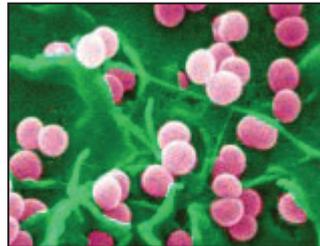


Laboratory Testing of the Residual Activity of Sychem Control

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PREPARATION OF INOCULUM

A methicillin resistant strain of *Staphylococcus aureus* (MRSA), NCTC 11940, was used as the test strain. An overnight culture of NCTC 11940 was grown up in buffered peptone water at 37°C and the final count ascertained by the surface drop (Miles and Misra) technique. This was repeated each week of the trial.



Staphylococcus aureus

TEST FOR RESIDUAL ACTIVITY

Sychem Control sanitising spray was sprayed onto the surface of six tiles and allowed to dry. A volume of 0.1 ml of an MRSA suspension containing a known number of colony forming units was spread over each tile to cover a defined area of 5 x 5 cm (25 cm²). For each concentration, one tile was swabbed 5 minutes after inoculation and a second tile swabbed 10 minutes after inoculation to determine the level of surviving organisms.

Each swab was then placed in 10 ml of TLTR neutralising solution (containing Tween 80, lecithin, sodium thiosulphate, ringers and maximum recovery diluent), vortexed vigorously then plated onto blood agar plates using 0.5 ml, 0.1 ml and 10µl volumes. Once the inoculum had been absorbed the plates were incubated at 37°C for 18-24 h and the number of colonies counted.

One week later and at further one week intervals for a total of three weeks each tile was re-inoculated in the same way using a fresh MRSA suspension and the procedure described above repeated. Four weeks after initial inoculation the same procedure was repeated but the tiles were swabbed 10 and 20 minutes after re-inoculation.

The same procedure was also used with tiles that had not been sprayed with Sychem Control to act as a control. In this case the swab suspension in TLTR solution was serially diluted before plating to ensure the presence of countable plates.

RESULTS

Control results showed an approximate 1 log reduction in bacterial levels which may be due to the recovery method rather than to the effects of desiccation. As expected from previous results, there was at least a 5 log reduction in count (6 log reduction in actual count less 1 log reduction seen in the control) immediately after applying the inoculum to the freshly sprayed tile.

When the tiles were re-inoculated one week later there appeared to be at least a 3 log reduction after 10 minutes of contact time but little or no reduction after 5 minutes when an inoculum level of 10⁶ per 25 cm² was used.

After 7 days using an inoculum level of 10⁴ per 25 cm² there appeared to be at least a 1 log reduction in count after 5 minutes of contact which did not increase significantly after a further 5 minutes.

Results for day 14 show a slight reduction (which may

not be significant) 5 minutes after reinoculation and a further slight reduction 10 minutes after re-inoculation.

Day 21 results suggest a very slight reduction in count 10 minutes after re-inoculation with the high and intermediate levels of inoculum only.

Results at day 28 indicate little or no reduction after 10 minutes but detectable reduction 20 minutes after re-inoculation, particularly with the intermediate and low level inocula.



RECOVERY OF MRSA AFTER COATING TILES WITH Sychem Control

| NUMBER OF DAYS FOLLOWING COATING | INOCULUM LEVEL OF MRSA | RECOVERY AFTER 5 MINUTES | | RECOVERY AFTER 10 MINUTES | |
|----------------------------------|------------------------|---------------------------|-------------|---------------------------|-------------|
| | | COLONY FORMING UNITS | % REDUCTION | COLONY FORMING UNITS | % REDUCTION |
| Day 0 | 1.2×10^7 | 20 | 99.9998 | <20 | >99.9998 |
| | 1.2×10^5 | <20 | >99.9998 | <20 | >99.9998 |
| | 1.2×10^3 | <20 | >99.9998 | <20 | >99.9998 |
| Day 7 | 2.2×10^8 | $>2.0 \times 10^5$ | - | $>2.0 \times 10^5$ | - |
| | 2.2×10^6 | 1.3×10^5 | 94.1 | 2.6×10^2 | 99.99 |
| | 2.2×10^4 | 7.4×10^2 | 96.64 | 4.7×10^2 | 97.86 |
| Day 14 | 1.2×10^7 | $>2.0 \times 10^5$ | - | $>2.0 \times 10^5$ | - |
| | 1.2×10^5 | 8.6×10^4 | 28.3 | 5.2×10^4 | 56.67 |
| | 1.2×10^3 | 5.9×10^2 | 50.83 | 5.4×10^2 | 55.0 |
| Day 21 | 8.7×10^6 | 8.2×10^6 | 5.75 | 5.4×10^6 | 37.93 |
| | 8.7×10^4 | 7.8×10^4 | 10.34 | 4.2×10^4 | 51.72 |
| | 8.7×10^2 | 2.8×10^2 | 67.82 | 4.9×10^2 | 43.68 |
| Control | 2.2×10^8 | 1.4×10^7 | - | 2.2×10^7 | - |
| | | RECOVERY AFTER 10 MINUTES | | RECOVERY AFTER 20 MINUTES | |
| Day 28 | 1.4×10^7 | 8.5×10^6 | 39.29 | 7.2×10^6 | 48.57 |
| | 1.4×10^5 | 1.9×10^4 | 86.43 | 4.5×10^3 | 96.79 |
| | 1.4×10^3 | 2.0×10^3 | 0 | 80 | 94.29 |

CONCLUSION

Results on day 0 after the first inoculation indicate that Sychem CONTROL spray coated onto a tile is effective in killing very high levels (10^7 colony forming units) of MRSA within 5 minutes.

Following reinoculation of MRSA at various levels at weekly intervals, residual activity could still be demonstrated 28 days after the Sychem CONTROL coating was applied to the tiles. At this time a 94 – 97% reduction was noted 20 minutes after re-inoculation with 10^5 or 10^3 MRSA colony forming units and a 48% reduction was noted with an inoculum of 10^7 MRSA colony forming units.



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