Survival of Gram-Negative Bacteria on Plastic Compounded with Hexachlorophene

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Gram-negative bacteria representing nine genera were screened for their ability to survive surface exposure to polyethylene sheet plastic containing chemically compounded hexachlorophene (0.25%). Subcultures were made at hourly intervals over a 6-hr period of time. An exceedingly large drop in viable cells beginning at the 1-hr exposure was noted for each genus except one tested on the hexachlorophene-plastic, whereas most nonadditive controls grew bacterial colonies too numerous to count.

The increased incidence of nosocomial infections with gram-negative bacteria within the last decade and the relative resistance of the bacteria to antibiotics and chemotherapeutic agents have alerted those interested in the need for hospital environmental control (11).

Because of their complexity, problems associated with the hospital environment do not lend themselves to solution by a single measure. We are, however, obliged to pursue critically any area which offers help in control of the hospital environment, especially its microbial ecology (13, 14).

A 0.25% hexachlorophene-containing polyethylene plastic (Medi-Gard plastic; supplied by Medical Plastics Corp. of America, Greensboro, N.C.) was tested for its antimicrobial activity against nine genera of gram-negative bacteria. Hexachlorophene migrates to the surface of the plastic until a surface saturation or equilibrium is obtained. When some hexachlorophene is removed from the surface, it is replaced from the reservoir in the mass of the plastic, thus providing a constantly replenished antibacterial surface over extended periods of time, months or years (1, 10).

The effective action of hexachlorophene and this plastic material against Staphylococcus aureus is well known (2-4, 7-9). Less is known about the antimicrobial surface action of hexachlorophene against gram-negative organisms. This study is concerned with determining this antimicrobial action in plastic compounded with hexachlorophene.

MATERIALS AND METHODS

Ten strains of gram-negative organisms freshly isolated from clinical specimens were used as the test organisms: Citrobacter freundii, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, P. morganii, Providencia stuartii, Pseudomonas aeruginosa, Salmonella panama, and Serratia marcescens.

The characterization of the Enterobacteriaceae, the taxonomic system, and the methodologies employed for the identification of these organisms, for the most part, were those commonly used by workers in this field (5, 6, 15).

Twenty-four hour Trypticase Soy Broth (BBL) cultures of the organisms were diluted to a concentration of approximately 6 × 10⁶ organisms per ml (MacFarland nephelometer no. 2).

Sheets of 0.25% hexachlorophene-compounded plastic and untreated plastic sheets were ruled into rows of six squares (2 by 2 inches (5.08 by 5.08 cm)). The test organisms were assigned a row of six squares, each representing 1 hr of time. A platinum wire loop calibrated to deliver 0.01 ml was used to transfer samples of the broth containing the test organisms to each of the time squares assigned to them.

The final concentration or challenging inoculum was approximately 6 × 10⁶ organisms per square. The same concentration of organisms was applied to the time squares of the control plastic sheets for each organism. At 1-hr intervals, a sterile cotton swab was moistened in a tube of sterile saline and pressed against the side of the tube to remove excess saline. The swab was first rolled over the surface of the test square to remove the bacteria; a subculture was made in the same way to the surface of an agar plate containing 5% sheep blood in Casman Medium Base (Difco) and was then streaked lightly across the surface with the tip of the same swab. It is necessary to use blood or some other inhibitor in the medium to neutralize any transfer of hexachlorophene, which

1 Present address: Director of Technical Services, Medical Plastics Corp. of America, 220 Atwell Ave., Greensboro, N.C.
### Table 1. Colony counts after exposure of gram-negative organisms to plastic with and without compounded hexachlorophene*  

| Organism                  | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr | 6 hr |
|---------------------------|------|------|------|------|------|------|
| ***Citrobacter freundii***|      |      |      |      |      |      |
| Hexachlorophene-plastic   | 105  | 0    | 0    | 1    | 0    | 0    |
| Untreated control plastic | TNTC | TNTC | TNTC | TNTC | TNTC | TNTC |
| ***Enterobacter aerogenes***|      |      |      |      |      |      |
| Hexachlorophene-plastic   | TNTC | 0    | 0    | 0    | 0    | 0    |
| Untreated control plastic | 310  | 95   | 95   | 95   | 95   | 95   |
| ***Escherichia coli***     |      |      |      |      |      |      |
| Hexachlorophene-plastic   | 0    | 0    | 0    | 0    | 0    | 0    |
| Untreated control plastic | TNTC | TNTC | TNTC | TNTC | TNTC | TNTC |
| ***Klebsiella pneumoniae***|      |      |      |      |      |      |
| Hexachlorophene-plastic   | 1    | 0    | 0    | 0    | 0    | 0    |
| Untreated control plastic | TNTC | TNTC | TNTC | TNTC | TNTC | 33   |
| ***Proteus mirabilis***    |      |      |      |      |      |      |
| Hexachlorophene-plastic   | 0    | 0    | 0    | 0    | 0    | 0    |
| Untreated control plastic | TNTC | TNTC | TNTC | TNTC | TNTC | 0    |
| ***P. morganii***          |      |      |      |      |      |      |
| Hexachlorophene-plastic   | 305  | 0    | 0    | 0    | 0    | 0    |
| Untreated control plastic | 324  | 280  | 280  | 280  | 280  | 280  |
| ***Providencia stuartii*** |      |      |      |      |      |      |
| Hexachlorophene-plastic   | 13   | 0    | 0    | 0    | 0    | 0    |
| Untreated control plastic | 97   | 9    | 9    | 9    | 9    | 9    |
| ***Pseudomonas aeruginosa***|    |      |      |      |      |      |
| Hexachlorophene-plastic   | 100  | 0    | 0    | 0    | 0    | 0    |
| Untreated control plastic | TNTC | TNTC | TNTC | TNTC | TNTC | TNTC |
| ***Salmonella panama***    |      |      |      |      |      |      |
| Hexachlorophene-plastic   | 0    | 0    | 0    | 0    | 0    | 0    |
| Untreated control plastic | TNTC | TNTC | TNTC | TNTC | TNTC | TNTC |
| ***Serratia marcescens***  |      |      |      |      |      |      |
| Hexachlorophene-plastic   | 320  | 2    | 2    | 2    | 2    | 2    |
| Untreated control plastic | TNTC | TNTC | TNTC | TNTC | TNTC | TNTC |

* Approximately 6,000,000 organisms per square.  
† Too numerous to count.

**DISCUSSION**

Two-inch (5.08-cm) squares were used as the inoculation area to provide an ample exposure surface for the heavy inoculum which was employed. An unrealistically high concentration of organisms was used to offer the plastic a challenge for the study. Since these procedures were conducted under ambient conditions, in an uncontrolled environment, indeterminate factors such as the effect of temperature and relative humidity described by McDade and Hall must be considered in the evaluation of these results.

**RESULTS**

The growth from subcultures of the test organisms after exposure to 0.25% hexachlorophene-plastic for 1 to 6 hr and results from the nonadditive control plastic squares are given in Table 1.

**might be carried over from the test plastic producing bacteriostatic conditions (4). Control squares were treated in the same manner. The procedure was repeated through the 6th hr of incubation at ambient conditions of 23 C and a relative humidity between 55 and 60%.**
(12). However, to estimate the effect of deleterious indeterminate factors, several screening tests were made with cultures of E. coli. Three of the tests were performed as described in this paper, except that the plastic test panels were placed in deep metal trays suspended on glass rods in the presence of water-soaked cotton towels. The trays were placed in plastic bags and tightly tied. An increase in humidity by this method did not affect the final result of the survival of E. coli from subcultures. Three other screening procedures were performed under ambient conditions (22 to 25°C and 40 to 60% relative humidity) without added moisture. Only 0 to 6 colonies were subcultured after a 1-hr exposure, regardless of which test environment was used. In each instance, subcultures from the control plastic grew E. coli colonies too numerous to count. As a result of these screening procedures, it was decided to conduct subsequent tests in the open laboratory environment under ambient conditions.

Although this surface subculture procedure does not attempt to be a quantitative one, the results of the growth from the hexachlorophene-plastic exposures, compared with those of the untreated plastic control exposures, show a definite diminished count of viable bacterial cells. These results closely parallel those of Davis (4).

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