Filtering Out Food Debris Before Microbiological Analysis

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Received 3 February 1981/Accepted 9 April 1981

Sterile disposable pipette "filter tips" capped with polyethylene mesh (111-μm pore size) removed bothersome debris from food suspensions before microbiological analysis. A study comprising 576 analyses of Escherichia coli and Staphylococcus aureus in lean and regular ground beef, chicken, cheddar and mozzarella cheeses, green and lima beans, rhubarb, and beef and turkey pot pies, showed that these filter tips did not reduce bacterial recovery.

Debris from food samples can be troublesome in microbiological analyses. The initial suspension may be unpipettable, colonies may be obscured, and an electronic counting may be impossible. We were particularly concerned about this last possibility when counting bacteria on hydrophobic grid-membrane filters (HGMF [2]).

Depth prefilters (paper, asbestos, etc.) clarify suspensions but can quickly be shown to dramatically reduce bacterial recoveries. We developed disposable pipette "filter tips" capped with polyethylene mesh; these clarified suspensions satisfactorily. This report compares the effect of these filter tips on bacterial recoveries; six foods containing Escherichia coli or Staphylococcus aureus were analyzed by (i) HGMF inoculated with prefiltered food suspension or (ii) and (iii) pour or spread plates inoculated with prefilttered and unfilttered suspensions.

MATERIALS AND METHODS

Filter tips. Filter tips are shown in Fig. 1. Short (25-mm) lengths of polyethylene tube (4-mm inside diameter; 1-mm wall) were capped with Spectrasmesh polyethylene mesh (nominal 111-μm pore size; Cole-Parmer Instrument Co., Chicago, Ill.) by using a welding device constructed in our laboratory. They were transferred to a tray-type dispenser and sterilized by ultraviolet irradiation.

HGMF. Sterile HGMF (90 by 60 mm, 0.45-μm pore size, 2,500 grid cells) were purchased from QA Laboratories Ltd., Etobicoke, Ontario, Canada. They were inoculated in a square filtration apparatus made in our laboratory.

Foods. Ground beef (lean and regular), chicken, cheese (cheddar and mozzarella), frozen beans (green and lima), frozen rhubarb, and pot pies (beef and turkey) were purchased locally. Two samples of each food were divided into eight subsamples and stored at -20°C in sterile pouches.

Microbial counts. Randomization schemes were designed to compare the performance of three methods of microbial analysis for six foods and two samples within these foods, totalling 576 analyses. Subsamples for the day's work were thawed each morning, spiked with very dilute suspensions of E. coli or S. aureus from overnight tryptic soy broth cultures, and stored as decimal dilutions. For E. coli, 1.0-ml samples measured in 1-ml pipettes fitted with filter tips were inoculated into violet red bile agar plates or onto HGMF which were laid on violet red bile plates. Unfiltered 1.0-ml samples were inoculated into violet red bile pour plates. Samples (0.5 ml) of S. aureus-contaminated suspensions were prefiltred or not, as required, and inoculated either onto HGMF or as spread plates on Baird-Parker agar. Plates (all in duplicate) were incubated at 35°C, 18 to 24 h for violet red bile and 24 to 28 h for Baird-Parker agars. Incubating the Baird-Parker plates for a further 24 h did not increase the number of colonies. Lactose-positive colonies on violet red bile agar were counted as E. coli; black shiny colonies showing typical egg yolk reactions on Baird-Parker agar were counted as S. aureus. Confirmation that the correct colonies were counted on HGMF was made by lifting the filter. Typical egg yolk reactions in the agar substrate.) HGMF scores were converted to the most probable number of growth units as previously described (3).

Statistical methods. The significance of the following sources of variability was studied by analysis of variance techniques: (i) method, (ii) method by food interactions, and (iii) method by sample (within food) interactions. The last two were included to determine whether performance depended on the food or sample type analyzed. A 5% significance level was used throughout unless otherwise noted; all analyses were conducted on log_{10} transforms of the data.

RESULTS

In the first experiment, in which ground beef, chicken and cheese were studied, there were no differences in recovery of E. coli by the three methods (Table 1). However, there were significant differences in S. aureus recovery. Subsequent multiple comparisons (Table 2) showed the cause to be significantly higher counts from
prefiltered HGMF and unfiltered spread plates compared with prefiltered spread plates. There were no other pairwise differences.

In the second experiment, recovery of *E. coli* from beans, rhubarb, and pot pie showed a significant method-by-food interaction. This resulted from the rhubarb counts, which were as expected on HGMF but were very low on both filtered and unfiltered plates. When this set of data was omitted from the analyses, the method-by-food interaction disappeared, and recoveries by all three methods were essentially the same for *E. coli*. Significant method differences were again noted in *S. aureus* recovery but no particular problem with rhubarb was noted. Multiple comparisons (Table 2) showed marginally significantly higher counts from prefiltered HGMF and prefiltered spread plates, relative to unfiltered spread plates. No other pairwise differences were observed.

The data set for *E. coli* counts in rhubarb was analyzed separately. Method differences were apparent; multiple comparisons showed that whereas recoveries from prefiltered and unfiltered pour plates were equivalent, they were significantly lower than recoveries from prefiltered HGMF (Table 2).

**DISCUSSION**

The use of disposable prefilters on pipettes greatly improved the clarity of food suspensions, facilitating the counting of colonies. Bacterial recoveries with and without prefiltration did not differ significantly in 5 of the 12 instances studied. In four of the remaining instances, the unfiltered method did not differ significantly from at least one of the methods using prefiltration. In the last three instances, the prefiltration methods showed the better recovery. We conclude that the prefiltration step did not adversely affect bacterial recoveries.

Growth inhibitors occurring in foods can reduce bacterial recoveries in conventional methods (1). One advantage of membrane filters in the microbiological analysis of foods is their ability to remove such substances (4). The apparently normal recovery of *E. coli* from rhubarb on HGMF when plate counts were very low probably reflects removal of acid or other inhibitors at the membrane filtration stage.

Our original intention was to carry out this study on naturally contaminated foods. How-
ever, these did not materialize in sufficient quantities to satisfy the statistical protocol. Some caution is, therefore, required in extrapolating these results to everyday situations.

ACKNOWLEDGMENTS

We thank George Jarvis for the experimental design and statistical analysis and Istvan Dudas for making the filter tips and square filtration apparatus.

LITERATURE CITED