

Institute of Progressive Medicine

10-26-2006

Mr. Carl Saporiti and Mr. Arne Rotne
INDUSCO Distributing of America, Inc.
801 Maplewood Drive, Suite 6
Jupiter, Fl, 33458

Dear Carl and Arne,

I am pleased to confirm that your product BioShield[®]75 strongly inhibited a reference strain of methicillin resistant *Staphylococcus aureus* (MRSA). The specific details of the experiments are provided below:

Materials:

MRSA Reference Strain obtained from American Type Culture Collection (ATCC) Manassas, Va. 20108. Catalogue number (strain) 43300.

Bacteria Culture Plates: DD Checker, MEC Agar for MRSA Screening from Denka Seiken. [9 mm square agar surface area, 1.25 square inches]. Provided in strips of 5 plates.

BioShield[®]75 as provided by INDUSCO, Containing 0.75% 3-(Trimethoxysilyl) propyldimethyloctadecyl ammonium chloride.

Spray bottle: 50 ml capacity (approximately 0.1 ml per spray) made by Classic Containers, Ontario CA 91781

Methods: Experiment 1.

Transferred 25 ml BioShield[®]75 to spray bottle

Sprayed individual MEC agar plates with two sprays from the spray bottle held 3 inches above each plate. Estimated 0.1 ml landed on each plate and 0.1 ml landing beyond the plate. A noticeable layer formed over the surface of the agar of the sprayed plates. A total of 5 plates were sprayed with 5 intervening plates unsprayed. The plates were left uncovered for 30 minutes.

Suspended a small colony of MRSA in 5 ml normal saline (Analytab Products, Plainview NY 11803. Using a sterile 0.2 ml micropipette (Beral, Chatsworth CA 91311) added one drop of the bacterial suspension to the center of each plate and gently rocked the plate to allow the suspension to spread across most of the plate.

Incubated the plates at room temperature and recorded observations.

Experiment 2.

MRSA bacteria from the above suspension added to 10 MEC agar plates. The plates were incubated at room temperature for 6 hours. Five of the plates were then exposed to two sprays of BioShield[®]75 and 5 plates were left untreated. All of the plates were subsequently examined.

Results:

Experiment 1. The plates were examined directly and with the aid of a dissecting microscope (Leica). At 24 hours, there were in excess of 1,000 distinct colonies of MRSA covering most of the surface of each of the 5 MEC agar plates not pretreated with BioShield[®]75. Only an occasional minute colony was seen in the plates pretreated with BioShield[®]75 and these tended to be towards the edge of the plates. No plate had more than 10 visible colonies. By day 2, the control plates showed confluent growth. Patchy growth began to appear on the BioShield[®]75 treated plates, but even after three days persisted mainly as individual colonies. Some confluence was seen as an outer ring.

Interpretation: The BioShield[®]75 formed a surface coating on the MEC agar plates that did not allow for effective growth of >99% of the MRSA bacteria subsequently added to the plate. The effect of the BioShield[®]75 persisted for more than 3 days inhibiting confluence of most of the residual bacterial growth.

Experiment 2. The control plates were similar to Experiment 1 with >1,000 colonies identified on day 1 and confluence seen on day 2 and thereafter. Very small colonies were noted on the BioShield[®]75 treated plates at day 1. Up to an average of 100 discrete colonies gradually appeared in the treated plates with very minimal confluence. The colonies tended to be unequally dispersed throughout the plates.

Interpretation: BioShield[®]75 was effective in inhibiting >90% of the ongoing growth of MRSA even on a nutritive, porous surface. The effect of the BioShield[®]75 persisted for several days inhibiting confluence of most of the residual bacterial growth.

In addition to these studies, I was able to show that BioShield[®]75 was effective in killing MRSA on plastic surfaces. No viable MRSA were recoverable from bacteria inoculated lids of unused MEC agar plates that were sprayed twice from 3 inches away with BioShield[®]75.

The data support a claim that BioShield[®]75 has bacteriocidal activity against a reference strain of MRSA. I will continue to advise you of additional results. Thank you for letting me experiment with your product. I have included my curriculum vitae for your reference. Please note that I have Specialty Certification in Medical Microbiology.

Sincerely,

W. John Martin, M.D., Ph.D.
Pathologist