CNS Isolates					
All CNS	69	22.9	232[§]	77.1	<.0001
Non-users	27	25.5	79[§]	74.5	<.0001
TCC users	25	24.5	77[§]	75.5	<.0001
TCS users	17	18.3	76[§]	81.7	<.0001
SA Isolates					
All SA	2	12.5	14[§]	87.5	0.003
Non-users	1	25.0	3	75.0	0.317
TCC users	0	14.3	7	85.7	
TCS users	1	20.0	4[§]	80.0	0.180

* Based on Chi-square
§ Contains 1-4 isolates with intermediate resistance

*Presented at the 2006 Annual Conference on Antimicrobial Resistance, Bethesda, MD

Table 9. Antibacterial MIC ranges for all isolates tested for susceptibility to TCC and TCS

User Group	TCC (ppm)	TCS (ppm)
CNS		
Non-users	0.0117-0.750	0.120-2.020
TCC-users	0.0234-0.750	0.004-2.020
TCS-users	0.0117-0.750	0.008-2.020
SA		
Non-users	0.0469-0.1875	0.510-2.020
TCC-users	0.0029-0.1875	0.120-1.010
TCS-users	0.0469-0.1875	1.010-2.020

Table 10. Antibacterial MIC values for CNS isolates most resistant to preferred drugs

ID	User Group	#of preferred drugs resistant to	MIC-TCC (ppm)	MIC-TCS (ppm)
0044-3	Non	4	0.0938	0.510
0062-3	Non	4	0.0938	1.010
0161-1	Non	4	0.1875	0.510
2045-1	TCC	5	0.0938	0.510
2124-3	TCC	4	0.0938	1.010
3012-2	TCS	5	0.1875	0.510
3035-1	TCS	4	0.1875	1.010
3046-1	TCS	4	0.0469	0.250
3080-1	TCS	4	0.1875	2.020

Table 11. Resistance to preferred drugs for CNS isolates most resistant to TCC

ID	User Group	MIC-TCC (ppm)	# of preferred drugs resistant to
0102-1	Non	0.750	1
2013-1	TCC	0.750	0
2033-2	TCC	0.750	2
2068-1	TCC	0.750	1
2079-2	TCC	0.750	2
3069-2	TCS	0.750	0
3091-1	TCS	0.750	0

Table 12. Resistance to preferred drugs for CNS isolates most resistant to TCS

CNS ID	User Group	MIC-TCS (ppm)	# of preferred drugs resistant to
0023-1	Non	2.02	0
0024-1	Non	2.02	0
0024-3	Non	2.02	3
0029-2	Non	2.02	0
0054-3	Non	2.02	1
0086-1	Non	2.02	0
0089-1	Non	2.02	2
0092-2	Non	2.02	1
0102-1	Non	2.02	1
0102-2	Non	2.02	0
0121-2	Non	2.02	2
0125-1	Non	2.02	0
0135-3	Non	2.02	1
0160-3	Non	2.02	1
0162-1	Non	2.02	2
0163-1	Non	2.02	1
0164-1	Non	2.02	0
0164-2	Non	2.02	0
0167-1	Non	2.02	0
0176-1	Non	2.02	1
0203-1	Non	2.02	0
0209-2	Non	2.02	0
2002-1	TCC	2.02	0
2002-2	TCC	2.02	2
2009-2	TCC	2.02	0
2013-2	TCC	2.02	0
2014-1	TCC	2.02	0
2027-1	TCC	2.02	0
2033-2	TCC	2.02	2
2067-2	TCC	2.02	0
2068-1	TCC	2.02	1
2079-2	TCC	2.02	2
2087-1	TCC	2.02	0
2087-3	TCC	2.02	0
2088-1	TCC	2.02	0
2088-2	TCC	2.02	1
2102-1	TCC	2.02	2
2109-1	TCC	2.02	2
2109-2	TCC	2.02	1
2119-1	TCC	2.02	1
2120-2	TCC	2.02	0
3034-1	TCS	2.02	1
3042-3	TCS	2.02	0
3070-3	TCS	2.02	0
3078-2	TCS	2.02	0
3078-3	TCS	2.02	1
3080-1	TCS	2.02	4
3080-2	TCS	2.02	3
3082-1	TCS	2.02	2
3085-3	TCS	2.02	0
3087-2	TCS	2.02	1
3091-1	TCS	2.02	0
3092-1	TCS	2.02	1
3094-1	TCS	2.02	0
3094-5	TCS	2.02	0
3095-1	TCS	2.02	1
3095-3	TCS	2.02	0
3101-3	TCS	2.02	0
3107-1	TCS	2.02	0
3108-1	TCS	2.02	1

Table 13. Resistance to preferred drugs for SA isolates most resistant to TCS

ID	User Group	MIC-TCS (ppm)	# of preferred drugs resistant to
0151-1	Non	2.02	0
3034-2	TCS	2.02	0
3078-1	TCS	2.02	0