Antibacterial Properties of Retail Sponges

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ABSTRACT

Seven different brands of cellulose sponges and one polyurethane variety were evaluated for inhibitory properties on twelve strains of gram positive and gram negative bacteria. Sponges were cut in 13 mm or 17 mm discs, autoclaved and aseptically placed on inoculated Tryptic Soy agar plates. The inhibitory effects of sterile sponges, unrinsed, and rinsed in distilled water, were measured. The zone of inhibition values were based on the average of the diameters of the clear zones on the inoculated plates. Polyurethane and EXPANDING CELLULOSE SPONGES were the only varieties which did not exhibit antimicrobial properties with any of the selected bacterial strains. A thorough rinsing procedure was often insufficient to remove the inhibitory agents from the sponges. Listeria monocytogenes strain Scott A and Staphylococcus aureus, both gram positive, were strongly inhibited.

Sponges have been used to detect Salmonella and other bacteria in environmental studies such as sampling of equipment surfaces, floors, walls and work benches and in quality control operations with eviscerated poultry and red meat carcasses (1-4). Quevedo et al. (3) presented some of the advantages of using sponges for surface sampling in slaughterhouses when compared with other microbiological sampling methods that use Rodac plates, cotton swabs and membrane filtration. These advantages included the ability to sample larger surfaces and detect lower levels of contamination; lower cost of operation; and easier sample collection. The need for glass containers and prepared media at the sample collection site was eliminated by using sponges.

In none of the published papers mentioned above was a source of supply given, nor were brand names stated. Also not stated was whether or not the sponges had been checked for inhibitory properties. When the need arose for doing surface sampling of carcasses with sponges, knowing that most readily available sponges have detergents to keep them soft and that many detergents are bactericidal, we purchased polyurethane foam and additional cellulose sponges to test. The test was the presence or absence of zones of inhibition that developed around discs cut from the sponges and placed on inoculated plates. Sponges that were inhibitory were rinsed and tested again.

Our work stresses the need for including a screening procedure for antimicrobial properties before selecting sponges for bacteriological studies.

MATERIALS AND METHODS

Sponges

Cellulose sponges were purchased at retail in local stores or ordered from catalogues. Polyurethane foam (1/2” thick) was cut to our specifications of 3” x 5”. They were obtained as follows:

3. SPONTEX PURE (100%) CELLULOSE SPONGE. Spontex, Inc., Columbia, TN.
5. Generic cellulose sponge (no brand name). General Services Administration.
6. EXPANDING CELLULOSE SPONGE. Nation/Ruskin Inc., Montgomeryville, PA.
7. E-Z ONE CELLULOSE SPONGE. Nation/Ruskin Inc., Montgomeryville, PA.
8. DUPONT PURE CELLULOSE SPONGE. Dupont Co., Wilmington, DE.

Sponge types #1, #2, #3, #4, #6, #7, and #8 were cut in circles of about 12-13 mm in diameter using a #9 cork borer. Sponge type #5 was also cut with a #9 cork borer; when moistened it expanded to an average diameter of 17 mm. The sponge discs were placed in separate pint mason jars and moistened with distilled water. The pH readings of the moistened sponges were recorded. The pint mason jars containing the sponges were autoclaved for 20 min at 121°C.

Test Cultures

The following cultures were on hand in the laboratory: Serratia marcescens, Pseudomonas putrefaciens, Salmonella newport, Salmonella typhimurium, Pseudomonas fluorescens, Escherichia coli ATCC 23355, Staphylococcus aureus ATCC 6538, Brochothrix thermosphaeta ATCC 11509, Enterobacter cloacae ATCC 23355, Aeromonas hydrophila, E. coli 0157:H7 strain 933 and Listeria monocytogenes strain Scott A.

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All cultures were kept refrigerated on Tryptic Soy Agar (TSA; Gibco) slants at 4°C. For each test, each bacterial culture was transferred to culture tubes containing 9 ml of Tryptic Soy Broth (TSB; Gibco) and incubated at 35°C overnight. A 0.1 ml sample from the first TSB tube was transferred to a second tube containing 4 ml of TSB. The second tube was incubated at 35°C overnight. The optical density of the cultures was adjusted to 0.5-0.6 (25-32%) T at a wavelength of 600 nm in a Bausch and Lomb Spectronic 20. Cultures were adjusted by either dilution with sterile TSB or by centrifugation and resuspension of the cell pellet in sterile broth.

Zones of inhibition testing

After the optical density of each bacterial culture was adjusted, a sterile swab was dipped into the culture. The tip of the swab was pressed against the inside of the tube just above the level of the broth, draining the excess liquid. The swab was used to spread the inoculum on the surface of a large (150 x 15 mm) plate containing 80 ml of TSA. The plate was swabbed in three directions to obtain a uniform lawn of growth. For each experiment, three plates were prepared for each bacterial strain. Two to three sponges per plate (each sponge from a different variety) were aseptically placed on each agar surface. All plates were incubated upright at 35°C for 22-24 h. This procedure was performed three times for each culture and sponge type. Since the zones of inhibition were not always perfectly round, their vertical and horizontal diameters were measured in millimeters.

Rinsed sponges

For the second stage of the experiment, sponges of each type were placed in separate stomacher bags. Two liters of distilled water were added to each bag. The sponges were processed in a Colworth Stomacher 3500 for two min. At the end of this step, the distilled water was decanted. This rinsing procedure was performed three times. The rinsed sponges were cut into discs and sterilized. The zones of inhibition testing procedures for the rinsed sponges were identical to those described above for the unrinsed sponges.

RESULTS

The inhibitory properties of each rinsed or unrinsed sponge against each bacterial species are shown in Table 1. These values represent the average diameters (in mm) of the zones of inhibition from all three experiments. The pH values for the moistened sponges before autoclaving were: Sponge type #1: 5.6; Sponge type #2: 5.8; Sponge type #3: 5.8; Sponge type #4: 5.6; Sponge type #5: 5.5; Sponge type #6: 5.3; Sponge type #7: 5.5; Sponge type #8: 5.6.

Polyurethane foam and EXPANDING CELLULOSE SPONGES had no inhibitory effect on any of the test cultures. The unrinsed sponge types with the highest inhibitory properties were sponge types #2, #3, and #7. Each of these sponge varieties had some type of inhibitory action on nine out of twelve challenge organisms. Zones of inhibition for unrinsed sponge types #2, #3, and #7, respectively, ranged from 0.7 to 16.5 mm, 0.8 to 11.3 mm and 0.3 to 9.3 mm. Unrinsed sponge type #4 showed some inhibitory action on a total of eight cultures. Zones of inhibition ranged from 0.3 to 6.7 mm. Unrinsed sponge type #5 inhibited the growth of seven cultures, and sponge type #8 inhibited four cultures.

For sponge types #2, #3, #4, #5, #7 and #8, a total of 72 rinsed versus unrinsed trials were compared. In 20 cases, the rinsing procedure reduced the diameter of the inhibition zone to zero mm. In 27 cases, the rinsing procedure usually reduced the diameter of the inhibition zone but did not totally remove the microbial inhibitors from the sponges. In the remaining 25 cases, the microorganisms were not inhibited by either the unrinsed or the rinsed sponges.

Salmonella newport and Salmonella typhiurium were not inhibited by any of the sponge types. The organism most susceptible to the inhibitory action of the rinsed or unrinsed sponges were Listeria monocytogenes strain Scott A, Staphylococcus aureus, Brochothrix thermosphacta, Aeromonas hydrophila, Pseudomonas putrefaciens and Pseudomonas fluorescens. E. coli (ATCC 23528 and 0157:H7), S. marcescens, and E. cloacae were slightly inhibited by some types of unrinsed sponges.

DISCUSSION

The results of this study indicate that retail cellulose sponges may be unsuitable for microbiological sampling procedures because of bacterial inhibitors. Rinsing was not totally effective.
often insufficient to remove the inhibitory agents from the sponges. Therefore, before the initiation of a microbiological surface sampling procedure, sponges should be tested for inhibitory properties.

The bacteria that were most susceptible to the inhibitory properties of the sponges are all of interest to food microbiologists and the pathogens, at least, might well be sought in environmental sampling to trace sources of contamination.

The only disadvantage encountered with polyurethane sponges was the lower capacity of absorbing liquids when compared with cellulose sponges. The polyurethane sponges as used in this study were not able to absorb more than 5 ml of buffered saline. The purchase cost of the only non-inhibitory cellulose variety was five times higher than the polyurethane brand we tested.