

## Limits In Assessing Microbiological Food Safety

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### ABSTRACT

Scientific information pertaining to the incidence of foodborne disease and the sources of pathogenic microorganisms is often limited in relation to the knowledge needed to make informed microbiological food safety decisions. Inherent limitations in the current epidemiological reporting system constrain its usefulness for ascertaining the true incidence of foodborne disease. Additionally, current detection methods are insufficient to make real-time decisions on the microbiological safety of products. An integrated approach that combines enhanced epidemiological data, improved detection methods, detailed knowledge of the behavior of pathogens in food systems, and development of techniques for making quantitative risk assessments is essential for the development of a comprehensive, cost-effective strategy for assuring microbiologically safe foods.

Basic understanding in food microbiology, particularly in relation to pathogen survival, growth, and toxin production, has increased dramatically over the past 30 years. Research has led to the development of a spectrum of sophisticated methods for detecting and controlling the microorganisms responsible for foodborne illness. However, professionals are increasingly called upon to defend the significance of their scientific achievements as observers note that the incidence of foodborne disease has remained steady or even increased during that same 30-year period. There is no doubt that much of this apparent lack of progress is illusionary, in that the statistics cannot help but reflect a doubling in the number of microorganisms which are recognized as causing foodborne illness. However, as the world has changed significantly over 30 years, so have the problems and expectations faced in controlling foodborne disease. Products and distribution systems are more intricate, consumer demands are more sophisticated, preservation systems are more complex, and competition in the marketplace is more intense.

Regardless of the progress made, there are also inherent limitations associated with the current approaches to identifying and controlling foodborne disease. Food safety professionals must be cognizant and capable of communicating these limits to prevent unrealistic expectations. Our intent is

to identify and discuss some of the limitations associated with two areas, the acquisition of epidemiological data, and the efficacy of microbial detection methodologies. In addition, an integrated assessment system that employs alternate sources of information to overcome some of the highlighted limitations is discussed.

### EPIDEMIOLOGY OF FOODBORNE DISEASES

The first step to solving a problem is knowing that a problem exists. In the case of foodborne disease, this is most often achieved through the acquisition and analysis of epidemiological data. There is no question that contaminated foods can be a significant vehicle for human disease (7,13,37-39). However, there are limitations associated with these data that must be considered when the information is used to set priorities for addressing microbiological food safety concerns.

In the United States, the primary means of gathering intelligence concerning foodborne disease is through the Centers for Disease Control's (CDC) National Foodborne Disease Surveillance System. Established in 1961, this program consolidates and reports outbreak data submitted from state and local public health agencies. While the program has provided reporting continuity, thus providing a basis for estimating the incidence and trends in foodborne disease, it does have significant limitations. The most obvious is that the incidence data for most foodborne diseases represent only a small fraction of the actual number of cases (5,13,18). There are several reasons for this under-reporting. Probably the most important is that the majority of foodborne diseases are mild, nonlife threatening, and self-limiting. Consequently, most individuals contracting the disease will not seek medical assistance. These cases go unrecognized and unreported. Even if individuals seek medical assistance, the attending medical staff must reach the conclusion that there has been a single source outbreak and mobilize the necessary resources to adequately investigate the outbreak to establish food as a vehicle. The initial identification often does not occur if the outbreak is small or the patients are not examined at a single location. Even if a single source outbreak is suspected, resource limitations often preclude conducting an adequate investigation, particularly in small municipalities. Finally, once finished the investigators must be willing to take the time to submit their findings to this voluntary reporting system. Considering these limitations, it is not surprising that for many foodborne diseases only 1 to 2% of the cases (usually the more severe ones) are counted among the CDC statistics (5,18).

Programs such as the National Foodborne Disease Surveillance System are targeted to the acquisition of outbreak data and do

not consider sporadic cases of foodborne disease. This focuses attention on diseases associated with large scale outbreaks, while reducing the importance of those that are sporadic in distribution. A good example is a comparison of the etiologies of gastroenteritis caused by *Salmonella* and *Campylobacter*. Relying on outbreak data alone suggests that the incidence of salmonellosis is 3 to 4 times that for campylobacteriosis (36). However, evaluations of clinical isolations from both epidemic and sporadic cases, by laboratories well-versed in the techniques for isolating both *Campylobacter* and *Salmonella*, provide a different picture. These studies indicate that the incidence rate for campylobacteriosis is more than double that for salmonellosis (8,21,31).

Passive surveillance systems that rely on the voluntary reporting of cases are insufficient to accurately assess the significance of foodborne transmission of diseases of a sporadic nature. Instead, active programs that aggressively investigate the incidence of specific diseases in target populations, including accompanying food histories and laboratory investigations to confirm a foodborne etiology are required. A good example is the recent CDC program to identify causes of sporadic listeriosis (28,32). The program coupled active reporting and investigation of cases with subsequent microbiological sampling of patients' foods to identify potential sources. However, this type of program is costly. In the absence of a clear threat, ongoing active surveillance systems on other than a research basis are difficult to justify, except possibly as part of a sentinel system for detecting new or reemerging microbial threats (24).

When epidemiological data are used to establish the relative importance of new microbial food safety concerns, "causality" must be established. The statistical correlation of the presence of a microorganism with a disease does not prove that the microorganism is the actual cause. Instead of being the etiologic agent, it is possible that an organism is present as a result of a disease. The traditional microbiological approach is to ensure that Koch's postulates have been fulfilled, with the isolated microorganism being shown to be capable of causing the disease. For example, extensive epidemiological data have implicated foodborne and waterborne *Aeromonas* as a cause of gastroenteritis (10). However, until isolates of this potential pathogen have been shown to cause gastroenteritis in humans or a close mammalian species, its specific role as a foodborne pathogen will remain unclear (25). Such scientific proof is increasingly more difficult to acquire; researchers are hesitant to become involved in the approval and reporting requirements associated with working with human volunteers. As will be discussed later, this is reinforced by concerns about significant sequelae with a number of foodborne diseases. Further, questions can be raised about the value of using healthy human volunteers as we increasingly deal with foodborne pathogens such as *Listeria monocytogenes* (17,30) or *Aeromonas hydrophila* (15,19,25) that primarily affect only specific high-risk subpopulations. Even with well-characterized pathogens such as *Salmonella*, there can be substantial differences in the levels of cells needed to produce a disease response in volunteers compared to the substantially lower levels that have been implicated in a portion of the foodborne and waterborne outbreaks.

Establishing causality has been an ongoing problem when assessing the role of raw foods, particularly those of animal origin, in transmission of foodborne diseases. Epidemiological investigations have implicated raw meat and poultry as important sources of pathogens in sporadic cases of foodborne diseases such as salmonellosis or campylobacteriosis. However, in many studies causality was not established because there was insufficient supporting microbiological data to definitively establish that the isolates from infected individuals were the same as those from raw food. Only recently have molecular biology techniques such as plasmid profiling, pulse electrophoresis, restriction mapping, etc. made it possible to determine unequivocally that isolates from

different sources are the same strain. When it comes to foodborne disease, a substantial amount of scientific detective work is often needed to identify potential cross-contamination caused by improper food handling practices and to determine the primary source of a pathogen (23).

Another problem faced by epidemiologists investigating foodborne disease is the effectiveness of their tools in relation to time. Epidemiological methods are most effective when the onset of symptoms closely follows the ingestion of a contaminated food. It is relatively easy to investigate an outbreak of staphylococcal food poisoning, where symptoms begin within a few hours. Investigations become much more complex when the incubation period for the disease stretches into days or weeks. For example, it is often difficult to trace the source of foodborne type A hepatitis outbreaks, where the onset time varies from 1 to 6 weeks. It is unlikely that patients will remember with any certainty the foods they consumed a month prior. It can lead to a reporting bias; diseases with longer onset times are less likely to be identified as foodborne.

A related limitation of epidemiological investigations has been their effectiveness in identifying the role of foodborne pathogens in chronic diseases. There is increasing medical data that suggest that many enteric bacteria, including those that cause acute foodborne diseases, are involved in the etiology of a number of chronic diseases (3,4). A relatively common sequelae for a small percentage of individuals involved in outbreaks of salmonellosis, campylobacteriosis, yersiniosis, etc. is the development of reactive arthritis, a painful inflammation of the joints which can last for several months. Other autoimmune diseases are suspected to involve enteric bacteria. For example, immuno-mimicacy of the histocompatibility HLA-B27 antigen with enteric bacteria is believed to play a role in the etiology of several rheumatoid diseases (3,27).

Individual susceptibility is increasingly recognized as an important factor in the etiology of foodborne disease. Classical food poisoning diseases typically have high attack rates. If nonimmunized individuals ingest sufficient levels of botulinal toxin, they will all develop symptoms. However, with other diseases, only a small portion of the population may be affected. Human listeriosis provides a timely example. Only a very small subpopulation is at risk for serious infections with this infectious agent (17,30). One of the food safety challenges during the past 5 years has been the development of techniques for identifying these high-risk populations and the subsequent development of realistic strategies for ensuring their protection. Being able to protect high-risk individuals is going to take on increasing importance as a greater portion of the population becomes less immunocompetent through aging, medical intervention, and disease. Likewise, individual susceptibility will have to be considered as we gain a better understanding of the relationship between foodborne bacteria and autoimmune diseases. For example, individuals that carry the HLA-B27 serum antigen have an increased susceptibility to a number of autoimmune diseases that may be associated with immuno-mimicacy for antigens present on the surface of foodborne pathogens. However, even here our understanding is incomplete since only a percentage of the HLA-B27 positive individuals actually develop symptoms after exposure (3). It is clear that any effective epidemiology program in foodborne diseases could be enhanced by overcoming current limits in assessing the impact of disease in high-risk subpopulations.

#### DETECTION METHODOLOGY

Another area where consumers and many food professionals often lack an appreciation of the limitations faced when attempting to assess microbiological safety is the microbiological examination of food products. Too often, tests are selected for the wrong reasons or the sampling protocols are insufficient to detect the microorganisms of concern. The only way to be absolutely sure that a food is free of pathogens would be to test all of the product just prior to

consumption. Because this is impossible, microbiological testing must be restricted to some realistic portion of the total food product. This and other methodological limitations must be dealt with each day when making decisions concerning the microbiological safety of foods.

#### *Limits of detection*

One of the biggest areas of confusion is the limits of detection associated with microbiological analyses. The term "zero-tolerance" often referred to in relation to infectious bacteria such as *Salmonella* and *Listeria* is a misnomer. What is really being said is that using a specified method there were no detectable pathogens in the sample being analyzed. For example, the "zero-tolerance" requirement for *L. monocytogenes* based on a 5-sample composite of 5 g each (total 25 g) is actually a requirement for no detectable *L. monocytogenes* per 5 g (2). The sensitivity of the test could be modified by increasing or decreasing the sample size. When establishing a sampling requirement, there has to be a balance between the level of concern and the practical limitations of sampling.

It is often useful when attempting to explain this concept to put it in terms that are more easily understood. For example, finding one *Salmonella* cell in a 2-kg sample that has 10,000 other bacteria per gram is the equivalent of trying to find one specific human in a 10-cubic mile space when there are 200,000,000 other people present.

Another characteristic of bacterial contamination of foods that severely limits the effectiveness of detection methodologies is the often nonhomogeneous distribution of microorganisms within the matrix. For example, one would not anticipate a problem detecting  $10^7$  *Salmonella* in 100 g of food. However, if all of the cells were present as a single colony within a 0.1-g portion, and only 1.0-g samples were being examined, there is strong probability that the pathogen would go undetected. To overcome this, techniques such as statistically based sampling plans must be used to provide the necessary degree of confidence, or at least allow the user to be aware of the inherent variability and reliability of the results.

A third factor that influences the limits of detection associated with detection methods is physiological injury to the target microorganism as a result of exposure to sublethal stresses. Such stresses can result from a variety of treatments associated with food processing operations such as thermal processing, exposure to acids, and the presence of antimicrobial agents. While noninjured cells of target organisms may grow readily on differential media, after cells are physiologically injured, they may no longer tolerate the media's selective agents (29). This results in quantitative recoveries that may be more than a 1,000-fold too low, giving unrealistic assessments of product safety. In extreme cases, bacterial cells become so damaged that revival requires special techniques, such as passage of the microorganism through a host animal. Such cells are referred to as being "viable but non-culturable". Not detecting injured cells can also have a large impact on the development of accurate food processing requirements as was recently observed with *L. monocytogenes* (12,22,30,33,34).

#### *Timeliness*

One of the most important practical limitations related to the usefulness of microbiological testing is its timeliness. In most instances, the need for rapid product turnover, either in terms of shelf life or storage capacity, precludes the routine use of microbiological assays to clear product. This reflects the fact that traditional analytical methods for specific pathogens can take 7 to 10 d. Even though there has been substantial progress during the last 20 years in the development of rapid methods, no method for the detection of low numbers of pathogens has successfully eliminated the need for a 24- to 48-h enrichment. Although improvements continue, at least for the immediate future, the timeliness of microbiological analyses will remain a major limitation.

#### *Moving target*

Another inherent limitation when dealing with microorganisms under conditions where there is the potential for microbial growth is that there is no guarantee that the results of microbiological tests performed today are going to approximate what will be seen tomorrow. The potential for growth coupled with the probability for mishandling means that unless absolute control of a product can be assured, microbiological status can change dramatically in a short time period. Difficulties in assessing the significance of microbiological test results are compounded by the practical consideration that most microbiological testing is performed at the plant; the point when the product should be at its best. Too often, there has been little validation of appropriateness of the microbiological tests and criteria selected in relation to various products (11). The establishment of effective criteria requires careful consideration of the relationship between the pathogenic or indicator microorganism being analyzed and the direct impact of the criteria on human health (26). Recently, there has been increased interest in new technologies, such as time/temperature integrators and predictive microbiology, that can be used to estimate the impact of changing storage conditions. However, these technologies are new and their effectiveness is still being evaluated.

#### *Which pathogen?*

A fundamental question when setting up a microbiological testing program is, what pathogens or indicator microorganisms should be included? While this seems self-evident, it is not a trivial question. It is impossible, both physically and fiscally, to test for all bacteria, fungi, viruses, protozoa, and parasites that are potential causes of foodborne diseases, let alone assay for microorganisms that may play a role in the microbiological quality of a product. Selecting and performing inappropriate tests is a waste of money and leads to a false sense of security concerning a product's safety. Ideally, microbiological testing for specific pathogenic species should focus on microorganisms that have been epidemiologically linked to a product. However, such information is often unavailable. An alternative is microbiological profiling, a technique that is discussed below.

#### *Virulence testing*

A concept that underlies current regulatory policy is that all isolates of a pathogen are equally pathogenic. However, it is well-known that this is not the case. For example, substantial differences exist among *Salmonella* species (35). This can include closely related members of the genus such as *Salmonella enteritidis*, which is moderately to highly pathogenic for humans, versus *Salmonella pullorum*, which is host-adapted to birds and only weakly pathogenic for humans (16,20). Our increasing ability to distinguish pathogenic isolates has made us aware of the need to consider genetic diversity. Probably the best example of how recent advances in our understanding of bacterial virulence has complicated the evaluation of microbiological analyses is *Escherichia coli*. Although it has been used for over 100 years as an indicator organism of enteric pathogens, it is now known that a percentage of *E. coli* isolates can cause one of several forms of gastroenteritis, at least one of which is potentially life-threatening (14). These pathogenic strains have arisen through the acquisition of extrachromosomal elements that include genes for various toxins and attachment factors. This means that to accurately assess product safety, analysis cannot be limited simply to determining the presence of *E. coli*. Additional analyses must be performed to determine the presence of virulence markers associated with the various classes of pathogenic *E. coli*. The differentiation of virulent and nonvirulent isolates of potential foodborne pathogens is likely to lead to interesting policy and legal questions. For example, should a food containing *Shigella*, the cause of bacillary dysentery, be deemed a risk to public health if the isolate lacks the large plasmid that is

required for the microorganism to be fully pathogenic? Our increased knowledge of bacterial virulence determinants and the ability of these microorganisms to transfer these genetic characteristics has emphasized another inherent limitation in assessing food safety. Even though an organism may not be considered pathogenic historically, no absolute guarantee can be made that it will not acquire the ability to cause disease in the future.

### MICROBIOLOGICAL PROFILING

It is evident that there can be significant limitations in the epidemiological and analytical information available for assessing microbiological food safety. This deficiency can be at least partially overcome by microbiological profiling, a process by which pertinent characteristics of a food are matched against the requirements of foodborne pathogens. The goal is to (a) identify the pathogens that are likely to be a problem in a food and (b) identify potential factors that can be manipulated to control the organism. The steps involved are:

- i. Determine quantitatively the characteristics of a food product in relation to the factors that influence microbial survival or growth. Examples include formulation and environmental factors such as temperature, pH, type of acidulant, water activity, type of humectant, sequesterants, atmospheric composition, antimicrobials, etc. These characteristics must be quantified both in terms of expected ranges and variability.
- ii. Once the pertinent characteristics have been identified, the next step is to assess the impact of each of the factors on the survival and/or growth of foodborne pathogens. In many foods, the data will have to be quantitative so that one can assess how quickly pathogens will grow. This step should include a realistic assessment of the potential for product abuse. In more sophisticated preservation systems employing multiple barriers, one may improve the predictive capability of the process if there are data available on how the various factors interact.
- iii. The third step in developing a microbiological profile is to consider the likelihood that a pathogen will occur in the food. For example, one can demonstrate in the laboratory that *Vibrio parahaemolyticus* can grow in temperature abused poultry meat. However, this estuarine organism is seldom isolated from that product and would be of minimal concern. Pertinent epidemiological or virulence data should also be factored into the assessment.
- iv. The final step in profiling a product is determining what options are available for successfully controlling the pathogens of concern. For example, if a thermal process is included, which of the pathogens are likely to survive and reinitiate growth? Do normal sanitation practices eliminate the organism? Can the product formulation be modified to better enhance pathogen control?

### INTEGRATED APPROACH TO ASSESSING MICROBIAL FOOD SAFETY

When employed alone, each of the three primary approaches for assessing the microbiological safety of foods, epidemiological investigations, microbiological analysis, and microbiological profiling has significant limitations. The three

approaches can be combined to develop an integrated strategy for acquiring and evaluating data from multiple sources that could eliminate many of the current limitations. However, to be fully effective, a fourth approach, risk assessment, needs to be integrated into the process. Such an integrated approach for assessing the microbiological safety of foods needs:

- i. *Enhanced medical and epidemiological intelligence related to the incidence, etiology, and sources of foodborne diseases.*
- ii. *Improved methods for the detection and enumeration of pathogens in foods.* Ultimately, quantitative data will be needed if effective risk assessments are to be realized. Ideally, research should focus on the development of more cost efficient, "multipathogen" tests similar to the multiresidue tests that have been developed for assessing the chemical safety of foods. A recent example is the development of a preenrichment formulation for the simultaneous detection of *Salmonella* and *Listeria* (6).
- iii. *Improved quantitative data on the behavior of pathogenic microorganisms in response to food formulation and storage variables.* Very little systematic quantitative data on the growth and survival characteristics of foodborne pathogens are actually available. It would be impossible to acquire quantitative data on each of the thousands of different foods available in the marketplace. However, recent advances in modeling techniques make it possible to estimate the behavior of pathogens using data generated in a limited number of "archetypal foods" selected to represent important classes of products (9). Increased availability of quantitative data will greatly enhance this new technology.
- iv. *Development techniques for making objective assessments of the relative risks associated with the various foodborne pathogens.* The data from epidemiological investigations, product evaluations, and microbiological profiling have to be integrated into an objective measure of relative risk. This type of risk assessment approach has been successfully employed for environmental toxicants but is only beginning to be explored in relation to foodborne diseases (1). Without the development of appropriate risk assessment techniques, efforts to establish priorities that address microbiological concerns will continue to be hampered. However, care must be taken in employing such an approach to ensure that the growth of pathogens and the integrity of the product until consumption are factored into the overall assessment. It would do little good to only estimate the impact of a specific level of *Salmonella* in a product at the time of manufacture on the incidence of salmonellosis. Consideration must be given to the likelihood that the product may be abused and the potential for the microorganism to increase in number as a result of that abuse.

The overall goal of an integrated food safety assessment would be to employ all available data from all available sources, and using risk assessment techniques, establish priorities in relation to microorganisms of concern for a process or product. However, the process does not stop with the assessment. The final step is to use this knowledge to allocate available resources in a cost efficient manner. This includes targeting products and processes that have an inherently

higher degree of risk, and redesigning product formulation and processes to realize more effective controls. In this regard, we can all learn from the experiences of the automobile industry. Quality and safety must be designed into a product; it cannot be inspected-in after the fact.

If the proposed integrated approach to assessing the significance of foodborne pathogens sounds familiar, it should. This is precisely the thought processes and information evaluation that should go into the hazard analysis phase of a good Hazard Analysis Critical Control Point program.

#### REFERENCES

- Albanese, R. A. 1992. Risk analysis and food safety J. Am. Vet. Med. Assoc. 201:245-249.
- Association of Official Analytical Chemists. 1986. FDA bacteriological analytical manual, 6th ed. Association of Official Analytical Chemists, Washington, DC.
- Archer, D. L. 1985. Enteric microorganisms in rheumatoid diseases: Causative agents and possible mechanisms. J. Food Prot. 48:538-545.
- Archer, D. L. 1987. Foodborne Gram-negative bacteria and atherosclerosis: Is there a connection? J. Food Prot. 50:783-787.
- Archer, D. L., and J. E. Kvenberg. 1985. Incidence and cost of foodborne diarrheal disease in the United States. J. Food Prot. 48:887-894.
- Bailey, J. S., and N. A. Cox. 1992. Universal preenrichment broth for the simultaneous detection of *Salmonella* and *Listeria* from foods. J. Food Prot. 55:256-259.
- Bean, N. H., and P. M. Griffin. 1990. Foodborne disease outbreaks in the United States, 1973-1987: Pathogens, vehicles, and trends. J. Food Prot. 53:804-817.
- Blaser, M. J., J. G. Wells, R. A. Feldman, R. A. Pollard, J. R. Allen, et al. 1983. *Campylobacter* enteritis in the United States: A multicenter study. Ann. Intern. Med. 98:360-365.
- Buchanan, R. L. 1992. Predictive microbiology: Mathematical modeling of microbial growth in foods. pp. 250-260. In J. W. Finley, S. F. Robinson, and D. J. Armstrong (ed.) Food safety assessment. American Chemical Society, Washington, DC.
- Buchanan, R. L., and S. A. Palumbo. 1985. *Aeromonas hydrophila* and *Aeromonas sobria* as potential food poisoning species: A review. J. Food Safety 7:15-29.
- Buchanan, R. L., F. J. Schultz, M. H. Golden, L. K. Bagi, and B. Marmar. 1992. Feasibility of using microbiological indicator assays to detect temperature abuse in refrigerated meat, poultry, and seafood products. Food Microbiol 9:279-301.
- Busch, S. V., and C. W. Donnelly. 1992. Development of a repair-enrichment broth for resuscitation of heat-injured *Listeria monocytogenes* and *Listeria innocua*. Appl. Environ. Microbiol. 58:14-20.
- Cliver, D. O. 1987. Foodborne disease in the United States, 1946 - 1986. Int. J. Food Microbiol. 4:269-277.
- Doyle, M. P., and V. V. Padhye. 1989. *Escherichia coli*. pp. 235-281. In M. P. Doyle (ed.) Foodborne bacterial pathogens. Marcel Dekker, Inc. New York.
- Dryden, M., and R. Munro. 1989. *Aeromonas* septicemia: Relationship of species and clinical features. Pathology 21:111-114.
- Dubos, R. J., ed. 1958. Bacterial and mycotic infections of man, 3rd ed. J. B. Lippincott Co., Philadelphia. pp. 378-379.
- Farber, J. M., and P. I. Peterkin. 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiol. Rev. 55:476-511.
- Hauschild, A. H. W., and F. L. Bryan. 1980. Estimate of cases of food- and waterborne illness in Canada and the United States. J. Food Prot. 43:435-440.
- Harris, R. L., V. Fainstein, L. Elting, R. L. Hopfer, and G. P. Bodey. 1985. Bacteremia caused by *Aeromonas* species in hospitalized cancer patients. Rev. Infect. Dis. 7:314-320.
- Jay, J. M. 1978. Modern food microbiology, 2nd ed. D. Van Nostrand Co., New York. p. 386.
- Johnson, K. E., and C. M. Nolan. 1985. Community-wide surveillance of *Campylobacter jejuni* infections: Evaluation of a laboratory-based method. Diagn. Microbiol. Infect. Dis. 3:389-396.
- Knabel, S. J., H. W. Walker, P. A. Hartman, and A. F. Mendonca. 1990. Effects of growth temperature and strictly anaerobic recovery on the survival of *Listeria monocytogenes* during pasteurization. Appl. Environ. Microbiol. 56:370-376.
- Kapperud, G., E. Skjerve, N. H. Bean, S. M. Ostroff, and J. Lassen. 1992. Risk factors for sporadic *Campylobacter* infections: Results of a case-control study in southeastern Norway. J. Clin. Microbiol. 30:3117-3121.
- Lederber, J., R. E. Shope, and S. C. Oaks, Jr. (ed.). 1922. Emerging Infections: Microbial threats to health in the United States. National Academy Press, Washington, DC. pp. 113-137.
- Morgan, D. R., P. C. Johnson, H. L. Dupont, T. K. Satterwhite, and L. V. Wood. 1985. Lack of correlation between known virulence properties of *Aeromonas hydrophila* and enteropathogenicity for humans. Infect. Immun. 50:62-65.
- National Academy of Sciences. 1985. An evaluation of the role of microbiological criteria for foods and food ingredients. National Academy Press, Washington, DC. pp. 65-131.
- Ogasawara, M., D. H. Kono, and D. T. Y. Yu. 1986. Mimicry of human histocompatibility HLA-B27 antigens by *Klebsiella pneumoniae*. Infect. Immun. 51:901-908.
- Pinner, R. W., A. Schuchat, B. Swaminathan, P. S. Hayes, K. A. Deaver, R. E. Weaver, B. D. Plikaytis, M. Reeves, C. V. Broome, and J. D. Wenger. 1992. Role of foods in sporadic listeriosis. II. Microbiology and epidemiologic investigation. J. Am. Med. Assoc. 265:2046-2050.
- Ray, B. (ed.) 1989. Injured index and pathogenic bacteria: Occurrence and detection in foods, water and feeds. CRC Press, Inc., Boca Raton, FL.
- Ryser, E. T., and E. H. Marth. 1991. *Listeria*, listeriosis, and food safety. Marcel Dekker, Inc., New York. pp. 45-65, 175-184.
- Sacks, J. J., S. Lieb, L. M. Baldy, S. Berta, C. M. Patton, M. C. White, W. J. Bigler, and J. J. Witte. 1986. Epidemic campylobacteriosis associated with community water supply. Am. J. Public Health 76:424-429.
- Schuchat, A., K. A. Deaver, J. D. Wenger, B. D. Plikaytis, L. Mascola, R. W. Pinner, A. L. Reingold, and C. V. Broome. 1992. Role of foods in sporadic listeriosis. I. Case-control study of dietary risk factors. J. Am. Med. Assoc. 267:2041-2045.
- Smith, J. L. 1990. Stress-induced injury in *Listeria monocytogenes*. pp. 203-209. In A. J. Miller, J. L. Smith, and G. A. Somkuti (ed.), Foodborne listeriosis. Elsevier. Amsterdam.
- Smith, J. L., B. S. Marmar, and R. C. Benedict. 1991. Influence of growth temperature on injury and death of *Listeria monocytogenes* Scott A during a mild heat treatment. J. Food Prot. 54:166-169.
- Starr, M. P., H. Stolp, H. G. Truper, A. Balows, H. G. Schlegel, (ed.) 1981. The Prokaryotes, vol II. Springer-Verlag, Berlin. p. 1151.
- Tauxe, R. V. 1992. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrial nations. pp. 9-19. In Nachamkin, Blaser, and Tompkins, (ed.), *Campylobacter jejuni*: Current Status and Future Trends.
- Todd, E. C. D. 1983. Foodborne disease in Canada - a 5-year summary. J. Food Prot. 46:650-657.
- Todd, E. C. D. 1989. Foodborne and waterborne disease in Canada - 1983 annual summary. J. Food Prot. 52:436-442.
- Todd, E. C. D. 1992. Foodborne disease in Canada - a 10-year summary from 1975 to 1984. J. Food Prot. 55:123-132.