Enterobacteriaceae and Pseudomonas aeruginosa Recovered from Vegetable Salads

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Klebsiella, Enterobacter, and Serratia were recovered frequently in high counts from vegetable salads. Pseudomonas aeruginosa, although isolated frequently, was in lower counts.

In studying the flora of foods served to patients in a hospital we recovered enteric bacteria and Pseudomonas aeruginosa from vegetable salads. Although others have recently reported isolation of bacteria from salads (8, 10) and vegetables (2, 3, 8), this investigation attempts a detailed examination of the frequencies of recovery and counts of various species of Enterobacteriaceae and P. aeruginosa.

Fresh vegetable salads were obtained from the hospital’s kitchen from trays prior to delivery to the wards and before addition of spices and/or dressings. The salads were prepared with vegetables which had been washed and rinsed with tap water in colanders and allowed to drain for not longer than 5 min until cut. The vegetables included tomatoes, radishes, celery, carrots, endive, cucumbers, and lettuce, most of which was received from Florida and central California. All samples were homogenized with an equal volume of sterile distilled water in a Waring blender for 1 min. A sample (0.1 ml) of the original homogenate and of 10-fold dilutions of the homogenate were plated in duplicate on eosin methylene blue agar, a modified brilliant green agar for isolation of Klebsiella (9), cetrimide agar (0.03% cetrimide [Sigma] in Mueller-Hinton [Difco]), Trypticase soy agar (BBL), and a deoxyribonuclease medium modified as follows for the isolation of Serratia: deoxyribonuclease agar with methyl green (Difco), 4.2%; L-arabinose, 1.0%; phenol red, 0.005%; and 1% methyl green, 0.4%. Antibiotics were added in the following final concentrations: ampicillin, 5 μg/ml; colymycin, 5 μg/ml; cephalothin, 10 μg/ml; and amphotericin B, 2.5 μg/ml (D. M. Berkowitz and W. S. Lee, Abstr. Annu. Meet. Am. Soc. Microbiol. 1973, M191, p. 105).

A portion (0.5 ml) of the original homogenate was added to each of two tubes containing 5 ml of acetamide broth (11). The cultures were incubated for 48 h as follows: eosin methylene blue agar and modified brilliant green agar at 37 C, cetrimide and acetamide media at 42 C, deoxyribonuclease at 32 C, and Trypticase soy agar at room temperature. Colonies from cetrimide plates which fluoresced under a Wood’s lamp were counted as P. aeruginosa and confirmed as such by the scheme of Kantor et al. (7). Acetamide broth cultures were transferred to cetrimide agar only when the original cetrimide plates were negative for fluorescent colonies of P. aeruginosa. Such positive acetamide broth cultures were the source of the low counts indicated in the range of 10^6 to 10^5. The enteric bacteria were identified by the schemes of Edwards and Ewing. (4).

The frequencies of recovery and counts of the bacteria recovered from salads are shown in Table 1. Enterobacter agglomerans was the organism most frequently recovered (85% of samples), with counts ranging from 10^2 to 10^6 colony-forming units/g. Other gram-negative bacteria isolated frequently and mostly in high counts were E. cloacae (48%) and Klebsiella (46%). E. aerogenes and the three species of Serratia, although in relatively high counts, were found in less than one-third of the salads examined. P. aeruginosa was recovered from 44% of the samples; however, its counts were lower (1 to 10^3 colony-forming units/g). Escherichia coli, Citrobacter, and Proteus were recovered in less than 4% of the salads.

Thus the bacteria found in salads most frequently and in highest counts belonged to the Klebsiella-Enterobacter-Serratia group of Enterobacteriaceae. These members of Enterobacteriaceae, although usually regarded as human pathogens, have also been considered inhabitants of soil and plants. In fact, E. agglomerans was considered a plant saprophyte prior to its recent recognition as a human pathogen (5). Taxonomists are of the opinion that Klebsiella and Enterobacter show a high degree of related-
ness to the plant bacteria *Erwinia* (1, 12).

We had earlier shown that salads were not contaminated with *P. aeruginosa* during the preparation in the hospital kitchen. Instead, the bacteria were already present on the vegetables (8). Concurrently, Green et al. (6) found *P. aeruginosa* in the soil of vegetable fields and suggested the pseudomonads may be acquired by vegetables from the soil. The high frequency of recovery and high counts of the *Klebsiella-Enterobacter-Serratia* group in our present results also indicate that these bacteria are natural flora of vegetables and are not necessarily contaminants from humans.

Thus vegetable salads may serve as a reservoir for the above bacteria for the colonization and infection of susceptible patients. The extent to which this occurs is currently under investigation.

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**LITERATURE CITED**