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Antimicrobial resistance: a microbial risk assessment perspective

Emma L. Snary[1]*, Louise A. Kelly[1,2], Helen C. Davison[1], Christopher J. Teale[3] and Marion Wooldridge[1]

[1]Centre for Epidemiology and Risk Analysis, Veterinary Laboratories Agency—Weybridge, New Haw, Addlestone, Surrey KT15 3NB; [2]Department of Statistics and Modelling Science, University of Strathclyde, Richmond St, Glasgow G1 1XH; [3]Veterinary Laboratories Agency—Shrewsbury, Kendal Road, Harlescott, Shrewsbury, Shropshire SY1 4HD, UK

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The emergence of antimicrobial-resistant microorganisms in both humans and food animals is a growing concern. Debate has centred on links between antimicrobial use in the production of food animals and the emergence of resistant organisms in the human population. Consequently, microbial risk assessment (MRA) is being used to facilitate scientific investigations of the risks related to the food chain, including quantification of uncertainty and prioritization of control strategies. MRA is a scientific tool that can be used to evaluate the level of exposure and the subsequent risk to human health relating to a specific organism or particular type of resistance. This paper reviews the recent applications of MRA in the area of antimicrobial resistance, and in particular, it focuses on the methods, assumptions and data limitations. Since MRA outputs are dependent on the quality of data inputs used in their development, we aim to promote the generation of good quality data by describing the properties that data should ideally possess for MRA and by highlighting the benefit of data generation specifically for inclusion in MRAs.

Keywords: data, models, uncertainty, targeted research

Introduction

For over 30 years, there has been much debate on links between antimicrobial use in the production of food animals and the emergence of resistance in the human population.[1] As a result of this, the use of several antimicrobial animal growth promoters has been banned in the European Union (EU) since the late 1990s.[2] The use of all remaining growth-promoting antimicrobials in food animals is due to be phased out within the EU in January 2006.[3] In addition to the growth promoters, the role of therapeutic antimicrobials in agriculture, e.g. fluoroquinolones, is also under examination. A ban on the use of such products could, however, have implications for animal welfare by limiting the availability of efficacious drugs to treat disease.[4]

Microbial risk assessment (MRA) is a scientific tool that can be used to evaluate the level of exposure and the subsequent risk to human health due to a specific organism or particular type of resistance. Such techniques are becoming increasingly used within the area of food safety because they facilitate scientific investigations of food-related risks including quantification of uncertainty and prioritization of control strategies.

Given suitable data, MRAs that investigate the emergence of resistance in any organism can, theoretically, be developed and hence used to provide decision-makers and the agricultural industry with information on which to base policies and codes of practice relating to food safety. MRAs are, however, highly dependent on data, and therefore data limitations can be a severe hindrance to their successful development. Poor quality input data will inevitably produce results with a large amount of uncertainty that can be overlooked by those commissioning MRAs. Policies based on such models could, in some circumstances, be counter-productive if the interpretation of the outputs of the model is incorrect.

This paper reviews the MRAs that have been recently applied to the area of antimicrobial resistance. In particular, issues such as the data limitations associated with the MRAs will be highlighted, as well as the methods used to overcome such data deficiencies. We aim to identify the types of data required for an MRA and hope this will help generate good quality data for future MRAs. One way in which that can be achieved is by collaborative projects, i.e. projects that have both a data collection and model development component. The identification of such data issues is key in determining the quality of

*Corresponding author. E-mail: e.l.snary@vla.defra.gsi.gov.uk

the MRA outputs and subsequent scientific advice generated from MRA.

Overview of antimicrobial resistance: a veterinary perspective

In the veterinary context, antimicrobials can be administered not just for the treatment of clinical illness, but also for several other purposes.[5] Overall, the usage of antimicrobials in animals can be separated into the following four categories: (1) therapy; (2) prophylaxis; (3) metaphylaxis and (4) growth promotion.

As in human medicine, clinically ill animals are treated for their symptoms on the basis of animal health and welfare, but also to prevent economic losses associated with death or decreased productivity as a result of illness.[2] Prophylactic treatment is used to prevent anticipated disease and, in the veterinary context, this is usually carried out by group administration. Likewise, metaphylaxis is also administered to a group, but to treat incipient disease in individuals and to prevent further outbreak in the group. Finally, antimicrobials can be used in low concentrations to increase the rate of growth and to optimize the feed conversion rate for the rearing of food-producing animals.

In human medicine, the majority of antimicrobial use is for the treatment of clinical disease on an individual basis. For these reasons, since 1969,[1] the use of antimicrobials in the production of food-producing animals has caused concern, particularly since many of the antimicrobials used in the veterinary field are important in the treatment of human illness and, furthermore, were being used for economic gain rather than therapeutic purposes. The use of animal growth promoters causes particular concern and the EU has taken steps to ban many growth promoters since the late-1990s based on the 'precautionary-principle', although some countries prohibited their use before this (e.g. Sweden).[6–9] The EU proposes to suspend the use of all remaining antimicrobial growth promoters (monensin, salinomycin, avilamycin and flavomycin) from the 1st January 2006.[3]

Attention has also been drawn to the use of fluoroquinolones (e.g. enrofloxacin) in food-producing animals since ciprofloxacin, a closely related drug, can be prescribed by general practitioners for gastroenteritis in humans.[10] Following investigations, which included an MRA, the Food and Drug Administration (FDA) in the USA is considering a ban on the use of the fluoroquinolone enrofloxacin in poultry.[11]

Antimicrobials are extensively used not only in the production of livestock and in human medicine, but also in aquaculture and in the treatment of companion animals. Coupled with these uses, the disposal of human sewage sludge and farm and abattoir waste may also be important in the dissemination of resistant bacteria. Vast quantities of such waste are spread over agricultural land, therefore potentially spreading resistant bacteria more widely in the environment, and in livestock and human populations. As a result of the widespread use of antimicrobials, be it for human or veterinary use, it is now clear that there exists a reservoir of resistant bacteria in humans, animals and the environment, and that this reservoir may impact on human health.

What is microbial risk assessment?

In the area of microbial risk concerned with human health, risk assessment is defined as a component of risk analysis; the other components being risk management and risk communication.[12] The Codex Alimentarius Commission (CAC), an international standard-setting organization for foods in international trade, and the EU Scientific Committee for Food has adopted the following four-step framework for risk assessment:[12] (1) Hazard Identification; (2) Exposure Assessment; (3) Hazard Characterization and (4) Risk Characterization.

Before the commencement of a risk assessment, a risk management question must be defined. This question will specify the microbiological hazard to be investigated and also the related consequence, for example, the risk of human infection. Defining the risk question is an important component of the whole process and must be given appropriate consideration before commencement of the risk assessment itself. The risk question will need to identify factors such as the country of concern, time period over which the risk is to be estimated and sequelae of interest. Once the risk question has been identified, the Codex framework can be used to outline the process that will identify and evaluate the consequences of the microbiological hazard.

The first qualitative step of the framework is Hazard Identification. In relation to antimicrobial resistance, the microbiological hazard may be a pathogen with resistance to a particular antimicrobial, a class of antimicrobials or a group of antimicrobials, for example vancomycin-resistant *Enterococcus faecium*, quinolone-resistant *Campylobacter* or multidrug-resistant *Salmonella* Typhimurium. Other possible microbiological hazards include the presence of a resistance gene, for example, the CMY-2 gene, which has been shown to confer cephalosporin resistance to *Salmonella*.[13,14]

Exposure Assessment aims to estimate the frequency and amount of the hazard to which a human or animal is exposed. It follows a number of steps, these are the development of the exposure pathway, collection of data and the development of the exposure model. Figure 1, taken from the WHO/FAO risk assessment for *Salmonella* in eggs and broiler chickens,[15] illustrates the typical exposure pathway used in an exposure assessment for a farm-to-consumption MRA. The prevalence of the hazard at all stages of the pathway is modelled, as is the quantity of the hazard. For a more detailed description of the exposure assessment step of a risk assessment, see Lammerding & Fazil.[16]

The consequence of a human or animal ingesting the hazard of interest is considered in the Hazard Characterization component of the risk assessment. The response from ingestion may focus on both the severity (for example, infection, illness, death) and the duration of the adverse effect; however there are many factors that will influence the response, and these can relate to the microorganism, the food and the host. Dose–response modelling is a quantitative approach to predicting the consequence of ingesting a certain amount of the hazard of interest and is typically generated from data from experimental feeding trials, outbreak investigations or trials involving surrogate hosts or pathogens.[17,18]

The final step, Risk Characterization, integrates the Exposure Assessment and Hazard Characterization components of the risk assessment to generate an overall estimate of risk. The overall estimate of risk is often quantified as the probability of infection per human per year or the annual number of cases per year.

Risk assessments can be qualitative or quantitative. However, regardless of type, the process is the same, i.e. the risk pathway must be identified, the data collected and the risk assessed. Qualitative risk assessment provides an estimate of risk in words, such as high, medium, low and utilizes all relevant data, including numerical data, in obtaining a conclusion. Qualitative assessments are normally

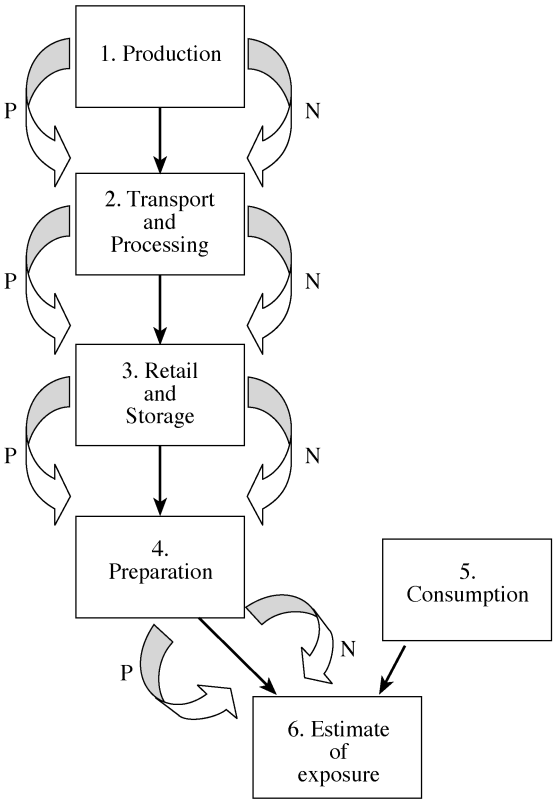

Figure 1. Modular pathway to describe the farm-to-consumption pathway. P: changes in prevalence; N: changes in numbers of organisms (reprinted from Ref. 15).

carried out before quantitative assessments and require fewer mathematical resources. The results generated from them can indicate whether or not a further quantitative assessment is required or possible. Quantitative risk assessments describe the biological processes using mathematical modelling techniques and therefore the estimate of risk is numerical. Again, all relevant data are used to build the model and the mathematical content of the MRA will be a direct consequence of the availability of data. The complexity of the model is, to a large extent, caused by the variability and uncertainty associated with the data, where variability describes the natural variation of the process (e.g. number of resistant bacteria per gram of cattle faeces) and uncertainty, the lack of knowledge (e.g. as a result of small sample sizes). Incorporating variability and/or uncertainty produces a final risk estimate with a variability or uncertainty distribution surrounding it, therefore providing further information, for example, confidence intervals.[19] The complexity of the model is also dependent on the risk question, with some questions requiring much less detailed analysis than others.

Although there may be significant uncertainties associated with the data or model, MRA can be used to prioritize data collection in order to reduce these uncertainties. Sensitivity or uncertainty analysis quantifies the degree to which the uncertainty associated with the parameters within the model affects the uncertainty of the output of the MRA.[20] Therefore, if the most sensitive model inputs have a large amount of uncertainty associated with them, the process will highlight where future resources for data generation should be directed in order to produce a risk estimate with a smaller degree of uncertainty, assuming the model is a good approximation to reality. Feeding the newly generated data into the model will normally reduce the uncertainty surrounding the final risk estimate, thus providing more precise information to decision-makers.

Microbial risk assessments applied to the area of antimicrobial resistance: methods, assumptions and data limitations

MRA, as predicted by the UK Advisory Committee on the Microbiological Safety of Food (ACMSF),[5] has played a role in the management of microbiological hazards, including antimicrobial resistance. As a result, a number of MRAs have been completed, or are ongoing, in the area of antimicrobial resistance. In particular, MRAs are being used to assess the possibility of a link between the veterinary use of antimicrobials in the production of animals and the emergence of resistant organisms in humans.

A list of antimicrobial resistance MRAs together with their key characteristics is given in Table 1.[21–38] In Table 2, the main data limitations associated with many of these risk assessments are summarized. Most of the assessments in Table 1 consider the link between the use of antimicrobials in animal production and resistant infections in humans; there are a few that do not consider this link[32,36,37] although these may be used to inform risk assessments that do. For example, Snary *et al.*[37] assessed the risk of the transfer of antimicrobial resistance genes in stored and spread animal waste, which could be built into a risk assessment investigating antimicrobial resistance bacteria on agricultural crops for human consumption.

From Table 1, it can be seen that, in order to manage the risks associated with the veterinary use of antimicrobials in animals, MRAs in the area of antimicrobial resistance are being commissioned by both Government and industry (i.e. pharmaceutical companies). In fact, in the USA, before the licensing of a new animal drug, the FDA proposes to request a qualitative farm-to-consumption risk assessment from the animal drug sponsor to investigate the microbiological effects of the drug on bacteria of human health concern.[39] As planned, the risk assessments will follow the Office International des Epizooties (OIE) risk framework[40] which is similar to the Codex framework[12] but categorizes the Hazard Identification step as a separate component.

Some of the risk assessments in the area of antimicrobial resistance have been carried out qualitatively, rather than quantitatively.[25–27] The MRAs carried out by Wooldridge[26] and Snary *et al.*[27] were qualitative assessments because the regulatory authorities needed MRAs at short notice to inform urgent policy matters. Several of the MRAs listed in Table 1 adopt the farm-to-consumption approach.[22–24,26,28,29] This approach is often taken if the aim of the MRA is to investigate the link between veterinary use of antimicrobials and resistance in humans. However, the data requirements for a farm-to-consumption MRA are normally extensive, since both prevalence and number of organisms are modelled, as are the biological or management factors that affect these levels, at all stages of the food production pathway. Furthermore, due to data limitations, it is often necessary to make important assumptions in undertaking this modelling; for example that the survival of resistant and susceptible organisms are equivalent under certain conditions. Lack of data, coupled with such generalized assumptions, means that farm-to-consumption modelling can result in large uncertainties associated with the description of risk.

If data are not available for the beginning of the farm-to-consumption pathway (i.e. the farm) an alternative approach is to

Table 1. MRAs applied to the area of antimicrobial resistance

Organism	Resistance	Country	Status	Funding	Key characteristics	Ref.
Campylobacter jejuni	fluoroquinolone	USA	complete	government	• quantitative • considers fluoroquinolone resistance that is attributable to the consumption of chicken • does not take a farm-to-consumption approach • clearly identifies assumptions and data limitations • details of validation not provided	21
Campylobacter jejuni	fluoroquinolone	USA	complete	industry	• quantitative • farm-to-consumption MRA, modelling both prevalence and microbial load • essentially an MRA for *Campylobacter* in chicken. Data on (human) FQ-resistance added in the last stages of the MRA • details of validation not provided	22
Campylobacter jejuni	fluoroquinolone	USA	complete	industry	• quantitative • as a result of lack of data an MRA that commences at retail • essentially an MRA for *Campylobacter* in beef. Data on (human) FQ-resistance added in the last stages of the MRA • model estimates compared to data from the Center for Disease Control (CDC) and US Department of Agriculture's Economic Research Service (USDA-ERS)	23
Campylobacter jejuni & *Campylobacter coli*	quinolone	UK	underway	government	• quantitative • MRA aims to investigate the contribution of different sources on human health • sources include chicken, pig, environmental routes, foreign travel and human-use of fluoroquinolones	24
Campylobacter jejuni & *Campylobacter coli*	erythromycin	generic approach	complete	not specified	• qualitative • uses erythromycin resistance as a marker to investigate the transmission of *Campylobacter* from pigs to man • details of validation not provided	25
Salmonella Typhimurium	fluoroquinolone	EU	complete	government	• qualitative • investigates the consequence of (fluoro)quinolone use in farm livestock on human health • details of validation not provided	26
Salmonella Newport	ACSSuT + full/intermediate resistance to 3rd generation cephalosporins	UK	complete	government	• qualitative • three components: (i) risk of importing multidrug-resistant *Salmonella* Newport (MRSN) into the UK from North America (ii) risk, if imported, of MRSN infecting livestock (iii) if livestock infected, risk of it spreading to other livestock • uses other *Salmonella* spp. as surrogate organisms • MRSN not detected in UK so difficult to validate	27
E. faecium	streptogramin	USA	complete	industry	• quantitative • preliminary farm-to-consumption MRA • details of validation not provided	28

Table 1. (*Continued*)

Organism	Resistance	Country	Status	Funding	Key characteristics	Ref.
E. faecium	streptogramin	USA	complete	industry	• quantitative • farm-to-consumption exposure assessment for SREf in broilers in the USA • identifies data deficiencies/limitations • use of surrogate organisms • acts as an input to the model developed by Smith *et al.*[31,32] • validates model to NARMS data[42] at the retail stage and also against community prevalence	29, 30
E. faecium	streptogramin	USA	complete	industry	• quantitative • related to Snary *et al.*[29] • conceptual, transmission model investigating the transmission of SREf within a US hospital • details of validation not provided	31, 32
E. faecium	streptogramin	USA	underway	government	• quantitative • feasibility study complete • will assess the human health impact of the development of the streptogramin-resistant *E. faecium* in humans that is associated with the use of virginiamycin in food-producing animals	33, 34
E. faecium	streptogramin	USA, Australia	complete	industry	• quantitative • does not take a farm-to-consumption approach • starts at the human level • investigates the consequence of a ban in the use of virginiamycin in Australia and the USA • details of validation not provided	35
Mixed species: Commensal *E.coli* and *E. faecium*	range (includes quinolones and avoparcin)	UK	underway	government	• quantitative • considers the persistence of antimicrobial resistance on a conventional broiler farm since resistant bacteria are still isolated after the veterinary use of the drug is suspended • parallel data collection	36
Mixed species: *S.* Typhimurium, commensal *E.coli* and *E. faecium*	conceptual model	UK	complete	government	• quantitative • cattle, pig and poultry waste • investigates the impact of the farm management of animal waste on the risk of resistance gene transfer in waste • parallel data collection • data not available for the validation of the models	37
Mixed species	range	UK	complete	not specified	• semi-quantitative • based solely on expert opinion • investigates the impact of antimicrobial resistance in bacteria and the contribution of animal sources to resistance in humans • details of validation not provided	38

Table 2. Key data limitations/issues affecting MRAs applied to the area of antimicrobial resistance

Data limitation/issue	Effect on MRA
Definition of resistance	• data from different sources are not comparable. May limit the amount of data available for the MRA
harmonization of MIC/disc-diffusion breakpoints required	
Microbiological methods	• the amount of data available for the MRA may be limited if the methods are not comparable
selective plating versus testing of one isolate from non-selective plate	• cannot compare selective plating against the testing of isolates without knowledge of the ratio of resistant to susceptible bacteria
enrichment versus non-enrichment, etc.	• if enriched, the number of organisms is increased and therefore cannot directly be used in the MRA
Multiple levels of the sampling framework	• large variability of sampling methods between studies. Therefore data from different sources may not be comparable; could limit the amount of data available for the MRA
Small sample sizes	• if the sample size is small at any level of the sampling framework, the uncertainty about the associated parameter will be large. This may contribute to a large uncertainty associated with the final risk estimate
Little data available on indicator organisms (resistant or susceptible) compared to pathogenic bacteria	• surrogate organisms, etc. may be used to overcome the data gap, thus increasing the level of uncertainty in the output of the model. This uncertainty may not be quantified
Sensitivity and specificity of the tests used	• MRA may overestimate or underestimate the risk
Causality unclear	• large assumptions made on the causality of antimicrobial resistance. This leads to a higher level of uncertainty in the model results, but which may be difficult to quantify
Lack of quantitative microbiological data	• microbial load of resistant bacteria in/on different sources is unknown, therefore either not modelled or key assumptions made
Little information on the use of antimicrobial agents for veterinary use (at animal and farm level), or human use	• causality difficult to consider. May lead to a large degree of uncertainty in the results of the model

start at a later point in the pathway. In particular, surveillance studies will often sample meat/meat products at retail.[41,42] The MRA for fluoroquinolone-resistant *Campylobacter* undertaken by Anderson *et al.*[23] started at retail for the reasons stated above. However, there is the potential for conflict here since, for example, a model that commences at retail may not be able to investigate the effect of an on-farm intervention such as the prohibition of a veterinary drug. Consequently, there needs to be a balance, given the available data, between what can be scientifically achieved and what is requested by the risk question. Anderson *et al.* did not consider the impact of on-farm interventions and therefore starting at the point of retail is adequate, particularly since there are data deficiencies before this point. If significant data deficiencies are identified, the primary aims of the risk assessment should be to use the model to identify data gaps and give insights into the overall process, rather than to produce predictions of risk.

If there are data gaps relating to the resistant bacteria, but data exist for the bacteria in general, another way to overcome these limitations is to develop an MRA for the specific bacteria, with the resistance component (and therefore resistance data) incorporated towards the end of the risk pathway. Both Anderson *et al.*[23] and Cox & Popken[22] used this approach for their MRAs for fluoroquinolone-resistant *Campylobacter*, in beef cattle and chickens, respectively. These authors essentially developed an MRA for *Campylobacter* and then introduced data from human clinical sources to assess the risk of fluoroquinolone-resistant *Campylobacter* from the food source.

The farm-to-consumption approach for establishing a link between the veterinary use of antimicrobials and human health is not the only approach that can be used to develop MRAs. The FDA-CVM model for fluoroquinolone-resistant *Campylobacter*[21] and the model by Cox & Popken for virginiamycin-resistant *Enterococcus faecium,*[35] both adopt an alternative approach. Focusing on the published FDA-CVM model, the overall aim of the model was to estimate the human health risk for different population bases. In essence, the probability of being affected by fluoroquinolone-resistant *Campylobacter* is given by the equation a/b, where a = the nominal mean number of fluoroquinolone-resistant *Campylobacter* cases attributable to chicken who seek care and are treated with a fluoroquinolone, and therefore affected by the fluoroquinolone resistance per year and b = the size of the population at risk. Different 'populations at risk' were considered; these were (1) a US citizen, (2) a US citizen with campylobacteriosis, (3) a US citizen with campylobacteriosis seeking care, and (4) a US citizen with campylobacteriosis seeking care and prescribed an antibiotic. Separating the population in this manner identifies the risk for the more vulnerable population, for example, a patient who seeks care and is prescribed an antibiotic. Even if a simpler approach such as this is taken, data limitations are frequently still a problem and many assumptions still have to be made.

For some antimicrobial resistance MRAs, good quality data for both the resistance and the organism *per se* are difficult to obtain. This is a particular problem with assessments for so-called 'indicator' organisms. Indicator organisms, such as commensal *Escherichia coli* and *E. faecium*, are of interest since they readily develop resistance to antimicrobials[43,44] and furthermore, they have the potential to disseminate their resistance genes to other bacteria, including patho-

genic bacteria.[45–47] Indicator bacteria can be transmitted to humans via the food chain in the same way as zoonotic food-borne bacteria, particularly since these bacteria will be present, potentially in high numbers, in most animal species. Especially at risk are hospital patients since enterococci commonly cause hospital-acquired infections.[48–51] The emergence of vancomycin-resistant *E. faecium* (VREf) is of concern since vancomycin is often used in hospitals to treat serious Gram-positive bacterial infections,[50] and is chemically related to the growth promoter avoparcin, which was used in Europe since the 1970s until its ban.[52] Likewise, the streptogramin virginiamycin, which was used as a growth promoter in Europe and the USA for more than 30 years, is related to quinupristin/dalfopristin, which can be used intravenously in humans to treat VREf infections.[52,53] For these reasons, indicator organisms have human health implications and, consequently, are frequently the subject of MRA (Table 1).

In comparison with pathogenic organisms, there are fewer suitable studies providing data for risk assessments of indicator organisms. This is particularly true in relation to quantitative microbiology, with information such as how indicator bacteria survive under certain conditions, microbial load and prevalence being severely limited. This is probably because, before the emergence of antimicrobial resistance, *E. faecium* was not of primary importance to human health since hospital-acquired infections could be treated with antibiotics such as vancomycin. Given these limitations, as discussed previously, unless alternative approaches to MRA are used (such as those used by Cox & Popken[35]), it may not be possible to develop an MRA without the use of surrogate organisms. Therefore, the availability of data for indicator organisms is a key data gap (Table 2).

A data limitation affecting many of the risk assessments summarized in Table 1 relates to the definition of resistance itself. This is an issue discussed by Davison *et al.*[54] The minimum inhibitory concentration (MIC) is the lowest concentration of antimicrobial that inhibits bacterial growth. For each organism and antimicrobial combination, there is an MIC interpretative breakpoint; if the MIC for an isolate is above this threshold, the isolate is defined 'resistant'; and if the MIC is less than (or equal to) the breakpoint, it is defined 'susceptible'. However, the breakpoints recommended by different national committees and regulatory authorities can vary, and are subject to debate.[55] Furthermore, although determination of MIC is a commonly used method, many published studies use a tablet or disc diffusion measure to define resistance.[56,57] Consequently, difficulties can occur when comparing the susceptibility of organisms to antimicrobials in different studies, and this can severely decrease the amount of data available for inclusion in an MRA. In recognition of this problem, efforts are now being made to standardize susceptibility tests, not just within the UK, but also on an international scale. Therefore before the commencement of an MRA, a definition of resistance should be established and, where possible, all data should be selected based on this definition.

Another key data limitation relates to the microbiological methods used at primary isolation. Two methods are commonly used. In the first, the culture substrate (e.g. faeces) containing bacteria of interest is inoculated onto or into primary isolation media containing an antimicrobial at a certain concentration (usually equivalent to the MIC breakpoint). If growth occurs on this selective medium, the sample is defined as positive for resistant organisms. In the second method, a single colony of the bacteria of interest is selected from a non-selective culture plate and tested for resistance (at the defined breakpoint). Each method is valid, but the results are not easily compared.[8]

The use of media containing an antimicrobial is common for the isolation of antimicrobial-resistant bacteria. This technique will commonly detect presence or absence of a specific resistant organism and, in many circumstances, this may be all that is necessary. Such microbiological techniques do not provide information on the overall population of the bacteria of interest in the sample.

In some studies,[41,58] the bacteria of interest will be cultured and a single colony (often of the predominant type) tested for antimicrobial resistance. Brun *et al.*[59] investigated the effect of within-sample diversity on antimicrobial resistance in faecal *E. coli* isolates in pigs. They concluded that, for information on antimicrobial resistance at the animal level (which is usually required by MRA), several isolates should be tested. This is because the single isolate method provides an estimate of the probability of an isolate being resistant. However, as before, without knowledge of the bacterial population within the sample, there is a potential for bias. Therefore, as highlighted by FDA-CVM,[21] data on the ratio between resistant and susceptible bacterial population are also necessary for this method of antimicrobial resistance testing in order to further our understanding of such data (Table 2). Although few data are available on within-sample variability, techniques have been published on both the percentage of resistant bacteria in a sample[60] and also the number of bacteria with different MICs within a sample.[61]

None of the MRAs listed in Table 1 make allowance for the sensitivity and specificity of the test. Such data (in any MRA) are often not available and rarely (if ever) given in studies relating to antimicrobial resistance. To provide such values would be problematic because of the many different organism–antimicrobial combinations. However, including sensitivity/specificity in the MRA leads to a more accurate risk estimate.

Sources of data for microbial risk assessment

Sources of data for MRAs applied to the antimicrobial resistance problem include published literature, data from on-going research projects and data collected by industry. Data can typically be divided into one of three groups; these are surveillance, observational research and experimental research.

Many countries operate surveillance systems in order to monitor the level of antimicrobial resistance in animals, foodstuffs and humans[41,56,62,63] and such surveys can be a valuable source of data for many reasons. First, if the sampling strategy is appropriate, it can give good indications of the level of antimicrobial resistance on a country level. There are many epidemiological factors that influence the level of antimicrobial resistance within a farm, region or country, and with a representative sample population these are incorporated into the data. Such data can be useful for validation of results from the MRA but not, for example, for estimating the likelihood of resistance acquisition for individual animals since this type of data would be obtained from a longitudinal study. If the microbiological tests and sampling regime are consistent over time, any trends in the level of antimicrobial resistance over time can be monitored.

Observational studies can provide information at different levels of interest (e.g. at the national, farm or animal level). In particular, longitudinal observational studies are useful since they provide information about incidence and trends over time. For example, Bager *et al.*[7] carried out a longitudinal study during which broiler chicken and pig farms were monitored for glycopeptide-resistant *E. faecium* following a ban of the growth promoter avoparcin in Denmark, and provided valuable information on the persistence of antimicrobial resistance at the farm level. Likewise, Welton *et al.*[64]

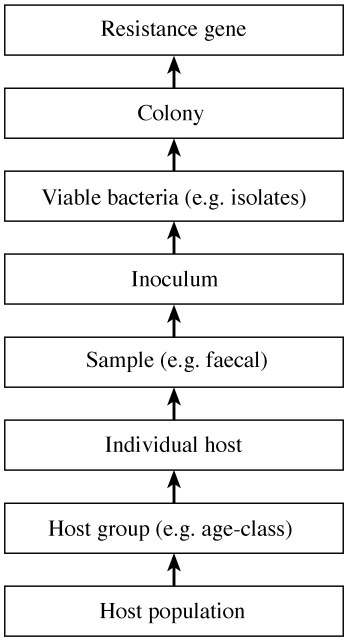

Figure 2. Multiple levels of a sampling framework to measure antibiotic resistance (reprinted with permission from Elsevier[54]).

fed virginiamycin to three flocks of turkeys (approximately 30 000 in each flock) on two farms and monitored the level of quinupristin–dalfopristin-resistant *E. faecium*.

Case–control studies are often carried out as field epidemiological studies and are good at generating hypotheses. They can be used in an MRA to investigate control strategies, and are therefore an important source of data.

Many data sets are obtained by experimental studies. For example, Jacobs-Reitsma *et al.*[65] administered the quinolones flumequine and enrofloxacin to groups of 15 *Campylobacter*-positive chickens in order to monitor the emergence of fluoroquinolone-resistant *Campylobacter* over 43 days. Studies such as these can give important data relating to the animal level rather than the general population, and are therefore extremely important in terms of input into MRAs. However, it should be noted that, in some circumstances, the experimental conditions can be artificial and so the degree to which the data can be extrapolated to the field situation is uncertain (e.g. when experimental work is undertaken in controlled laboratory conditions). Consequently, such studies may add additional uncertainty to the MRA.

Data collection for antimicrobial resistance MRAs is more complex than for non-resistance assessments because there is an extra level within the sampling framework which investigates the resistance profile of the sample. The additional data required by an antimicrobial resistance MRA can therefore include (depending on the risk question) the proportion of animals that are carrying resistant organisms, the proportion of tested isolates that are resistant, the ratio of resistant isolates to susceptible isolates, the presence of a certain resistance gene and data on the MICs of the isolates. The additional level to the sampling framework for antimicrobial resistance testing is demonstrated in Figure 2 and the paradigm equally affects surveillance, observational and experimental studies. The extra level involved in a sampling framework for antimicrobial resistance means that there is more variability of sampling methods between studies, thus increasing the difficulty associated with the comparison of different studies. Many researchers are now starting to recognize how their research may be useful in a future risk assessment, and therefore obtain advice on the sampling issues during the design stage.

With any type of data, sample size is an important consideration. Data input into a risk assessment can have its uncertainty and/or variability described by a probability density function. However, if the sample size is small at any level of the sampling framework, the uncertainty about the associated parameter will be large, which may contribute a large uncertainty associated with the final risk estimate. Both the sample size and the sampling framework provide limitations into the data available for antimicrobial resistance MRAs; this is highlighted in Table 2.

Overcoming data limitations/deficiencies in microbial risk assessment

There are many reasons why there may be limitations or deficiencies in the data required for MRA. In the area of antimicrobial resistance, there are many organism–antimicrobial–animal combinations that could be considered and therefore a limited number of studies for some combinations. Data availability can be further reduced by the incomparability of many of these studies, due to differences in the definitions of resistance or the microbiological methods used. Finally, the data may be labour intensive to obtain (for example, the ratio of resistant to susceptible bacteria) and therefore expensive. There are several ways in which data gaps or data deficiencies can be overcome, and these are summarized in Table 3. However, regardless of which method is selected, it is essential that the approach is appropriate, justifiable and transparent.

If there are no data, or data are sparse, one method of gathering information is the elicitation of expert opinion. This approach was used in numerous MRAs listed in Table 1.[23, 27–29,38] For example, Anderson *et al.*[23] used data generated by expert opinion[66] to estimate the intensity of illness from the ingestion of *Campylobacter jejuni*. An MRA could actually be based solely on expert opinion. For example, Bywater & Casewell[38] used such an approach to assess the impact of antimicrobial resistance in different bacterial species and of the contribution of animal sources to resistance in human infections. Any method used to elicit expert opinion should not only minimize any bias, but should also allow any bias to be understood.[20] Ideally, the estimates will incorporate both uncertainty and variability: uncertainty from the expert's level of knowledge and variability associated with the natural variation of the process, but this is not always possible. Consulting multiple experts enables the uncertainty to be quantified with more confidence.[20,67]

Surrogate organisms can also be used if limited or no data are available. The selection of a surrogate organism should be based on its biological characteristics (e.g. its ability to survive/multiply/die at certain temperatures) in comparison with those of the organism of interest. The idea of surrogate organisms can be extended to MRA in the area of antimicrobial resistance since susceptible bacteria have been used (directly or indirectly) as surrogates for resistant bacteria.[22,23,27] Likewise, in the absence of any selection pressure, a different type of antimicrobial resistance can be used. It is important to ensure that, if data for a surrogate organism is to be utilized, experts are consulted in order to have reasonable confidence that the choice of surrogate is appropriate.

Table 3. MRAs applied to the area of antimicrobial resistance: methods to overcome data gaps/deficiencies

Method	Comment
Expert opinion	• need to ensure that the method of elicitation minimizes bias • use of expert opinion to be clearly identified
Surrogate data different organism susceptible organism different antimicrobial resistance	• essential to consult with microbiologists to ensure that the chosen surrogate organism is appropriate • use of surrogate data to be clearly identified
Start at a later point in the risk pathway	• simplifies the model • may be difficult to investigate the effect of control strategies before this point
Predictive mathematical modelling	• increases the complexity of the model • beneficial for the investigation of control strategies

An alternative way to overcome data deficiencies or limitations in MRA is to change the approach. For example, as discussed above, due to no data at the farm and processing stage of the farm-to-consumption pathway, Anderson *et al.*[23] started their risk assessment at retail, the point at which data became available. Another approach is to develop a predictive mechanistic model to estimate the missing data. Such an approach was adopted by Hartnett *et al.*[68] in the farm module of a farm-to-consumption risk assessment for *Campylobacter* in chickens, where the within-flock prevalence (i.e. the probability of a random bird within a *Campylobacter*-positive flock being colonized with *Campylobacter*) was required. Not only did this overcome the data gap but it also gave added value to the risk assessment since the transmission model could be used to assess control strategies associated with within-flock transmission.

Although data limitation and deficiencies are often seen as a weakness of MRA, the identification of such limitations is an important output. In particular, by highlighting such data limitations or deficiencies, MRA can help to prioritize future research in the area of antimicrobial resistance. In practice, however, once the deficiencies have been identified and prioritized, it is frequently the case that no additional data generation occurs for these model parameters.

Validation and verification of MRAs in the area of antimicrobial risk assessment

Once the risk has been assessed, the MRA should ideally be validated against any suitable surveillance or observational data; however, in practice, this is difficult to do. As seen in Table 1, very few MRAs provide details of a validation step, which may be indicative of the problems associated with this step. Firstly, data, for the reasons described above, may not be available to compare against the risk estimate produced by the MRA (e.g. indicator organisms), although it may be possible to validate the MRA at intermediate steps, e.g. for a farm-to-consumption exposure pathway, the model could be validated at the retail stage. Secondly, if some data are available, they may not be directly comparable with the results obtained from the MRA. For example, surveillance data may provide the percentage of isolates on a food product that were resistant, whereas the MRA may estimate the percentage of food products that are contaminated with one or more resistant organisms. Likewise, an MRA will consider the prevalence and number of resistant bacteria from one particular source of interest (e.g. UK chicken); whereas data used to validate MRAs cannot specify the original source of the bacteria or whether the food-product was contaminated by other types of food-product (e.g. added ingredients) during processing or via food handlers. Finally, the validation of qualitative MRAs is not easy since it would require a numerical interpretation of the descriptive outcome of the MRA. Consequently, although recognized as a highly important step in the MRA process, many of the MRAs listed in Table 1 do not provide details of any type of validation.

All MRAs should be presented to interested parties (including regulators, stakeholders, microbiologists, veterinarians, etc.) and fully discussed in order to facilitate a review process. Throughout its development, people with appropriate expertise should review the risk assessment to ensure that the biological data used are the best available and that the underlying assumptions of the MRA are those currently accepted as correct. For some MRAs, e.g. FDA-CVM,[21] draft reports are available on the internet and a period of public consultation takes place. Furthermore, if the MRA is quantitative, an external risk assessor is often asked to review the mathematical methodologies on which the model is based. Therefore, although potentially difficult to validate MRAs with data, most MRAs are verified via peer review. Consequently, to allow the scrutiny of data, underlying assumptions and mathematical methodology, it is essential that the risk assessment is transparent at all stages of the development process. Once reviewed, the MRA can be updated and the process repeated, thus fully utilizing the iterative nature of MRA.

Current and future data collection for risk assessment

Every MRA requires data, and these can be of many different types. Many of the data requirements are given by Salisbury *et al.*,[69] who listed the key requirements according to the type of hazard (e.g. antimicrobial resistance gene). Although many researchers consult with a risk assessor before the start of a project, there still exists uncertainty relating to the types of data that are required for MRA. Unfortunately, there is no generic list of data requirements for an antimicrobial resistance MRA because the types of data required depend on the risk question. However, the way in which data are published can be improved in order to increase their usefulness.

There is now a wealth of published literature focusing on the topic of antimicrobial resistance. With respect to MRA, fundamental requirements for published data include the full details of the study design, data collection and microbiological methods, and the presentation of more detailed resistance data, e.g. quantitative micro-

biological data. In addition to this, access to the raw data is desirable because this will provide insight into the variability distribution of the input for which the data are required. Unfortunately, on occasions, the available data are old or qualitative, microbiological methods are not consistent, and the sample size is inadequate. Furthermore, the observed effects are confounded. For example, the variable that the MRA wishes to investigate (e.g. withdrawal of antimicrobials) is affected by another variable (e.g. change in biosecurity measures) so that there is an apparent (but untrue) association between the variables of interest or that a true association is masked. Consequently, for all of the occasions given above, either the data cannot be included in the risk assessment or it is necessary to make further assumptions or estimations.

From conversations with many researchers, it is clear that most would like their data to be suitable for use in MRA. However, without appropriate data, the construction of MRAs is hindered. Some of the data limitations identified in this paper are recognized not only by risk assessors, but also by the antimicrobial resistance research community. For example, the harmonization of antimicrobial resistance testing is a recognized issue. The implications of such data limitations have been outlined here in order to encourage the consideration, and hence generation, of good quality data for MRA in the area of antimicrobial resistance.

In some cases, MRAs are being developed in conjunction with laboratory and field-based research work.[36,37] This enables the findings of this research work to be put into context in relation to the risk to animals or humans. Interactive projects such as these enable the risk assessor to be involved at the planning stage of the laboratory or field-based work, and to continually advise on the type of data required to parameterize an MRA. This is the ideal situation since it should ensure that all data generated is suitable for inclusion in an MRA.

Conclusion

The reservoir of antimicrobial-resistant microorganisms in humans, food-producing species and the environment remains an issue in the 21st century. However, as in other areas of animal and human health, scientific investigations of this problem can be facilitated by the use of MRA. Given suitable data, MRAs to investigate the emergence of resistance in any organism can, theoretically, be developed and hence be used to provide decision-makers and industry with information on which to base policies and codes of practice relating to food safety.

The precision of MRAs is a direct result of the data available to parameterize them, and therefore, data limitations can be a severe hindrance to their successful development. Although abundant, data relating to antimicrobial resistance can be difficult to obtain, difficult to compare, uncertain and not fully documented. However, decision-makers and industry may not appreciate the extent of deficiencies in data quality that exist, and may request MRAs to be undertaken that will consequently generate risk estimates with large uncertainties. It is important that the uncertainties are understood and quantified using sensitivity analysis since, where a large degree of uncertainty exists, basing policies on MRA results alone could prove unwise.

Although there are ways in which data deficiencies can be overcome, these methods can potentially introduce additional uncertainty or bias into the model. Here we have described the properties that data should ideally possess for inclusion within MRAs, and by doing this we hope to have promoted the generation of good quality data.

Ideally, risk assessment should be an iterative process; importantly, it is able to identify data needs and then, as a result, the models should be routinely updated with the acquisition of new data. Such an interactive approach ensures the appropriate data for the model is generated; but more importantly focuses research. Through multidisciplinary projects (involving microbiologists, epidemiologists and risk assessors), and the willingness of funders to provide funding for field and laboratory work, key data for MRAs could be generated. As a consequence, this would provide decision-makers and industry with more complete scientific information on which to base policies and codes of practice. Such issues need to be fully recognized by both decision-makers and data providers if risk assessment is to progress in the area of antimicrobial resistance.

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