# Potential Role for Triple Antibiotic Ointment (TAO) Against Mupirocin-Resistant Staphylococci: A USA and Australian Clinical Isolate Sample

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## ABSTRACT

**Background:** Mupirocin (MUP) topical preparations are used to control staphylococcal colonization worldwide, leading to the emergence of resistance (R) at high (> 256 µg/ml) or low (16 - 256 µg/ml) levels. TAO, used OTC since the 1950's, contains neomycin (NEO), polymyxin B (PB) and bacitracin (BAC) at high concentrations providing activity against Gram-positive and -negative pathogens. TAO was assessed against a recent two-nation collection of MUP-R *S. aureus* (SA) and coagulase-negative staphylococci (CoNS).
**Methods:** SA/CoNS from the USA (200/40) and Australia (AUST; 200/40) were tested by CLSI broth microdilution methods and 90 MUP-R strains were detected. Other comparators were oxacillin (OXA), linezolid (LZD), Synercid®, vancomycin, gentamicin (GENT) and fusidic acid (AUST only). S-breakpoints tentatively used were equivalent to conc./100 of the topically applied NEO (≤ 50 mg), PB (≤ 50 IU) and BAC (≤ 4 IU) alone or combined per gm.
**Results:** MUP-R was greatest in USA strains (SA, 5 - 18%; CoNS, 39 - 47%; two phase study) versus AUST (SA, 3%; CoNS, 5%). OXA- or GENT-R strains had greater MUP-R rates, but no R was noted to LZD or glycopeptides.

| | Cum. % inhibited at TAO MIC (µg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sample (no. tested) | ≤1.2 | 2.4 | 4.9 | 9.8 | 20 | (39) | (78) | % S |
| USA/SA (33) | 21 | 21 | 21 | 21 | 21 | 70 | 100 | 100 |
| USA/CoNS (49) | 51 | 59 | 73 | 94 | 96 | 98 | 100 | 100 |
| AUST/SA (6) | 33 | 33 | 33 | 67 | 67 | 83 | 100 | 100 |
| AUST/CoNS (2) | 50 | 50 | 50 | 100 | - | - | - | 100 |

All MUP-R staphylococci had TAO MICs at ≤ 78 µg/ml (approx. 1:100 of TAO concentrations) and CoNS strains were more TAO-S ($MIC_{90}$, 9.8 µg/ml vs 78 µg/ml). TAO was also active against nearly all Enterobacteriaceae and non-fermentors (not MUP).
**Conclusions:** TAO, composed of older rarely used antimicrobials, remains highly active against MUP-R staphylococci endemic in the USA and AUST. The older topical could be economically used where MUP-R rates are high and where tolerated.

## INTRODUCTION

The use of the Triple Antibiotic Ointment (TAO) containing neomycin, polymyxin B and bacitracin was initially described in the mid-1950's although each component had been available in various geographic locations since 1943 - 1949. TAO products may have different formulations, however the TAO described in this report contains neomycin-polymyxin B-bacitracin, that product utilized for decades in the United States (USA). TAO has been promoted as a topical "first-aid" agent to minimize/prevent infections in superficial wounds or burns (21CFR Part 333). By combining antimicrobial agents with narrower spectrums targeting either Gram-positive or -negative pathogens, a wide spectrum of preventative activity has been achieved that includes: staphylococci, pyogenic streptococci, Enterobacteriaceae (*Escherichia coli*, *Klebsiella* spp., etc.), *Pseudomonas aeruginosa* and several other organisms of environmental origin commonly observed as co-pathogens or opportunists of cutaneous infection. The advantage of these compounds, applied as non-prescription topicals, has been the lack of wide spread use by the parenteral route. Neomycin was withdrawn as a clinical agent approximately four decades ago following the discovery of wider-spectrum, less toxic aminoglycosides (gentamicin, tobramycin and kanamycin). Similarly, polymyxins (polymyxin B or colistin) were utilized sparingly due to side-effects when required to treat at-risk patients with documented systemic infections, mainly caused by resistant Gram-negative bacilli. Bacitracin has never been a serious candidate for systemic infections since its discovery during World War II.

TAO use in the Over-The-Counter (OTC) environment presents minimal resistance-based compromise to the subsequent therapy of any prominently used antimicrobial or class. In fact, the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards [NCCLS]) withdrew all in vitro testing criteria from their disk diffusion and MIC test standards in the early 1980's as these drugs were no longer applied to systemic infection therapy nor routinely tested in medical centers in the USA. This fact presents problems for accurate monitoring for continued potency of these agents in the USA, since limited information exists from direct testing of any TAO component.

Breakpoints utilized for susceptibility to TAO components were not rigorously validated in the past and were selected for the prediction of success versus systemic infections, not for topical formulations with very high concentrations (3.5 or 5.0 mg neomycin base; 5,000 IU polymyxin B; 400 U bacitracin/gm). The historical breakpoints for neomycin have varied from ≤ 3 µg/ml (*S. aureus*) to ≤ 10 µg/ml (all pathogens); for polymyxin B the breakpoint has been ≤ 2 IU/ml, without an intermediate susceptibility definition; and for bacitracin a breakpoint was placed at ≤ 2 IU/ml. Debate continues about appropriate breakpoints for susceptibility to topical agents, but a safe or conservative definition of the TAO concentration should be used, but only for comparing topically-applied products. An example of a topical product with a widely published breakpoint has been mupirocin (susceptible at ≤ 4 µg/ml and high-level resistance at > 256 µg/ml). Many experts believe that only the high-level mupirocin resistance may have clinical significance, and this breakpoint corresponds to an approximate 1:100 dilution of the marketed product (2.0%). These facts will be taken into consideration in this presentation.

The purpose of this multi-phased study was to: 1) determine the contemporary resistance or co-resistance patterns of neomycin compared to the routinely assessed aminoglycosides, gentamicin, when testing USA pathogens and its relationship to TAO and mupirocin resistance; and 2) establish the level of TAO activity against contemporary clinical isolates of mupirocin-resistant staphylococci from medical centers in Australia. The results should indicate the long-term effects of TAO-OTC use in the USA and establish the level of susceptibility of staphylococci in Australia to TAO and its components. The use of such in vitro surveillance information and continued monitoring should provide a mechanism to detect significant trends toward resistances as TAO or other topical antimicrobials move to OTC use worldwide, and existing topical agents such as mupirocin becomes less usable in clinical settings.

## MATERIALS AND METHODS

Susceptibility tests. Susceptibility testing used the M7-A6 reference broth microdilution method and interpretations by CLSI M100-S15, where available. Historical interpretations of susceptibility/resistance for TAO components used ≤ 3 or ≤ 10 µg/ml for neomycin; ≤ 2 IU/ml for bacitracin and polymyxin B.

Quality control (QC) used NCCLS recommended QC strains including: *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *E. coli* ATCC 25922; and *P. aeruginosa* ATCC 27853. All QC results were within published/established ranges for the tested agents. The commercial producer of the frozen, reference panels (TREK Diagnostics, Cleveland, Ohio, USA) also obtained acceptable QC results prior to distribution to investigator sites in Iowa (USA) and Australia.

Organism collection. For the determination of antimicrobial activity of TAO against pathogens taken from USA isolates collected from 1997 through 2002, aminoglycoside resistance was emphasized (potential increased rates of neomycin resistance) and then adjusted to the true resistance rates for TAO and components by comparing to the observed gentamicin rates within each species in longitudinal surveillance trials (SENTRY Antimicrobial Surveillance Program). The strains used were approximately 40 samples per year (224 total strains) including *S. aureus* (30) and CoNS (10) from community-acquired infections with no more than one strain per geographic city or medical center per species. The mupirocin non-susceptible subsets were specifically analyzed for TAO activity.

For the determination of neomycin, polymyxin B, bacitracin and TAO rates of resistance in year 2002 - 2003 "community-acquired" isolates from Australia (AGARS Program), these strains were sampled: *S. aureus* (200 strains, 100 oxacillin-resistant [MRSA] and 100 oxacillin-susceptible [MSSA]) with clinical epidemic strains minimized by sampling no more than five strains per medical center; CoNS (40 strains, 30 MR-CoNS and 10 MS-CoNS) with a maximum sample of two strains per site.

## RESULTS

- Using a staphylococcal collection enriched for potential TAO resistance (aminoglycoside [gentamicin]-resistant), resistance to TAO components was observed, but TAO MIC results at ≤ 78 µg/ml inhibited nearly all (99 - 100%) of USA strains (Table 1).
- Similarly, clinical strains of *S. aureus* and CoNS from Australia were 98 (oxacillin-resistant CoNS) to 100% inhibited by TAO at ≤ 78 µg/ml.
- Mupirocin resistance in both countries among staphylococci was greater than TAO resistance (Tables 1 and 2).
- Table 3 shows the TAO activity against 90 strains of mupirocin-resistant staphylococci. All mupirocin-resistant staphylococci were susceptible to TAO at ≤ 78 µg/ml with $MIC_{90}$ results ranging from 9.8 (CoNS) to 78 µg/ml.

**Table 1.** Summary of United States isolate results for six agents or combinations tested against a selected group of strains with elevated gentamicin MIC values (≥ 8 µg/ml).[a]

| | | MIC (µg/ml) | | % by category[b] | |
|---|---|---|---|---|---|
| Organism (no. tested) | Antimicrobial agent | 50% | 90% | Susceptible | Resistant |
| *S. aureus* (159) | Neomycin | 20 | 78 | 36 | 48 |
| | Polymyxin B | 157 | 157 | -[c] | - |
| | Bacitracin | 50 | 400 | 0 | - |
| | TAO | 39 | 78 | 36 | 48 (1)[d] |
| | Mupirocin | ≤0.5 | 1024 | 82 | 11 |
| | Oxacillin | >2 | >2 | 24 | 76 |
| CoNS (65) | Neomycin | 2.4 | 20 | 89 | 6 |
| | Polymyxin B | 39 | 78 | - | - |
| | Bacitracin | 50 | 100 | 0 | - |
| | TAO | 2.4 | 20 | 89 | 6 (0)[d] |
| | Mupirocin | ≤0.5 | >1024 | 62 | 19 |
| | Oxacillin | >2 | >2 | 23 | 77 |

a. Subset with highest probability of TAO resistance via elevated neomycin MIC values.
b. Susceptibility as defined by the CLSI [2005] or cited criteria.
c. - = no criteria have been published.
d. TAO tested as the topical formulation using the neomycin component as the MIC. Percentage susceptible or resistant was based on the coverage of the most active component. The percentage in parenthesis is for the proposed topical breakpoint of resistance at > 78 µg/ml (1:100 dilution of clinical formulations).

**Table 2.** Antimicrobial activity of triple antibiotic ointment (TAO), its components and two topical comparison agents tested against endemic Gram-positive pathogens isolated from patients in Australia (240 strains).

| | | MIC (µg/ml or IU/ml) | | |
|---|---|---|---|---|
| Organism (no. tested) | Antimicrobial agent | 50% | 90% | % susceptible[a] |
| *S. aureus* | | | | |
| oxacillin-susceptible (100) | Neomycin | ≤1.2 | ≤1.2 | 96 |
| | Polymyxin B | 157 | 157 | - |
| | Bacitracin | 25 | 50 | 0 |
| | TAO[b] | 2.4 | 2.4 | 100 (100) |
| | Mupirocin | ≤0.5 | ≤0.5 | 99 |
| | Fusidic Acid | ≤1 | 8 | 88 |
| oxacillin-resistant (100) | Neomycin | ≤1.2 | 39 | 85 |
| | Polymyxin B | 157 | 157 | - |
| | Bacitracin | 25 | 50 | 1 |
| | TAO | ≤1.2 | 9.8 | 94 (98) |
| | Mupirocin | ≤0.5 | ≤0.5 | 95 |
| | Fusidic Acid | ≤1 | 8 | 81 |
| Coagulase-neg. staphylococci | | | | |
| oxacillin-susceptible (10) | Neomycin | ≤1.2 | ≤1.2 | 100 |
| | Polymyxin B | 39 | 78 | - |
| | Bacitracin | 50 | 50 | 0 |
| | TAO | ≤1.2 | ≤1.2 | 100 (100) |
| | Mupirocin | ≤0.5 | ≤0.5 | 100 |
| | Fusidic Acid | ≤1 | ≤1 | 100 |
| oxacillin-resistant (30) | Neomycin | ≤1.2 | 4.9 | 100 |
| | Polymyxin B | 39 | 157 | - |
| | Bacitracin | 50 | 50 | 0 |
| | TAO | ≤1.2 | 9.8 | 100 (100) |
| | Mupirocin | ≤0.5 | ≤0.5 | 93 |
| | Fusidic Acid | ≤1 | 32 | 67 |

a. Susceptibility criteria of the CLSI [2005], where available. Breakpoints for TAO (breakpoint of the most active component; ≤ 1:100 dilution in parenthesis), neomycin (≤ 10 µg/ml), polymyxin (≤ 2 IU/ml), bacitracin (≤ 2 IU/ml), and fusidic acid (≤ 2 µg/ml) were applied for comparison purposes only.
b. TAO = triple antibiotic ointment diluted ($log_2$) from the commercial product mixture of 5000/5000/400 µg/ml or international units/ml. Only the neomycin sulfate component is listed as the MIC.

**Table 3.** Activity of TAO versus 90 mupirocin-resistant staphylococci isolated in the United States and Australia.

| | Cum. % inhibited at TAO MIC (µg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Nation/organism (no. tested) | ≤1.2 | 2.4 | 4.9 | 9.8 | 20 | 39 | (78)[a] | % susceptible |
| Australia | | | | | | | | |
| *S. aureus* (6) | 33 | 33 | 33 | 67 | 67 | 83 | 100 | 100 |
| CoNS (2) | 50 | 50 | 50 | 100 | - | - | - | 100 |
| United States | | | | | | | | |
| *S. aureus* (33) | 21 | 21 | 21 | 21 | 21 | 70 | 100 | 100 |
| CoNS (49) | 51 | 59 | 73 | 94 | 95 | 98 | 100 | 100 |

a. Equivalent to a 1:100[th] concentration of each TAO component.

## CONCLUSIONS

- TAO remains highly active in vitro against both Gram-positive and -negative pathogens because of the combined potencies of its components (neomycin, polymyxin B, bacitracin), with synergy noted at higher concentrations (data not shown).
- Mupirocin-resistant strains were routinely detected in the USA and Australia, usually greater in USA medical centers.
- Mupirocin-resistant strains of staphylococci were inhibited by TAO at a concentration equal or less than 78 µg/ml (a 1:100 dilution of the topical TAO formulation).
- Older agents (TAO components and combined), rarely used in parenteral human practice, could have an increasing role as an alternative topical agent for inhibiting mupirocin-resistant Gram-positive cocci. The combination of three agents also provides broader-spectrum coverage and economic advantages in the USA and Australia.

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