



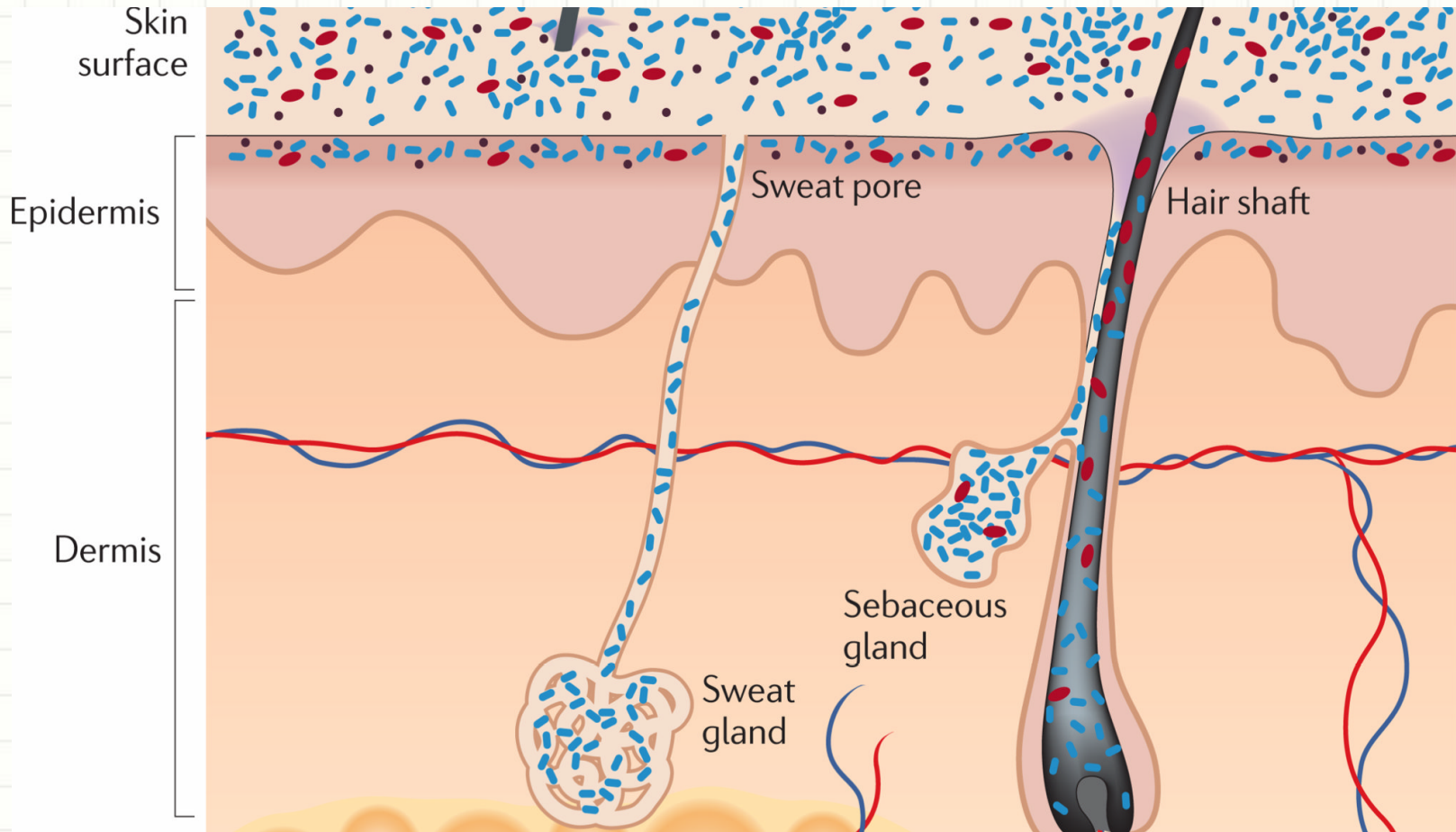
REDUCTION IN THE BACTERIAL LOAD ON THE SKIN IN A CLINICAL SETTING

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The Skin Microbiome

From Grice and Segre Nat Rev Micro 2011 9(4): 244-253.



Rationale of Study Design

Hypothesis: *In view of the mechanism of action of HOCl and its rapid biocidal activity in solution, the removal of bacteria on the surface of the skin would be measurable after a single application*

Primary Objective: *To quantitatively determine the bacterial load on the surface of the skin just below the lower eyelid before and 20 minutes after a single application of Avenova.*

Aspects of Design and Demographic Data

- To be eligible, subjects had lid inflammation and for whom daily lid hygiene would be recommended
- Microbiological specimens were collected OU
 - Before and 20 minutes after first HOCl application
- Demographic data
 - 36 subjects – 71 of 72 ocular specimens were processed
 - Female - 22; Male - 14
 - Average age was 63; range 19-88 yrs

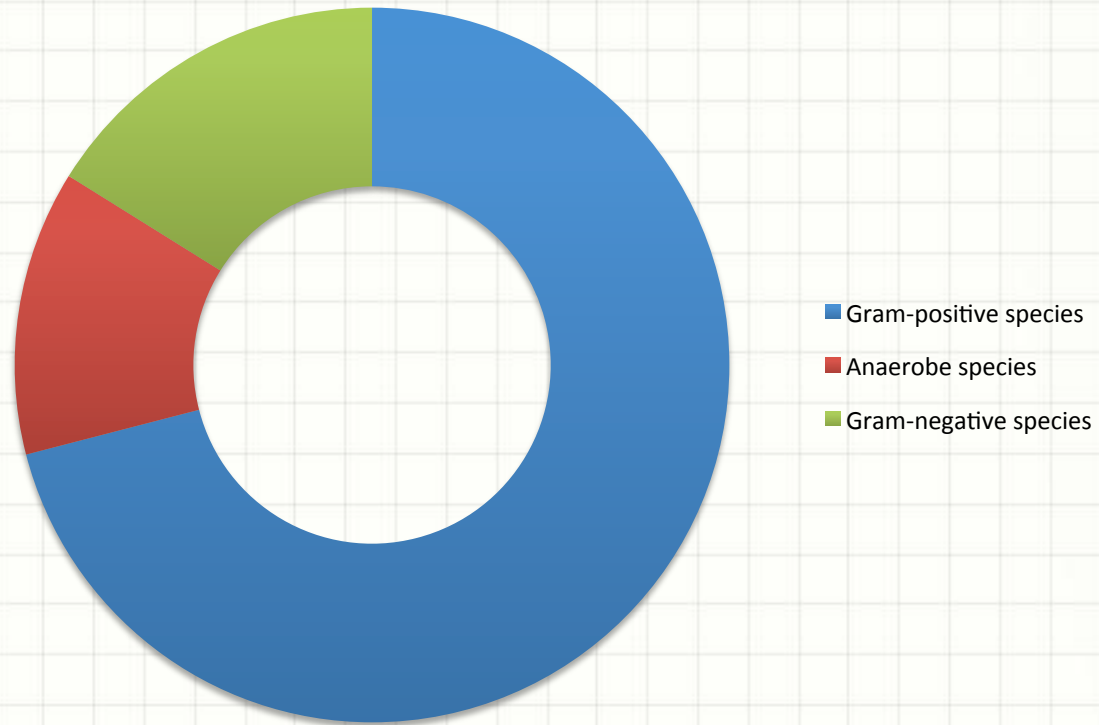
Recovered Bacterial Species

- **Staphylococcus:** *S. aureus*, *S. capitis*, *S. caprae*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. lugdunensis*, *S. pasteurii*, *S. simulans*, *S. warneri*
- **Corynebacterium:** *C. accolens*, *C. bovis*, *C. confusum*, *C. macginleyi*, *C. propinquum*, *C. tuberculostearicum*
- **Bacillus:** *B. circulans*, *B. pumilus*
- **Enterococcus:** *E. faecalis*
- **Micrococcus:** *M. luteus*
- **Rothia:** *R. dentocariosa*
- **Streptococcus:** *S. mitis*
- **Propionibacterium:** *P. acnes*, *P. avidum*, *P. granulosum*
- **Prevotella:** *P. oris*
- **Enterobacter:** *E. aerogenes*
- **Moraxella:** *M. osloensis*
- **Neisseria:** *N. flavescens*
- **Pantoea:** *P. agglomerans*
- **Pseudomonas:** *P. aeruginosa*, *P. oryzihabitans*

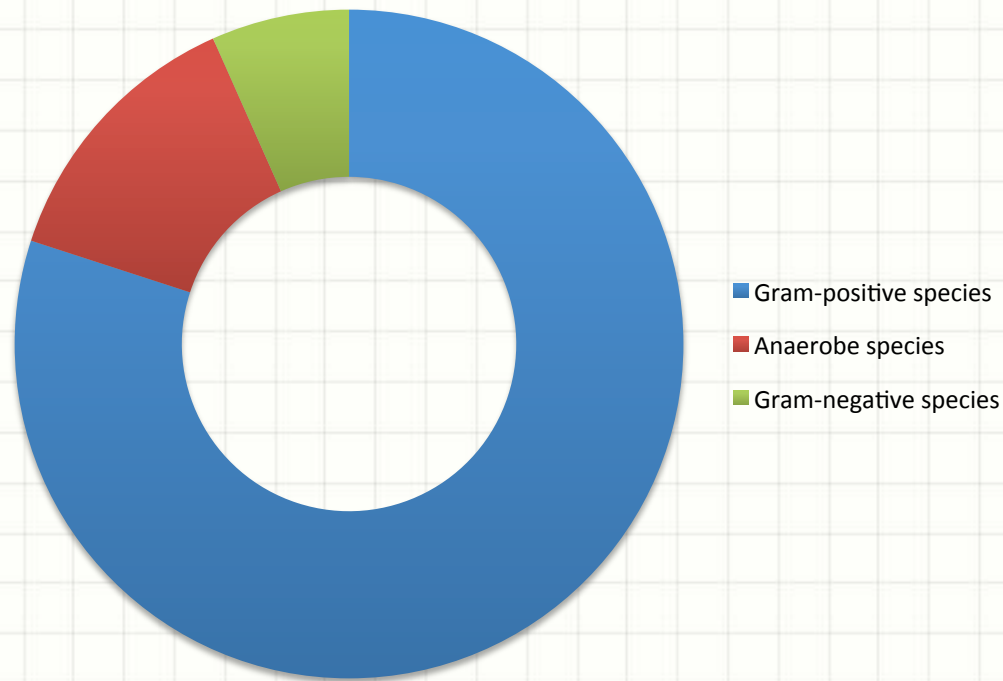
Bacterial Diversity on the Skin below the Eyelid

| Bacterial Groups | Number of Species | | Number of Strains | |
|------------------|-------------------|---------------|-------------------|---------------|
| | Time = 0 | Time = 20 min | Time = 0 | Time = 20 min |
| Gram-positive | 22 | 12 | 117 | 35 |
| Anaerobes | 4 | 2 | 39 | 23 |
| Gram-negative | 5 | 1 | 6 | 1 |

Diversity of Recovered Bacteria (before treatment)



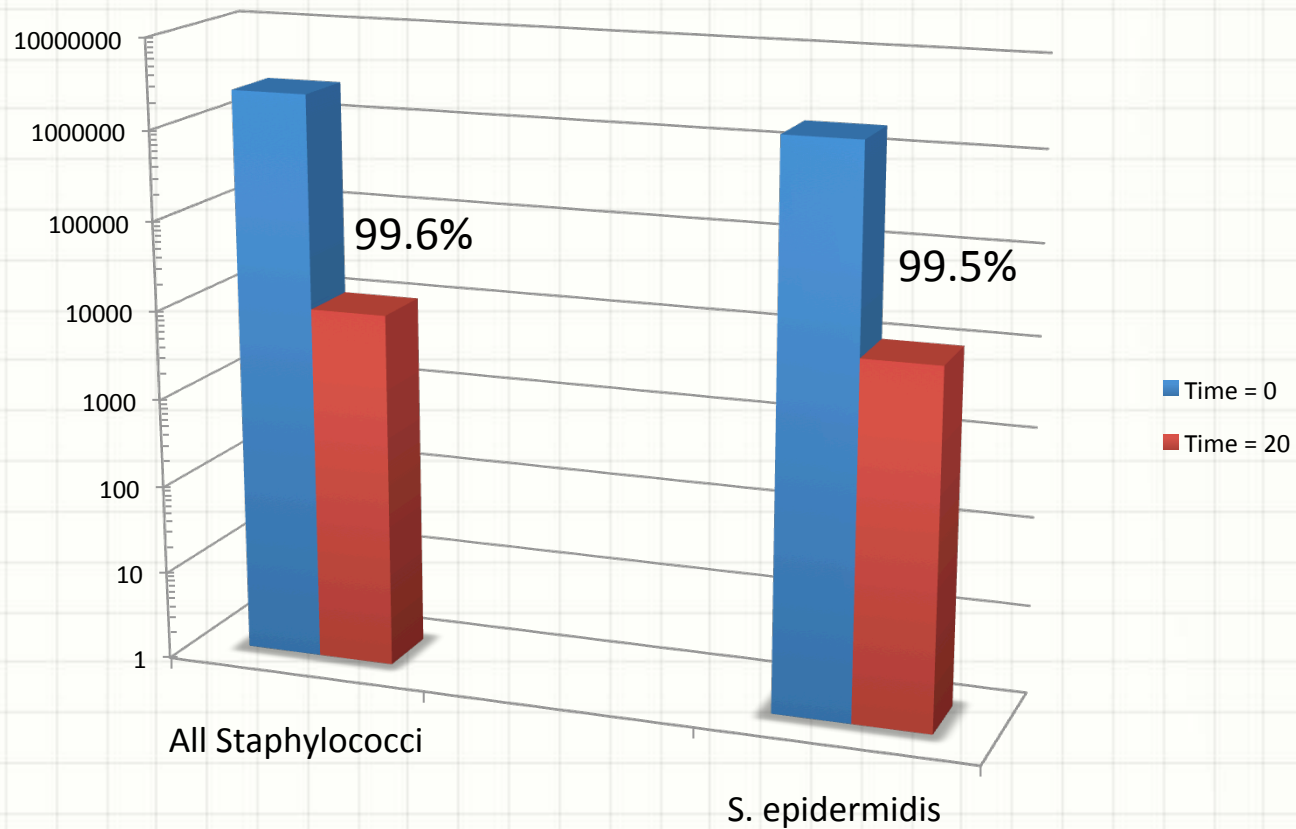
Diversity of Recovered Bacteria (after treatment)



Bacterial Load on the Skin below the Eyelid

| Bacterial species* | Number of strains | | CFUs | |
|--|-------------------|---------------|-----------|----------------|
| | Time = 0 | Time = 20 min | Time = 0 | Time = 20 min |
| Staphylococci | 98 | 24 | 2,362,786 | 10,094 (0.43%) |
| <i>S. epidermidis</i> | 60 | 18 | 1,830,774 | 9,898 (0.54%) |
| <i>P. acnes</i> * | 37 | 23 | 12,582 | 896 (7.1%) |
| | | | | |
| *Strain level analysis was not performed | | | | |

Reduction of Staphylococcal Load in 20 minutes



Susceptibility Profile of *S. epidermidis*

| Antibiotic | Susceptible/Resistant Isolates | | Percent Resistant Isolates | |
|--------------------------------------|--------------------------------|---------------|----------------------------|---------------|
| | Time = 0 | Time = 20 min | Time = 0 | Time = 20 min |
| <i>Oxacillin (Methicillin) >2</i> | 43/17 | 13/5 | 28% | 28% |
| <i>Penicillin >1</i> | 33/27 | 8/10 | 45% | 56% |
| <i>Tobramycin >1</i> | 44/16 | 12/6 | 27% | 33% |
| <i>Clindamycin >1</i> | 54/6 | 15/3 | 10% | 10% |
| <i>Erythromycin >1</i> | 32/28 | 9/9 | 47% | 50% |
| <i>Ciprofloxacin >1</i> | 44/16 | 12/6 | 27% | 33% |
| <i>Tetracycline >2</i> | 55/5 | 15/3 | 8% | 17% |
| <i>Trimethoprim >4</i> | 43/13 | 14/4 | 22% | 22% |
| <i>Sulfamethoxazole >64</i> | 7/53 | 3/15 | 88% | 83% |

Summary of Primary Findings

- The diversity of microbial species is not changed significantly after treatment with HOCl
 - Staphylococcal isolates were 60% of the total isolates recovered prior to treatment
 - *S. epidermidis* isolates were 36% of the total isolates recovered prior to treatment
 - *P. acnes* isolates were 22% of the total isolates recovered prior to treatment
- Total staphylococci recovered were reduced by 99.6% 20 minutes after treatment with HOCl
 - Total *S. epidermidis* recovered were reduced by 99.5%
- Susceptibility profile of *S. epidermidis* isolates revealed that one HOCl treatment reduced the the bacterial load of isolates with resistant phenotypes equally well as those with susceptible phenotypes

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- Now, for the rest of the story.....

Species/Isolates Emerging Post-HOCl

| Bacterial Groups | Original Species | | Species | Number of Unique Isolates | | "New" Isolates |
|------------------|------------------|---------------|---------------|---------------------------|---------------|----------------|
| | Time = 0 | Time = 20 min | Time = 20 min | Time = 0 | Time = 20 min | Time = 20 min |
| Gram-positive | 22 | 12 | 12 | 117 | 35 | 54 |
| Anaerobes | 4 | 1 | 2 | 39 | 23 | 6 |
| Gram-negative | 5 | 0 | 1 | 6 | 0 | 1 |

| Bacterial species | Number of Unique Isolates | | "New" Isolates |
|--------------------------------------|---------------------------|---------------|----------------|
| | Time = 0 | Time = 20 min | Time = 20 min |
| Staphylococci | 98 | 24 | 44 |
| <i>S. epidermidis</i> | 60 | 18 | 31 |
| <i>P. acnes</i> * | 37 | 23 | 6 |
| *Strain level analysis not performed | | | |

Susceptibility Profile of “new” *S. epidermidis* Isolates

| Antibiotic | % Resistant strains | | % Resistant strains |
|--------------------------------|---------------------|---------------|---------------------|
| | Time = 0 | Time = 20 min | At 20 min |
| <i>Oxacillin (Methicillin)</i> | 28% | 28% | 23% (7/31) |
| <i>Penicillin</i> | 45% | 56% | 35% (11/31) |
| <i>Tobramycin</i> | 27% | 33% | 29% (9/31) |
| <i>Clindamycin</i> | 10% | 10% | 16% (5/31) |
| <i>Erythromycin</i> | 47% | 50% | 45% (14/31) |
| <i>Ciprofloxacin</i> | 27% | 33% | 16% (5/31) |
| <i>Tetracycline</i> | 8% | 17% | 10% 3/31) |
| <i>Trimethoprim</i> | 22% | 22% | 10% (3/31) |
| <i>Sulfamethoxazole</i> | 88% | 83% | 77% (24/31) |

CONCLUSIONS

- The diversity of microbial species is not changed significantly after treatment with HOCl
- Total *S. epidermidis* recovered were reduced by 99.5% 20 minutes after treatment with HOCl
- HOCl treatment reduced the bacterial load of isolates susceptible and/or resistant phenotypes to various antibiotics equally well
- Skin near lid margin will be recolonized after HOCl treatment rapidly by isolates from surrounding skin. This data supports the need for multiple treatments with HOCl per day for optimal benefits
- The resumption of the clinical study will assess diversity and the bacterial load after two weeks of HOCl applications



THANKS TO ALL FOR YOUR ATTENTION
HAPPY TO ANSWER YOUR QUESTIONS