Source Attribution and Risk Assessment of Antimicrobial Resistance

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Source Attribution Models and Risk Assessment

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Summary

Source attribution and microbial risk assessment methods have been widely applied for the control of several foodborne pathogens worldwide by identifying i) the most important pathogen sources, and ii) the risk represented by specific foods and the critical points in these foods' production chain for microbial control. Such evidence has proved crucial for risk managers to identify and prioritize effective food safety and public health strategies. In the context of antimicrobial resistance (AMR) from livestock and pets, the utility of these methods is recognized but a number of challenges have largely prevented their application and routine use. One key challenge has been to define the hazard in question: is it the antimicrobial drug use in animals, the antimicrobial resistant bacteria in animals and foods, or the antimicrobial resistant genes that can be transferred between commensal and pathogenic bacteria in the animal or human gut or in the environment? Other important limitations include the lack of occurrence and transmission data, and the lack of evidence to inform dose-response relationships. We present the

main principles, available methods, strengths and weaknesses of source attribution and risk assessment methods, discuss their utility to identify sources and estimate risks of AMR from livestock and pets, and provide an overview of conducted studies. In addition, we discuss remaining challenges and current and future opportunities to improve methods and knowledge on the sources and transmission routes of AMR from animals through food, direct contact or the environment, including due to improvements in surveillance and developments on genotypic typing methods.

1. Introduction

Antimicrobial use in humans and animals has been identified as a main driver of AMR, and bacteria harboring resistance to antimicrobials can be found in humans, animals, foods and the environment. As a consequence, humans can be exposed to antimicrobial resistantbacteria through a wide range of sources and transmission pathways. To inform policies aimed at reducing the burden of AMR from animals and foods, risk managers need evidence on the most important sources and transmission routes, and the critical points throughout the production chain for the prevention and control of AMR. While this process is complex and deeply reliant on the integration of surveillance data from humans, animals and foods, it is supported by scientific disciplines that have evolved rapidly in the last decades, including *source attribution* and *quantitative risk assessment*.

Source attribution is a relatively new discipline that has been developed to assist risk managers to identify and prioritize effective food safety intervention measures. It is defined as the *partitioning of the human disease burden of one or more foodborne illnesses to specific sources, where the*

63 *term source includes reservoirs and vehicles* (1). A variety of source attribution methods is
64 available to estimate the relative contribution of different reservoirs or vehicles of foodborne
65 pathogens, including methods relying on data on the occurrence of the pathogen in sources and
66 humans, epidemiological studies, intervention studies or expert elicitations. These methods have
67 been applied to inform food safety policy-making at national or international level, particularly to
68 inform *Salmonella* and *Campylobacter* intervention strategies (see e.g. (2–6)). Source attribution
69 methods differ in their approaches and data requirements, and as a consequence they attribute
70 disease at different points along the food chain (points of attribution), i.e. at the point of reservoir
71 (e.g. animal production stage, environment emissions) or point of exposure (end of the
72 transmission chain) (Figure 1). The application and utility of each method, therefore, depends on
73 the risk management question being addressed and on the availability of data.

74

75 Figure 1. Routes of transmission of zoonotic pathogens and points of source attribution. Adapted
76 from (7).

77 Microbial risk assessment is a systematic and science-based approach to estimate the risk of
78 microbial hazards in the production-to-consumption chain (8, 9). Microbial risk assessment can
79 be used to detect critical control points along the food chain and for the assessment of control and
80 intervention strategies. It is a well-established discipline that has been widely applied to estimate
81 the risk of an extensive variety of pathogen-food commodity pairs, and it is also systematically
82 applied to inform food safety risk management in many countries and international bodies such
83 as the European Food Safety Authority (EFSA) (e.g. (10–12)). In coordination with source
84 attribution studies, it is particularly useful to focus on the production chain of the most important
85 source(s) of the hazard of interest (as identified in the source attribution step), identify the steps

86 in the food chain that are critical for hazard control, and identify and suggest strategies for
87 reduction of the risk to humans.

88 While source attribution and risk assessment have been widely used to provide evidence that can
89 support strategies to reduce the burden of a number of foodborne pathogens, the transmission and
90 spread of pathogens carrying resistance to antimicrobials adds an extra layer of complexity to this
91 integrated food safety paradigm. On one hand, virtually any foodborne pathogen can acquire
92 resistance to antimicrobials, which may lead to prolonged and more severe disease and even be
93 life-threatening, when antimicrobial therapy is required but fails to succeed due to resistance
94 towards the prescribed drug(s). On the other hand, the potential transfer of antimicrobial
95 resistance genes (i.e. the gene(s) carrying the resistance trait) between pathogenic and commensal
96 bacteria in the human gut can amplify the public health impact of foodborne AMR (13). As a
97 consequence, it is not only challenging to estimate the direct risk posed by resistant foodborne
98 pathogens, but also to quantify the relative contribution to risk of the transfer of AMR genes, e.g.
99 from commensals originating from animal reservoirs to human pathogens.

100 This chapter describes the overall concepts and methods within source attribution and microbial
101 risk assessment, provides the state-of-the art of their application in the area of AMR, and
102 discusses current challenges and future perspectives for the development of methods to inform
103 policies to reduce the disease burden of AMR in human populations.

104 ## 2. Source attribution

105 ### 2.1. Source attribution of antimicrobial resistance

106 The purpose of applying source attribution methods to antimicrobial resistant pathogens (i.e. a
107 pathogen that has acquired resistance to at least one antimicrobial drug) or AMR genes is to

identify the most important sources and transmission routes for human exposure to AMR. It is widely recognized that one of the main drivers of resistance in zoonotic bacteria is antimicrobial use in livestock production (i.e. in the reservoirs) (14). Identifying the most important reservoirs for human exposure to AMR is hence critical to direct policy making aimed at reducing antimicrobial use at the primary production level. In addition, knowledge on the transmission routes from reservoirs to humans is crucial for the prioritization of risk management along the food chain.

While a range of source attribution methods attributing disease to the original reservoirs or to exposure routes of foodborne pathogens exists, only a few studies have applied these in the context of AMR, and the relative importance of transmission pathways of resistance remains a critical knowledge gap.

Challenges of applying source attribution methods for AMR include the fact that virtually any pathogen can become resistant to antimicrobials and that most zoonotic pathogens can be transmitted to humans via a variety of foodborne and non-foodborne routes. Thus far, source attribution typically focused on a single pathogen (e.g. *Salmonella* or *Escherichia coli*), and on resistance profiles found among that pathogen in different sources (15–17). In addition, antimicrobial resistance genes are often located on plasmids, which can be transferred between bacterial species (plasmid-mediated horizontal gene transfer) and therefore also from commensal bacteria to human pathogens (e.g. *Klebsiella* spp.). Focusing on a single bacterial species is therefore likely to underestimate the overall exposure and thus the risk posed by AMR.

To address this challenge, source attribution of the AMR determinant may be more efficient. Such studies require knowledge and data on the prevalence, abundance and transmission of genes, and on horizontal gene transfer rates, which is still being gathered (e.g. in the European

Union project EFFORT - Ecology from Farm to Fork Of microbial drug Resistance and Transmission; http://www.effort-against-amr.eu/).

2.2. Existing source attribution approaches

2.2.1. Microbial subtyping

The microbial subtyping approach involves characterization of the hazard by subtyping methods (e.g., phenotypic or genotypic subtyping of bacterial strains), and the principle is to compare the subtypes of isolates from different sources (e.g. animals, food) with the subtypes isolated from humans. The subtyping approach attributes illness at the point of reservoir and is enabled by the identification of strong associations between some of the dominant subtypes and a specific reservoir or source, providing a heterogeneous distribution of subtypes among the sources (1).

Microbial subtyping methods for source attribution include frequency matched models and population genetic models. While the frequency matched methods are based on the comparison of human strain types and the distribution of those types in the sources, the population genetic models are based on modelling the organism's evolutionary history (18). In the frequency-matched models, subtypes exclusively or almost exclusively isolated from one source are regarded as indicators for the human health impact of that particular source, assuming that all human cases caused by these subtypes originate only from that source. Human cases of disease caused by subtypes found in several reservoirs are then distributed relative to the prevalence of the indicator types (2, 3, 19). Population genetics approaches use genotyping data to infer evolutionary and clonal relationships among different strains, including the occurrence of novel (combinations of) alleles in strains from humans that are unobserved in source populations (20).

All microbial subtyping models require a collection of temporally and spatially related isolates from various sources, and thus are facilitated by an integrated foodborne disease surveillance programme providing a collection of isolates from the major animal reservoirs of foodborne diseases. These models do not require prevalence data, and can rely on the distribution of the isolates' subtypes in the different sources and in humans.

Either type of models has been applied to attribute foodborne pathogens to sources in a variety of countries. Microbial subtyping approaches have been particularly successful to attribute *Salmonella* and *Campylobacter* infections (see e.g. (3, 21–24)). The method has also been applied to other pathogens (namely *Listeria monocytogenes* and shiga toxin-producing *Escherichia coli* (25, 26)), even though less frequently due to lack of available surveillance data in most countries.

The microbial subtyping approach has seldom been used to estimate the relative contribution of sources of antimicrobial resistant pathogens to AMR in humans. To our knowledge, two frequency-matched studies have been conducted, both using antimicrobial susceptibility patterns as a typing method for *Salmonella* (15, 16). Both studies demonstrate that AMR data can be used to characterize pathogen subtypes in a microbial subtyping source-attribution model, and discuss its utility in terms of discriminatory power, but do not focus on the source origin of specific AMR genes.

Microbial subtyping methods are recognized as one of the most robust data-driven methods for source attribution. They have the advantage of attributing illness to the reservoirs of the pathogens, thus informing risk-management strategies closest possible to the original sources and preventing further spread to other routes or sources of transmission (1). Another advantage of this approach is that it does not require data on the prevalence and concentration of the pathogen in

the different sources (which is often difficult to obtain), or on the exposure frequency in the population. Still, these methods are often limited by the requirement of comparable subtyping data originating from an operative integrated surveillance of human cases and food/animals. In addition, the methods cannot distinguish between different transmission routes from a specific animal reservoir to humans.

2.2.2. Comparative exposure assessment

Comparative exposure assessments determine the relative importance of the known transmission routes by estimating the human exposure to the hazard (e.g. pathogen) via each route. For each known transmission route, this approach requires information on the prevalence and/or dose/concentration of the pathogen in the source, of the changes of the prevalence and quantity of the pathogen throughout the transmission chain, and of the frequency at which humans are exposed by that route (e.g. consumption data). Exposure doses are then compared, and the relative contribution of each of the various transmission routes to human exposure in the population is estimated, proportionally to the size of each exposure dose.

The data requirements of the comparative exposure assessment approach will depend on the overall transmission groups considered in the model (i.e. foodborne, environmental and/or contact with animals), as well as on the point in the transmission chain where the "origin" of the pathogen is set. In general, contamination data for each source, information on the main steps in the transmission chain and data on the effects of these on contamination, and exposure data are needed. If transmission via contact with live animals is considered, the exposure model needs to be expanded and consider different possibilities for direct and indirect contact with a contaminated animal.

197 Exposure assessments have been used with different degrees of success to source attribute disease
198 by several microbial agents, namely *Listeria*, *Campylobacter*, VTEC (and *Toxoplasma gondii*,
199 and by chemical hazards - aflatoxins, cadmium and lead(27–34).

200 In the context of AMR, this approach is particularly useful to address a widely-recognized
201 knowledge gap, which is understanding the relative contribution of the exposure routes of AMR
202 from animals to humans. Specifically, it can be used to estimate the relative importance of the
203 food chain, companion animals and the environment for exposure of the general population to
204 antimicrobial resistant bacteria or AMR genes. Thus far (and to our knowledge), two comparative
205 exposure assessments have been applied to estimate the relative contribution of different types of
206 meat to the exposure of consumers to extended spectrum beta-lactamases (ESBL)/and AmpC
207 beta-lactamases producing *Escherichia coli* in the Netherlands(17) and in Denmark (35).

208 An important drawback of this approach is that, due to data limitations and gaps (e.g. in food
209 preparation habits and the effect of these in the contamination of foods), exposure estimates for
210 microbial pathogens are likely to present wide uncertainty intervals. Furthermore, in the context
211 of AMR, these studies focus on specific antimicrobial resistant pathogens, and do not address all
212 concomitant transmission routes contributing to overall transmission of resistance to humans (e.g.
213 same AMR determinant present in other members of the meat bacterial community), which adds
214 to the uncertainty of the relative exposure estimates.

215 *2.2.3. Epidemiological approaches*

216 Epidemiological approaches for source attribution include analyses of data from outbreak
217 investigations and studies of sporadic infections; both approaches attribute illness at the point of
218 exposure. An outbreak is here defined as (1) the occurrence of two or more cases of a similar

illness resulting from the exposure to a common source (36), or (2) a situation in which the observed number of cases exceeds the expected number and where the cases are linked to the same food source (37). Sporadic cases represent cases that have not been associated with known outbreaks (38). Even though outbreak-associated cases are more likely to be captured by public health surveillance systems, an unknown proportion of cases classified as sporadic may be part of undetected outbreaks.

Many outbreak investigations are successful in identifying the specific contaminated source or ingredient causing human infections. A simple descriptive analysis or summary of outbreak investigations is useful for quantifying the relative contribution of different foods to outbreak illnesses. However, these implicated foods may be composed of multiple ingredients, and thus outbreak data does not always allow pinpointing the actual source of infection. Probabilistic models using outbreak data to estimate the total number of illnesses in the population attributable to different foods provide a useful way to generalize outbreak data to a broader population of foodborne illnesses. These models are not only used to generalize the results of outbreak investigations, but also to estimate the contaminated sources in composite or "complex" foods.

Analyses of data from outbreak investigations benefit from detailed data on each reported outbreak, and require the adoption of a food categorization scheme for classification of implicated foods (see e.g.(39)). Composite foods will be assigned to two or more food categories depending on the number and nature of their ingredients. By assigning a probability to each ingredient corresponding to the likelihood that it was the source of the outbreak, outbreak data, including data about both simple and complex foods, can be used to attribute foodborne illnesses to sources.

241 Several analyses of outbreak data for source attribution have been published in recent years, most
242 of them modelling (40–42) or summarizing (43, 44) data from multiple pathogens. The strength
243 of this method is that it uses data that is readily available in many countries worldwide, and thus
244 its use is not restricted to countries with integrated foodborne disease surveillance programmes.
245 Also, it attributes foodborne illnesses at the point of exposure, which means that it is particularly
246 useful to identify which foods (including processed foods) most frequently cause disease, as well
247 as which risk factors contribute more for contamination of foods at the end of the food chain (e.g.
248 cross contamination). This type of information is valuable to define interventions at the
249 processing and consumption level, but does not provide evidence to inform risk management
250 strategies at the origin of the pathogen (reservoirs).

251 Several outbreaks caused by antimicrobial resistant pathogens have been reported and
252 investigated in the last decades (see e.g. (45, 46)). A review of outbreak data has also been used
253 for source attribution of antimicrobial resistant *Salmonella* in the United States, suggesting that
254 antimicrobial susceptibility data on isolates from foodborne outbreaks can help determine which
255 foods are associated with resistant infections (47). Even though few countries or regions are
256 likely to have sufficient data for a robust source attribution analysis using AMR-related
257 outbreaks, summarizing available information may provide evidence on the relative contribution
258 of different foods for infection with antimicrobial resistant pathogens.

259 Another epidemiological approach that can be used for source attribution of foodborne disease is
260 the case control study of sporadic cases. Case-control studies are a valuable tool to identify
261 potential risk factors for human illness, including sources and predisposing, behavioral or
262 seasonal factors (48). In addition to individual case-control studies, a systematic review of
263 published case-control studies of sporadic infections of a given pathogen can provide an

264 overview of the relevant exposures and risk factors for that disease, and a summary of the
265 estimated population attributable fractions for each exposure (49). A systematic review follows a
266 rigorous search strategy to identify all potentially relevant peer-review case-control studies for a
267 hazard, studies being conducted in a variety of countries and time periods, designed with
268 different settings, and potentially focused on specific age groups within the population. A meta-
269 analysis is then performed to compare and combine information from different studies. To do
270 this, risk factors may be stratified according to source-categorization schemes, location of
271 exposures and, if appropriate, frequency of exposure. An overall population attributable fraction
272 derived from a meta-analysis or weighted summary of several case-control studies of a certain
273 hazard can be combined with estimates of the burden of disease caused by that hazard to estimate
274 the burden of disease attributed to each exposure.

275 This method is particularly useful for hazards that do not frequently cause outbreaks but that have
276 been extensively studied (50). In addition, it is valuable to attribute illness at a regional or global
277 level when data are scarce in most countries. A number of case-control studies have been
278 conducted to investigate risk factors for infection with foodborne pathogens resistant to
279 antimicrobials (see e.g. (51, 52)). However, the utility of a meta-analysis of case-control studies
280 to investigate the relative contribution of different sources and risk factors for infection with
281 antimicrobial resistant pathogens may be limited if a low number of case-control studies focused
282 on specific antimicrobial resistant pathogens or AMR genes has been conducted.

283 *2.2.4. Other approaches*

284 Other approaches for source attribution of foodborne pathogens include intervention studies and
285 expert elicitations. Intervention studies are large-scale, well-structured prospective studies that

286 are specifically tailored to evaluate direct impacts of a specific intervention on the risk of disease
287 in a population. While they would be the gold-standard of an attribution study, they have the
288 disadvantages of being resource-demanding, expensive, and difficult to implement because other
289 concurrent factors may affect occurrence of disease.

290 Expert elicitations can be designed as structured methods to gather and analyze knowledge from
291 experts, which are communicated with a measure of uncertainty. They are particularly useful to
292 attribute the burden of foodborne diseases to main transmission pathways (i.e. foodborne,
293 environmental, direct contact), for which data-driven methods are typically insufficient(50).
294 There are numerous methods used for expert elicitation, including methods that are based upon
295 iteration and finding consensus among a small group of experts (e.g. the Delphi method). Expert
296 judgments are subjective by nature and may be biased by the specific background and scientific
297 expertise of the respondents, and several methods to evaluate the expert's performance have been
298 described. Several expert elicitation studies have been conducted for source attribution of
299 foodborne disease (e.g. Havelaar et al. 2008; Ravel et al. 2010). The World Health Organization's
300 Initiative to Estimate the Global Burden of Foodborne Diseases (WHO-FERG) has undertaken a
301 large-scale and successful expert elicitation to attribute disease by 19 foodborne hazards to main
302 transmission groups at a global, regional and sub-regional level (55). The study applied
303 structured expert judgment using Cooke's Classical Model (56) to obtain estimates for the
304 relative contributions of different transmission pathways for several foodborne hazards.

305 ## 2.3. Applications and results

306 Despite the increased recognition of the importance of source attribution of foodborne pathogens
307 to direct risk management strategies, and the growing use of these approaches in several countries

and research groups, source attribution of AMR is still in its infancy. There are few published examples of the different methods here described, and the identified challenges are still being addressed. The two microbial subtyping studies published are both frequency-matched studies that used antimicrobial susceptibility patterns as a typing method for *Salmonella* (15, 16). These studies use AMR profiles as a typing method (i.e. to characterize pathogen subtypes) but do not focus on the source origin of specific AMR genes. Still, they are able to estimate the distribution of AMR in human cases attributed to different sources, as is done routinely in the Salmonella source attribution activities in Denmark (57). Similarly, the two comparative exposure assessments that have been applied to estimate the relative contribution of different types of meat to the exposure of consumers to AMR have focused on the same causative agent, this time extended spectrum beta-lactamases (ESBL)/and AmpC beta-lactamases producing *Escherichia coli* (17, 35). These studies demonstrate that the method could be extended to other countries and agents. The recent review of outbreak data for source attribution of antimicrobial resistant *Salmonella* in the United States suggests that antimicrobial susceptibility data on isolates from foodborne outbreaks can help determine which foods are associated with resistant infections (47). This method could be applied in countries that have sufficient data, or to regional data in an attempt to gather information from multiple countries. Numerous epidemiological studies of sporadic infections (case-control or cohort studies) investigating risk factors for of antimicrobial resistant infections in humans demonstrate these methods usefulness to identify routes of AMR (e.g. (58–60). While their use focusing on foodborne or direct or indirect contact to animals' transmission has been limited, available studies still provide information for food safety risk management (51, 52).

2.4. Strengths and weaknesses

Source attribution of AMR genes and of antimicrobial resistant pathogens is a research area under active development. The application of the methods here described remains a challenge, for reasons that depend on each method considered.

For the application of subtyping frequency-matched studies, two of the main challenges are the limited availability of animal, food and human AMR data from established surveillance systems, and the difficulty to define number of antimicrobial resistance profiles highly specific to a particular source/transmission route, a cornerstone of this method. Furthermore, the fact that the method does not determine the actual transmission route from each specific reservoir to humans represents another limitation for the use of frequency-matched models. Due to the public health need for understanding the transmission of AMR, population genetics approaches may eventually be a good complement to frequency-matched models, especially considering the increasing availability of whole genome sequencing and metagenomics data, which describe occurrence of AMR genes in populations. For instance, population genetics can help identifying reservoir-specific AMR genes' patterns that can then be used in frequency-matched models. New generation sequencing data may also contribute to unravel details that contribute to a more accurate source-attribution, such as the evolution of AMR patterns over time in different sources, and resistance in humans that is not transmitted from animals or foods.

While single genomics and metagenomics may support the development of novel subtyping source-attribution methods, they may hinder the application of comparative exposure assessment. Information on prevalence and quantity of AMR genes or antimicrobial resistant pathogens in each source, as well as their changes throughout the transmission chain, are difficult to assess from those data and impaired by a high degree of uncertainty.

Epidemiological methods of source-attribution, e.g. based on outbreak investigation, have the advantage of not relying on a sophisticated, data abundant and integrated surveillance system, encompassing animal reservoirs, foods and humans. However, they require consistent AMR investigation on food sources and human cases, based at least on bacterial isolation and phenotypic susceptibility testing. Eventually, new generation sequencing may overtake traditional diagnostic methods in outbreak investigation (14, 61), which will also require modification of the current epidemiological approaches.

Intervention studies have, in the context of AMR, the same limitations as when applied to bacterial pathogens. It is difficult to evaluate the exact impact of a specific intervention (e.g. reducing antimicrobial use at the farm level) on the population where disease is attributed (e.g. AMR occurrence in humans). Control measures that reduced antimicrobial use in primary production have been successfully implemented with the aim of reducing AMR in animals (e.g. the antimicrobial growth promoter intervention, the voluntary ban on the use of cephalosporins and the yellow card antimicrobial scheme in swine herds in Denmark (62–64)). However, to assess the real success of such measures in terms of public health impact, it is necessary to collect data prior to and following the intervention (14), at all dimensions of AMR transmission to humans, i.e. also including other transmission routes such as environment and antimicrobial use in humans.

3. Risk assessment

3.1. Microbial Risk Assessment (MRA) of antimicrobial resistance

Risk assessment is the process of estimating the likelihood that exposure to a biological, chemical or physical hazard will result in an adverse health effect in exposed individuals. Microbial risk assessment has been established as a part of the food safety risk analysis paradigm by international and national bodies in the last decades, with harmonized guidelines being proposed and widely adopted worldwide (8, 65). In the context of AMR, risk assessments are useful to inform regulatory decision making for the mitigation of potential health consequences in both humans and animals (66). While the importance and need for AMR risk assessments have been recognized for decades (67), its application has been complicated by several knowledge gaps. Challenges of the development of AMR risk assessment include:

- The nature of the hazard is difficult to identify and will determine the nature of the adverse consequence of the exposure. In the context of AMR risk assessment, different hazards can be considered (68, 69). For example, Salisbury et al.(2017) (68)discussed three interrelated hazards that can be assessed separately: the antimicrobial drug, the antimicrobial resistantbacteria, and the AMR determinant, leading to three different health consequences, respectively - development of resistance, infection and treatment failure and transference of resistance. Similarly, Manaia (2017)(69) describes that resistome-associated risks have been discussed considering the microbial community, the genome and transmission of resistance.
- The nature of the risk posed by antimicrobial use and AMR to human health is inherently complex and logically linked to the nature of the hazard, as mentioned above. In other words, while the likelihood that humans will be infected by pathogens that are resistant to

one or several antimicrobials can be estimated, the resulting adverse health consequences can be one or several of the following: development of disease due to infection with the pathogen; failure of treatment of the infection due to resistance to the used drug(s); and spread of AMR genes to commensal bacteria in the human host (which can amplify the risk and extend the impact of an isolated exposure in time).

- There are numerous factors in the process of selection and spread of resistance in bacterial populations, between and within animal species, humans and the environment, and within different bacterial populations in those same reservoirs. These factors include the several drivers for the emergence and spread of AMR in the food production, specifically at the farm. At this level, antimicrobial use is recognized as the most important driver, but not always necessary (if for example co-resistance and co-selection occur), and not always sufficient; additional drivers are e.g. poor prevention and control of infectious diseases leading to increased antimicrobial use and the spread of clones that have established themselves in the herd/environment, and keep selective pressure, even if antimicrobial use is interrupted. These factors, among many others, influence the development of exposure assessment in microbial risk assessment.
- Additionally to the challenges described above, estimating the likelihood of adverse health effects, given exposure to an antimicrobial resistant pathogen or determinant, is difficult due to the absence of a well-defined dose-response effect for AMR, and the existence of various possibilities of adverse effect.

Recognizing the need for AMR risk assessments to identify strategies aimed at preventing and reducing the disease burden of AMR transmitted through foods, a number of reviews and scientific articles have proposed frameworks for such risk assessments in the late 90's and early

418 2000's (67, 68, 70). Even though such proposals were comprehensive and structured to address
419 the challenges identified at that time, they were not widely adopted, mostly due to remaining
420 knowledge and data gaps in the AMR transmission and impact. More recent frameworks apply
421 current available data and either are mostly qualitative or semi-quantitative (see e.g. (71, 72)),
422 take a linear approach (e.g. (73)), and/or focus on marketing authorization applications for
423 antimicrobial veterinary medicinal products for use in food producing species (74).

424

425 ## 3.2. Description of the four steps of microbial risk assessment focusing on AMR

426 The microbial risk assessment process is, as described by the *Codex Alimentarius* guidelines (8),
427 constituted by four main components: hazard identification, hazard characterization, exposure
428 assessment and risk characterization.

429 In an AMR risk assessment, the hazard can be the antimicrobial drug, the antimicrobial resistant
430 pathogen or the AMR determinant. Ultimately, the *identification of the hazard* of interest will
431 depend of the risk-assessment question to be addressed. In a traditional microbial risk assessment
432 (i.e. focused on a pathogen-food pair, without considering resistance to antimicrobial drugs) the
433 hazard identification step consists of the qualitative description of the hazard, including the
434 evaluation of the presence of the pathogen in a food product available for consumption in a
435 population and the host interface (types of disease caused, susceptible populations). In the context
436 of AMR, this step is complicated by a number of factors: i) selection of resistance in a pathogen
437 can occur by multiple mechanisms (namely mutation and horizontal gene transfer of mobile
438 genetic elements containing AMR genes (HGT)) (75); ii) one or more genes may be necessary for
439 development of AMR; iii) AMR genes can be located in chromosomal or extra-chromossomal

DNA such as plasmids (75), and iv) several bacterial species or strains can harbor and serve as a reservoir for resistance.

The *hazard characterization* step of a risk assessment consists of the review and collection of information on the relationship between the dose of the hazard and the onset of disease in the exposed individuals (i.e. infectious dose), and the relationship between different doses and the probability of occurrence of disease (i.e. dose-response). The response of a human population to exposure to a foodborne pathogen is highly variable, reflecting the fact that the incidence of disease is dependent on a variety of factors such as the virulence characteristics of the pathogen, the numbers of cells ingested, the general health and immune status of the hosts, and the attributes of the food that alter microbial-host interaction (76). Thus, the likelihood that any individual becomes ill due to an exposure to a foodborne pathogen is dependent on the integration of host, pathogen, and food matrix effects. Again, in AMR risk assessment, the required data to assess a dose-response relationship will depend on the hazard considered; it can be one of the three: dose level of the antimicrobial for observing resistance usually expressed by minimum inhibitory concentration (MIC) breakpoint (75), or any other factor that can affect the development or amplification of resistance, the dose of the pathogen needed to cause disease, or any factor related to the stability and transfer potential of the AMR gene in a bacterial population (68).

In the *exposure assessment* step, the likelihood that an individual or a population will be exposed to a hazard and the numbers of the microorganism that are likely to be ingested are estimated (77). The exposure assessment requires data on the prevalence and concentration of the hazard in the food source(s), as well as information on the potential changes of the pathogen load throughout the food processing chain (e.g. growth, reduction) (78); in addition, it requires data on

463 the frequency and amount of food consumed by individuals of the population. As mentioned
464 above, numerous factors influence the process of selection and spread of resistance, consequently
465 influencing the final exposure of the consumer to AMR genes or antimicrobial resistant
466 pathogens. These factors are either still unknown or there are limited data reporting their
467 influence on AMR transmission throughout the food chain.

468 In the last component of a risk assessment, *risk characterization*, the final risk to the consumer is
469 estimated by integrating the previous three components. Specifically, the measure of exposure
470 (i.e. the likely dose an individual is exposed to in a given food consumption/exposure event) is
471 integrated with the dose-response relationship to estimate the likelihood of adverse health effect.
472 In the context of AMR microbial risk assessment, even after an appropriate definition of the risk
473 question and the targeted hazard identification (which determine the adverse effect to be
474 assessed), and the estimation of the likelihood of exposure to the hazard of interest,
475 characterizing the risk in the absence of an appropriate and comprehensive hazard
476 characterization step remains a challenge. A "dose-response" step becomes particularly
477 demanding when "dose" at exposure is expressed in genotypic terms (by use of genomics or
478 metagenomics AMR data) and "response" must be expressed in phenotypic terms (e.g.
479 expression of resistance in a pathogen or horizontal transfer of an AMR gene between
480 commensal and pathogenic bacteria).

481 ### 3.3. Applications and results

482 A number of risk assessments focused on specific antimicrobial resistant pathogens-food/animal
483 pairs have been conducted since the publication of the different proposed guidelines. These
484 include qualitative, semi-quantitative and quantitative risk assessments, performed by food

485 authorities, academia or industry. Here we provide examples of the three-types of risk assessment
486 that have been important to highlight the challenges and limitations they still face, the
487 applications of their results and the need for further studies.

488 *Qualitative risk assessments*

489 One of the first studies published assessed the health impact of residues of antibacterial and anti-
490 parasitic drugs in foods of animal origin and was published over two decades ago (79). It was a
491 qualitative and comprehensive review that focused on residues of a variety of drugs in multiple
492 foods, and an important step for the recognition of several of the challenges described in this
493 chapter. More drug- and pathogen-focused qualitative assessments have been conducted since
494 then, including in recent years, such as the qualitative risk assessment focused on Methicillin
495 resistant *Staphylococcus aureus* (MRSA) conducted by a multi-sectorial and interdisciplinary
496 expert group in Denmark (80). This study is a good example of an applied risk assessment,
497 conducted upon request from the food and veterinary authorities with the aims of 1) assessing the
498 risk of livestock MRSA based on the existing knowledge and the results of veterinary screening
499 studies conducted in herds, and 2) providing recommendation for control measures to reduce the
500 spread of MRSA from the affected herds to the surrounding environment and community. The
501 method consisted of a comprehensive evaluation of all available data on the prevalence of MRSA
502 in animals and humans, as well as on the risk factors for infection by livestock MRSA from the
503 environment, from meat, from occupational activities (e.g. risk for slaughterhouse or farm
504 workers) and from the community. The risk assessment consisted of a descriptive evaluation of
505 the risk of these types of transmission in the Danish population.

506 Another recent study has applied the risk assessment framework developed by the European
507 Medicines Agency (74) to assess the AMR risk to public health due to use of antimicrobials in

508 pigs, using pleuromutilins as an example (81). Livestock-associated methicillin-resistant
509 *Staphylococcus aureus* of clonal complex 398 (MRSA CC398) and enterococci were identified as
510 relevant hazards. This framework followed the International Organization for Animal Health's
511 (OIE) approach to risk assessment and consisted of four steps describing the risk pathway,
512 combined into a risk estimate. The study applied a qualitative approach, where the output of each
513 step was defined in a scale. Likewise, the level of uncertainty was described qualitatively in the
514 different steps and the output (as high, medium or low). The authors discuss the value of
515 mathematical modeling as a tool to simulate pathways and identifying ways of reducing
516 resistance. Still, they stress that the relationship between reducing consumption of antibiotics and
517 reducing resistance is not necessarily linear, and defend that this relationship needs to be better
518 established for modeling to have full value (81). Despite the fact that this study is recent at the
519 point of writing of this chapter and thus could build on all newly available evidence on AMR
520 mechanisms, it still dealt with substantial data and knowledge gaps that enhanced uncertainty
521 around outputs (81).

522 Another example of a qualitative assessment is the WHO's list of Critically Important
523 Antimicrobials (71). The list applies criteria to rank antimicrobials according to their relative
524 importance in human medicine. The purpose of this assessment is to provide clinicians,
525 regulatory agencies, policy-makers and other stakeholders' information to develop risk
526 management strategies for the use of antimicrobials in food production animals globally. The first
527 WHO list of Critically Important Antimicrobials was developed in a WHO expert meeting in
528 2005, where participants considered the list of all antimicrobial classes used in human medicine
529 and categorized antimicrobials into three groups of *critically important*, *highly important*, and
530 *important* based on two criteria that describe first the availability or not of alternatives to the

antimicrobial for treatment of serious bacterial infections in people, and second if the antimicrobial is used to treat infections by (1) bacteria that may be transmitted to humans from nonhuman sources, or (2) bacteria that may acquire resistance genes from nonhuman sources. The output of the qualitative assessment is a list of classes of drugs that met all three of a set of defined priorities. Since its original publication, the assessment has been revised several times and is now in its 5th edition.

Semi-quantitative risk assessments

One example of a semi-quantitative assessment is the study integrating a probabilistic quantitative risk assessment conducted in Denmark to assess the human health risk of macrolide-resistant *Campylobacter* infection associated with the use of macrolides in Danish pig production (82). This model was able to account for exposure through imported and domestic meat (i.e. that could be a vehicle for antimicrobial resistant bacteria as a consequence of antimicrobial drug use in animal production in the country) and used evidence available at the time. One important feature of this study is that, while it measured exposure probabilistically and thus reflected model and data uncertainty, the final step of the risk assessment –risk characterization – used an ordinal scale and thus risk was described in a qualitative scale.

Quantitative risk assessments

Several quantitative risk assessments have been published since the early 2000's. These include the high profile assessment of fluoroquinolone-resistant *Campylobacter* from chicken in the United States (US) (83), which ultimately prompted the Food and Drug Administration to propose withdrawal of the approval of the new animal drug applications for fluoroquinolone use

552 in poultry, an action that would prohibit fluoroquinolone use in chickens and turkeys in the
553 country (84).

554 Another early study employed probabilistic methodology to analyze the potential public health
555 risk from *Campylobacter jejuni* and fluoroquinolone-resistant *C. jejuni* due to fresh beef and
556 ground beef consumption (85). The model focused on the beef product at retail and modelled
557 consumer handling in the kitchen, processing and consumption. The model estimated first the
558 risk of *Campylobacter* infection through consumption of beef, and then the risk of treatment
559 failure given infection, concluding an increased health impact due to resistance.

560 In another study, a risk assessment followed the US Food and Drug Administration's Center for
561 Veterinary Medicine Guidance (86) and was commissioned by a pharmaceutical company to
562 estimate the risk of human infection treatment failure associated with the use of an AM drug in
563 food animals (87). The deterministic model included all uses of two macrolides in poultry, swine,
564 and beef cattle. The hazard was defined as illness (i) caused by foodborne bacteria with a
565 resistance determinant, (ii) attributed to a specified animal-derived meat commodity, and (iii)
566 treated with a human use drug of the same class. Risk was defined as the probability of this
567 hazard combined with the consequence of treatment failure due to resistant *Campylobacter spp*.
568 or *Enterococcus faecium*. At the time, this microbial risk assessment had the advantage of being
569 quantitative and thus more transparent when compared to previous assessments focusing on
570 AMR. Thus, the authors highlighted several limitations, particularly with regards to data gaps on
571 the probability of treatment failure due to the antimicrobial resistant bacteria and the probability
572 of resistant determinant development. In contrast to many evidence and risk assessments
573 conducted elsewhere, the results of this study lead the authors to conclude that current use of

574 macrolides in cattle, poultry, and swine create a risk much lower than the potential benefit to food
575 safety, animal welfare, and public health (87).

576 The same author published another risk assessment a few years later, applying a similar approach
577 to estimate the risk of a different combination of antimicrobial-pathogen - fluoroquinolone-
578 resistant *Salmonella* and *Campylobacter* in beef in the US (88). This approach was able to
579 provide a better measure of uncertainty but was similar in its findings, concluding that the risk of
580 health consequences in humans was minimal.

581 The most recent quantitative risk assessment study published is also the more novel and
582 promising of the AMR studies here reviewed (89). It considered the existence of environmental
583 compartments resulting from sewage-treatment plants, agriculture production and manufacturing
584 industries, and assessed their role in the maintenance, emergence and possible dissemination of
585 antibiotic resistance. This study used probabilistic methods to assess the risks of antibiotic
586 resistance development and neutralizing antibiotic pressures in hotspot environments.
587 Importantly, this study presents a modelling approach to assess the selective pressure exerted by
588 antibiotics in bacterial communities and to calculate antibiotic resistance development risks.
589 While the described approach was exemplarily used to model antibiotic resistance risks in an
590 intensive aquaculture production scenario of south-east Asia, it has potential to be applied to
591 other cases, including other types of animal production, settings and drugs.

592 ### 3.4. Strength and weaknesses

593 Microbial risk assessment is a science-based tool with proven benefits in supporting food safety
594 authorities in policy making. It is hence aspired to continue its use in assessing the consequences
595 for the consumer of the transmission of AMR genes /pathogens throughput the food chain. The

fact that it is a well-defined, stepwise-structured method facilitates its adaptation to the food safety challenge of AMR. However, several limitations have already been identified and require the joint focus of the scientific community, risk assessors and authorities. Examples of a few critical challenges are:

- The definition of antimicrobial resistance is critical for the four steps of microbial risk assessment, and needs therefore to be well-established at the very start of a risk assessment study. Martínez et al. (2014) (75) explains the existence of several possible definitions of resistance, (namely clinical, epidemiological and operational), and two definitions of resistance gene (ecological and operational). The adoption of standard concepts and terminology is a requisite for the transparency of microbial risk assessment and an important part of its development. Although transmission of AMR genes and antimicrobial resistant bacteria may be perceived and have been defined as two separate hazards, it has also been recently suggested that the risk of AMR transmission to humans cannot be estimated unless the AMR gene pool and the presence and quantity of antimicrobial resistant bacteria that are able to colonize and multiply in the human body are both taken into consideration (69).
- Exposure assessment often relies on available knowledge of the changes in the microbial hazard levels throughout the food chain, due to e.g. growth or inactivation. In the context of microbiomes and resistomes, it is difficult to model these changes, as the very composition of the microbial population (and corresponding AMR genes) may significantly change between "farm" and "fork" (90, 91). Consequently, microbial risk assessment for AMR is highly dependent on data collected at several points of the transmission pathway, both from the source(s) of AMR and from exposed human subjects.

- While new generation sequencing attractively provides a broad characterization of the presence and abundance of AMR genes in a particular pathogen or in the microbiome from a particular reservoir, it remains a challenge to determine variability of the resistome and of the potential to exchange AMR genes (i.e. presence of phage recombination sites, plasmids, integrons or transposons) between different pathogen strains (69). This knowledge is crucial, respectively, to assign the AMR genes detected with metagenomics to the corresponding bacterial hosts, and to account for the occurrence of horizontal gene transfer between commensal and pathogenic bacteria in a population.
- Furthermore, an important challenge for the integration of metagenomics data in MRA is the harmonization of languages between the "omics" and the food microbiology communities (92).
- Risk characterization requires knowledge of the relationship between a "dose", resulting from exposure assessment, and a "response", i.e. the adverse health effect of exposure. However, the infective dose and the modes of transmission of most of the antimicrobial resistant bacteria of relevance are still unknown (69), which represents an important knowledge gap for the development of microbial risk assessment for antimicrobial resistance.
- Finally, a major limitation of the current microbial risk assessment frameworks is that they do not allow estimating the long-term impact of exposure to AMR. Particularly serious public health consequences of AMR arise when multiresistant bacteria emerge and become widely spread. There is therefore the need to develop microbial risk assessment methods that include a different characterization of the risk of AMR. In addition to immediate consequences to human health due to a single exposure to a antimicrobial resistant pathogen, it is necessary to estimate the likelihood that such exposure (eventually together with past and subsequent

641 ones, to the same or other types of AMR) will lead to the development of antimicrobial multi-
642 resistance in the future. Also, it is necessary to assess the potential of multi-resistance spread,
643 to characterize the severity of the consequences of exposure to multi-resistance and to
644 estimate the time from initial exposure to those consequences.
645

646 ## 4. Discussion and future perspectives

647 Several position and stakeholder papers have stressed the need for improved quality and
648 increased amount of data for risk assessment of AMR (see. e.g.(93)). These include e.g. data on
649 antimicrobial use in animal production, AMR surveillance data in animals, foods and humans,
650 and gene transfer and spread of AMR genes. All data requirements apply for most source
651 attribution studies, and thus are transversal to the methods described in this chapter. Likewise,
652 many of the challenges to the application of these methods in the context of AMR are common to
653 source attribution and risk assessment approaches (Table 1).

654 Table 1. Definition, overview of methods and main challenges of source attribution and microbial
655 risk assessment approaches.

	Source attribution	**Microbial risk assessment**
Definition	Partitioning of human cases of illness to the responsible sources (e.g. foods, animal reservoirs)	Systematic and science-based approach to estimate the risk of microbial hazards in the production-to-consumption chain
Methods	• Microbial subtyping	• Qualitative RA*

	• Comparative exposure assessment
	• Outbreak-data analysis
	• Case-control studies
	• Expert elicitations
	• Intervention studies
		• Semi-quantitative RA
		• Quantitative RA
		  ○ Deterministic
		  ○ Probabilistic

Main challenges in the context of AMR	• Hazard identification, e.g. the antimicrobial drug, the antimicrobial resistant pathogen or the AMR determinant • Lack of occurrence/prevalence data • Definition of the health outcome, i.e. infection with antimicrobial resistant agent, treatment failure (in case treatment is needed) or spread of resistance determinant between commensal and pathogenic organisms	
	• Lack of epidemiological data	• Establishment of dose-response relationship • Determining variability of the resistome and of the potential to exchange AMR genes between different pathogen strains

*RA: risk assessment

658 The studies here described all show the importance of knowledge on 1) the most important
659 sources and routes of transmission of antimicrobial resistant bacteria or AMR genes, 2) the
660 actual risk for human health, and 3) the points in the transmission chain where interventions
661 could be effective to reduce this risk. While all findings so far have been crucial to direct policies
662 and raise awareness to the public health impact of AMR in animals and foods, they are
663 insufficient for a complete understanding of the underlying transmission mechanisms and the real
664 impact of AMR. Several challenges have been addressed, including the fact that emergence and
665 spread of AMR is complex. From an epidemiological point of view, the risk of AMR most
666 probably follows the "sufficient-component causes" principle (94) . The sufficient-component
667 causes is an epidemiological causal modeling approach that can be used to explain diseases, or
668 conditions like AMR, characterized by many causes, none of which alone is necessary or
669 sufficient. The relations among the causes are described in a way that a *sufficient cause* is a set of
670 minimal conditions that will definitely lead to the outcome (e.g. antimicrobial resistant infection),
671 and a *component cause* is one of the minimal conditions included in a sufficient cause (94). For
672 example, a particular resistance gene can be a component cause of an antimicrobial resistant
673 infection, but the sufficient cause of the latter includes other conditions, such as the bacterial
674 strain carrying that particular gene, that pathogen causing infection, treatment of the infection
675 with antimicrobial(s) for which resistance is encoded in the gene, and actual expression of that
676 resistance gene. The future of microbial risk assessment for antimicrobial resistance may
677 therefore include defining the components sufficient to cause AMR transmission from
678 animals/foods/environment to humans followed by treatment failure of infections by
679 antimicrobial resistant pathogens.

Recent developments in “omics” technologies (whole genome sequencing and metagenomics, transcriptomics, proteomics, metabolomics, fluxomics) provide unique opportunities to fill in some of our knowledge gaps. It is now widely recognized that these “omics” technologies have advantages compared to traditional phenotypic culture-based methods for characterizing microorganisms (92, 95).

Brul et al (2012)(92) described in detail how “omics” can be integrated in each step of microbial risk assessment, contributing to a mechanistic insight into the interaction between microorganisms and their hosts, new perspectives on strain diversity and variability and physiological uncertainty, and overall more robust risk assessments. Den Besten et al. (2017)(95) discussed the utility of “omics” technologies applied by the food industry, to help identify the influence of different bacterial ecosystems on both pathogen survival and growth – information that can eventually contribute to the future definition of Food Safety Objectives (FSO).

A particular advantage of metagenomics is that it provides a picture of the whole microbial community and its resistome, which is key to understanding AMR emergence and spread in a population. Importantly, these new “typing” techniques have been rapidly followed by new bioinformatics and new statistics/modelling tools that allow for the analysis and sense-making of such (big) data (92, 96). For example, machine learning has the potential to be applied on the analysis of omics data. Combining machine learning approaches with metagenomics and farm specific data could allow for describing e.g. health, production efficiency, and the relative abundance of AMR genes, based on the identification of (clusters of) genetic factors in the farm microbiome. In addition, such techniques could be used to examine the predictive importance of (clusters of) genetic factors in order to characterize 1) a ‘healthy farm microbiome’ or 2) AMR genes in a specific animal reservoir. They can also be used to identify (combinations of) specific

703 husbandry practices that are associated with e.g. a particular resistome or a 'healthy farm
704 microbiome'. The latter could lead to recommendations on how to shift the farm microbiome in
705 order to improve the overall health of the farm, and consequently on the long term, to reduce the
706 level of antimicrobial use and antimicrobial resistant bacteria. It is possible that promoting a
707 'healthy farm microbiome' will have a more long-term impact on the overall reduction of AMR,
708 than focusing exclusively on the farm resistome. Metagenomics and other "omics" technologies
709 have hence enormous potential for the future development of source attribution and microbial
710 risk assessment of AMR through foods. To explore their full potential, different technologies
711 shall be combined. For example, genomics studies should be coupled with proteomics, as gene-
712 expression studies do not always reflect the actual protein levels (92). Also, genomic similarities
713 may not imply similarities in behavior, as the surrounding environment (food matrix, bacterial
714 ecosystem, etc) also plays a role (95) . Furthermore, "omics" data are not sufficient without
715 accompanying epidemiological data that allow for the identification of risk factors for AMR.

716 ## *5.* Concluding remarks

717 Recent developments in source-attribution and microbial risk assessment of AMR are promising
718 and have significantly contributed to the evolution of each of these methods. However, the
719 adaptation to the "omics" big data era is happening at a much slower pace than the speed at
720 which these data are becoming available. This is due to the many challenges encountered when
721 interpreting those data.
722 Antimicrobial resistance at the animal reservoir, food, environment and human levels is
723 increasingly described by the characterization of the resistomes of single bacteria isolates (by
724 whole genome sequencing) or the bacterial whole community (by metagenomics) representing
725 each of those populations. Gradually, AMR surveillance will convert from phenotypic to

genotypic (e.g. PulseNet International is already on its way to standardize whole genome sequencing-based subtyping of foodborne disease (96). For a successful transition, it is crucial to pair genomic data with phenotypic data and relevant explanatory epidemiological data.

This transition will require a parallel adaptation of the existing analysis methods, which will include the development of new source-attribution and microbial risk assessment modelling approaches. It is therefore with great expectation that we foresee in the near future a surge of influencing and inspiring scientific output in both fields.

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