THE PATHOGEN/COMMENSAL PARADIGM; CAN TAXONOMY PREDICT RISK?

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The ability to determine the risk of contracting a food borne illness is dependent upon the ability to detect and quantitate the pathogen in the environment. Risk is the probability of an exposure to a specific hazard to cause harm. Hazard, then is the potential of the organism to cause harm, and under this definition, all strains of an organism do not represent the same hazard or the same risk. The use of conventional culture based methods to enumerate and identify pathogens has tremendous limitations in this capacity. These limitations can be overcome using molecular ecology techniques based on sequence comparisons of nucleic acids (DNA and RNA) and can be used to provide a molecular characterization, which can be used to predict virulence determinants and phylogenetic relationships. The application of a variety of nucleic acid probes and the polymerase chain reaction (PCR) can potentially provide a complete description of an organism's genetic composition, the extent to which these activities are expressed, as well as taxonomic information. Thus this molecular approach serves to evaluate the presence of specific sequences in the environment and provide a link between knowledge obtained in pure culture and the microbial populations they represent in the environment. Three enteric community examples are presented for which genomics based approaches have been used to address genetic relationships and disease or risk associations. First, is the example of the commensal-turned-pathogen, vancomycin-resistant *Enterococcus faecalis* described by Paulsen et al. (2003). *E. faecalis* is a prominent member of the commensal gastrointestinal microbiome. However, a subgroup of *E. faecalis* isolates harbor a virulence island that contains vancomycin-resistance genes a well as genes involved in virulence and infectivity. These *E. faecalis* isolates represent a hazard and as such detection and quantitation of risk must be specific for this genotype and not others that lack the virulence island. A second example relates to the enteric pathogen *Salmonella*, for which there are over 2,200 serovars. The majority of disease causing isolates (for warm-blooded animals) fall into subspecies I, which also represents more that 60% of all *Salmonella* strains identified. It is also clear that genotype and serotype are not congruent (Weigel et al., 2004). Therefore, as with *E. faecalis*, the target for predicting risk or hazard must be pathogen specific, not organism specific. The invA gene is the target of many of these methods because it not only is specific to the *Salmonella* genus, but it is also found in all known serovars of *Salmonella* (Chiu and Ou, 1996; Galan et al., 1992; Rahn et al., 1992; Swamy et al, 1996). However, even though this is a specific target, when Salmonella prevalence is low in a sample set, a pooled sample PCR may be needed as an indicator of the samples that are likely to be Salmonella culture-positive and reduce the amount of work associated with those samples likely to be culture-negative (Singer et al., 2006). Finally, *Escherichia coli* O157:H7 has been analyzed by octomer-based genome scanning which has demonstrated that there are two genetically distinct O157:H7 populations, one that causes illness and another that may be incapable of causing illness in humans or that is not easily

transmitted to humans from cattle (Kim et al., 1999, 2001). This group has also identified more than 100 genetic markers for the different populations some that may be used as markers in fast, simple tests to identify different O157:H7 populations in samples from humans and animals (Yang et al., 2004). Again this illustrates the paradigm of strains that are commensal and not pathogenic and those that are pathogens. Thus development of markers for risk must address the hazard and not merely the taxonomy of a microbial group.

References:

Chiu, C.H., and J. T. Ou. 1996. Rapid identification of *Salmonella* serovars in feces by specific detection of virulence genes, invA and spvC, by an enrichment broth culture-multiplex PCR combination assay. J. Clin. Microbiol. **34**:2619–2622.

Galan, J.E., C. Ginocchio, and P. Costeas. 1992. Molecular and functional characterization of the Salmonella invasion gene invA: homology of InvA to members of a new protein family. J. Bacteriol. **174**:4338–4349.

Kim, J., J. Nietfeldt, and A.K. Benson. 1999. Octamer-based genome scanning distinguishes a unique subpopulation of *Escherichia coli* O157:H7 strains in cattle. Proc. Natl. Acad. Sci. USA. **96**:13288-13293.

Kim, J., J. W. Nietfeldt, J. Ju, J. Wise, P. Desmarchelier, N. Fegan, and A.K. Benson. 2001. Ancestral divergence, genome diversification, and phylogeographic variation in subpopulations of sorbitol-negative, b-glucuronidase negative *Escherichia coli* O157. J. Bacteriol. **183**:6885-6897.

Paulsen, I. T., L. Banerjei, G. S. A. Myers, K. E. Nelson, R. Seshadri, T. D. Read, D. E. Fouts, J. A. Eisen, S. R. Gill, J. F. Heidelberg, H. Tettelin, R. J. Dodson, L. Umayam, L. Brinkac, M. Beanan, S. Daugherty, R. T. DeBoy, S. Durkin, J. Kolonay, R. Madupu, W. Nelson, J. Vamathevan, B. Tran, J. Upton, T. Hansen, J. Shetty, H. Khouri, T. Utterback, D. Radune, K. A. Ketchum, B. A. Dougherty, and C. M. Fraser. 2003. Role of mobile DNA in the evolution of vancomycin-resistant *Enterococcus faecalis*. Science **299:**2071 – 2074.

Rahn, K., S. A. De Grandis, R. C. Clarke, S. A. McEwen, J. E. Galan, C. Ginocchio, R. Curtiss III, and C. L. Gyles. 1992. Amplification of an invA gene sequence of Salmonella typhimurium by polymerase chain reaction as a specific method of detection of Salmonella. Mol. Cell Probes **6**:271–279.

Singer, R. S, C. L. Cooke, C. W. Maddox, R .E. Isaacson, and R. L. Wallace. 2006. Use of pooled samples for the detection of Salmonella in feces by polymerase chain reaction. J. Vet. Diagn. Invest. **18**:319–325.

Swamy, S. C., H. M. Barnhart, M. D. Lee, and D. W. Dreesen. 1996. Virulence determinants invA and spvC in Salmonellae isolated from poultry products, wastewater, and human sources. Appl. Environ. Microbiol. **62**:3768–3771.

Weigel, R. M., B. Qiao, B. Teferedegne, D. A. Barber, R. E. Isaacson and B. A. White. 2004. Comparison of pulsed field gel electrophoresis and repetitive sequence polymerase chain reaction as genotyping methods for detection of genetic diversity and for making inferences about transmission of *Salmonella*. Vet. Microbiol. 100:205-217.

Yang, Z., J. Kovar, J. Kim, J. Niefteldt, D.R. Smith, R. A. Moxley, M. E. Olson, P. D. Fey, and A. K. Benson. 2004. Identification of common subpopulations of sorbitol-non-fermenting, beta-glucuronidase-negative *Escherichia coli* O157:H7 from bovine production environments and human clinical samples. Appl. Environ. Microbiol. **70**:6846-6854