Difficulties establishing causality in biological systems are abundant—and they affect efforts to assess risks of importance in microbiology, such as likelihoods of infectious disease or development of antibiotic resistance. Consider the case in which von Pettenkofer openly challenged Robert Koch on whether Vibrio cholerae causes cholera by drinking some of the cholera bacillus (Microbe, May 2006, p. 223). When von Pettenkofer failed to develop symptoms of cholera, he concluded that V. cholerae does not cause the disease. Of course, subsequent experimental research established that this microbe causes cholera, and von Pettenkofer merely proved that exposure to the pathogen alone is not sufficient to cause this illness.

A basic principle of risk assessment is that multiple factors, including the ingested or inhaled dose of a pathogen, determine the likelihood and severity of adverse effects. More generally for both chemical and microbial hazards, there is a widely accepted framework for risk assessment that encompasses four main elements: hazard identification, exposure assessment, dose-response assessment, and risk characterization. One key point is that dose-response predictions are uncertain, as exemplified in the von Pettenkofer case.

Assessing Risk of Infectious Diseases Proves a Complex Undertaking

With our understanding of host-pathogen interactions and infectious diseases, one could pose several alternative hypotheses to explain why von Pettenkofer did not develop cholera after ingesting V. cholerae. For instance, possible explanatory factors include host defenses, the virulence and physiological state of organisms in the ingested dose, the environment of the flask and gastrointestinal tract, or some combination of those factors. Perhaps von Pettenkofer was resistant to factor or highly tolerant of that pathogen. Thus, any one or several combined factors could have miti-

Summary

- Difficulties establishing causality are abundant, and they affect efforts to assess the most likely sources of microbiological and other biological or chemical risks of importance.
- Risk assessments of antimicrobial resistance that might be attributable to food animal sources model possible, not necessarily causal, scenarios. The suggestion that Monte Carlo simulation can convert possibilities to probabilities is a misleading oversimplification that complicates efforts to communicate about risk and uncertainty.
- The available scientific data are inadequate for predicting with certainty the likelihood and severity of adverse effects from infectious agents such as those responsible for bovine spongiform encephalopathy, avian flu, and diseases from intentional or accidental releases of biothreat agents.

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gated the biological effectiveness of his ingested dose.

A formal dose-response assessment for cholera would present evidence for the factors that cause or control pathogenesis and virulence, typically described in terms of the infectious disease triangle (interactions among the host, pathogen, and environment). Koch could have miscalculated the dose needed to cause illness in a human because his experience was based mainly on how pathogen preparations affected animals.

The von Pettenkofer example illustrates the difficulty in demonstrating causality in the absence of a systematic framework—in this case, sophisticated knowledge of how to verify infectious disease mechanisms through experiments. Even now, data for predicting infectious disease outcomes are generally correlative in nature, and often do not meet criteria for causality such as Koch’s postulates (Microbe, January 2006 p. 223). In more general terms, many nuances of proving or refuting causality escape the notice of even experienced scientists and practitioners of risk assessment.

### Problems with Risk Assessments in the Context of Antimicrobial Resistance

Risk assessment frameworks (e.g., CADDIS, http://cfpub.epa.gov/caddis/; the work of Harry Marks at the U.S. Department of Agriculture [USDA] in Washington) could provide a formal means for attributing causality, and thus strengthen the scientific basis of inferences. Officials at the Center for Veterinary Medicine (CVM) within the Food and Drug Administration (FDA) did not have an established framework for demonstrating causality when they focused on antimicrobial resistance in the past decade.

The issue they considered is whether and to what extent antimicrobial resistance may develop in bacteria that colonize food animals exposed to antimicrobial drugs, which are used to promote growth. Bacterial resistance genes can develop in the bacteria associated with such animals and may be transferred to other bacteria that colonize the human gastrointestinal tract, possibly leading to the clinical failure of drugs used in human medicine. CVM describes a possible causal pathway for human health impacts from food animal uses of antimicrobials and then uses Monte Carlo simulations to estimate that risk and attendant uncertainty.

This scenario for transmission of antibiotic resistance was discussed twice in last year’s Microbe magazine (March 2006, p. 115; July 2006, p. 303). In the first case, we think that Scott Hurd of Iowa State University in Ames confuses expert opinion or belief about hypothetical possibilities with scientific methods of establishing causality. For instance, we find his statement that simulation can convert “possibilities” into “probabilities” confusing and potentially misleading. Simulating a sequence of possible events does not provide sufficient demonstration of cause-and-effect relationships, particularly when this effort depends on subjective judgments to assign parameter values. In the second case, we observe that Peter Collignon of Australia National University in Woden also applies subjective assumptions and presumes causality in another possible scenario.

Although particular events may appear to be associated or correlated, they may not be causally related. Thus, Hurd’s conclusion that a particular Monte Carlo simulation of antibiotic resistance risks can convert “possibility” into “probability” oversimplifies. In our opinion, presenting one sequence of events as if it were the true causal chain rather than one possibility overstates the rigor of the available evidence. Indeed, both Hurd and Collignon simulated events representing little more than a series of hypotheses or beliefs regarding a scenario poorly defined by the available data. Neither perspective fully addresses uncertainty or objectively assesses the impact of alternative assumptions, even though definitive scientific data from direct experimental observations along the hypothetical pathways are lacking. Alternative scenarios are possible and selection of probable scenarios frequently engenders debate not only on parameter assumptions, but also on the forms of the models.

In general, risk assessments can provide objective estimates of risk and uncertainty when they characterize appropriate alternative scenarios. Recall that risk analysis is an “analytical-deliberative” process—one that describes probable behavior based on systematic testing of possible behavior. To work effectively, any such analysis depends on linkage to mechanistic information, not correlative associations, as a “reality check.”
Practices when Conducting Microbial Risk Assessments

Researchers and regulatory officials have been conducting risk assessments for decades. Typically, those efforts address a standard set of questions, including what can go wrong, how likely it is to go wrong, and what the consequences would be. Although these seem to be simple questions, the analytic process is not. It involves compiling and validating evidence and models, developing assumptions and extrapolations, making predictions for complex systems, and assembling interdisciplinary teams whose members exercise a good deal of judgment.

Risk assessments of adverse effects from infectious agents conducted by scientists at regulatory agencies are typically team efforts taking several years. These efforts depend on carefully developed principles and processes, including the principle that such efforts should be transparent and that the process should provide opportunities for stakeholders to comment. However, guidance is meager for providing sufficient transparency to distinguish objective inputs (scientific data) from subjective inputs (assumptions and judgments) or results of hypothetical simulations for different groups of stakeholders.

While general risk assessment principles are fairly well established, the quantitative methods used for assessing, managing, and communicating chemical, physical, and microbial risks are evolving as knowledge increases. The adequacy of current methods to distinguish between subjective opinion or belief and objective data or evidence continues to be controversial, particularly given the hypothetical nature of many microbial risks. Methodologic improvements are needed to improve decision-making under uncertainty.

Some Specific Challenges of Assessing Risk of Infectious Diseases

Ideally, risk assessment includes weighing bodies of scientific evidence relevant to a problem and appropriately addresses uncertainty within the biological system of concern. For instance, documenting plausible dose-response relationships can be a major challenge when assessing microbial risks. Variability in dose-response relationships, for example, is typically understated because data on host defenses, including innate and adaptive immune functions, are not routinely available. Moreover, making comprehensive models simple, reliable, and easy to update with new evidence or interpretation is also a monumental challenge.

Consider the risk assessment for bovine BSE that officials of the USDA commissioned in 1998. The nature of the agent that causes BSE, which is a transmissible, neurodegenerative, and fatal brain disease of cattle, remains a matter of scientific controversy. Although an infectious agent is present in the brains and other tissues of animals with BSE, not everyone agrees with scientists such as Stanley Prusiner of the University of California San Francisco that the causative agent is a prion. He describes prions as aberrantly folded proteins—in the BSE case, containing 250 amino acids but no RNA or DNA.

Uncertainties regarding the BSE infectious agent in the USDA risk assessment were attributed to many possible sources, including: (i) spontaneous mutations in cattle that develop symptoms; (ii) importation of infected cattle, meat products, and feed; (iii) transmission to cattle from domestic sheep with scrapie; (iv)
transmission to cattle from deer, elk, mink, or pigs infected with chronic wasting diseases; (v) transmission to cattle from domestic feed; and (vi) consumption of materials from prion-contaminated bovine carcasses.

USDA officials continued to draw on that BSE risk assessment model when they dealt with the first confirmed cases of BSE in U.S. cattle. Although the model was updated, it still stops short of claiming a causal relationship between those possible sources of risk and BSE. The major finding from the formal risk assessment is that these diseases, namely BSE and similar encephalopathies that affect humans, are not understood sufficiently to predict the likelihood of possible future cases from consuming beef products from BSE-infected animals in the U.S. food supply.

**Principle of Iterative Risk Assessment**

Risk assessments are by nature iterative, based on the available body of evidence and judgments. If direct evidence existed, risk could be calculated directly, without having to extrapolate to scenarios of particular interest to regulators. However, knowledge is nearly always incomplete, indirect, and ambiguous. Thus, risk assessment assumptions, models, and results need to be re-assessed as knowledge advances.

Recall how von Pettenkofer failed to become sick after consuming the agent that causes cholera. Three volunteers among a more recent challenge group of 38 healthy individuals administered 100 million bacterial cells of a virulent El Tor strain of *V. cholerae* successfully withstood that challenge and did not develop illness, according to Myron Levine and his collaborators at the University of Maryland School of Medicine in Baltimore. Any risk model for cholera therefore should include variables for host resistance to account for this outcome.

 Integrating advances in methodologies for host-pathogen interactions, such as In Vivo Induced Antigen Technology (IVIAT) applied by Stephen Calderwood of Massachusetts General Hospital in Boston and his collaborators, require development of more advanced methodologies in microbial risk assessment. Such detailed knowledge about interactions of hosts and infectious agents suggests future advances in risk assessment modeling beyond current empirical dose-response models. This level of analytical-deliberative process is essential to strengthen the scientific basis of predictions of resistance and susceptibility to adverse effects following exposures to infectious agents.

**ACKNOWLEDGMENTS**

The perspective represents the opinions of the coauthors and not policy of the Food and Drug Administration or the Oregon Department of Environmental Quality. The authors appreciate review of drafts of this article by Jeff Fox, *Microbe* Features Editor, and D. Anthony Gray of Syracuse Research Corporation’s Environmental Science Center.

**SUGGESTED READING**


