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Review

# Pet food safety: emerging bacterial hazards and implications for public health

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Bacterial hazards in pet food, especially in raw diets, are a current public health issue to both pets and humans. The most substantial body of evidence and consequent risk stems from food-borne pathogens such as Salmonella and bacteria resistant to last-resort antibiotics (e.g. colistin, third generation of cephalosporins, linezolid). State-of-the-art methods, particularly whole-genome sequencing, have been fundamental to link bacterial pathogens from pet food to human cases across different countries. While there are limited data on antimicrobial resistance, it is becoming increasingly evident that pet food can harbor multidrug-resistant bacteria, calling for increased vigilance within a One Health perspective, namely, by identifying transmission routes of pathogens and antimicrobial-resistant bacteria to pet food. A concerted action involving veterinarians, regulatory agencies, pet food industry, and other stakeholders is required to promote the awareness of pet food potential hazards to mitigate public health risks.

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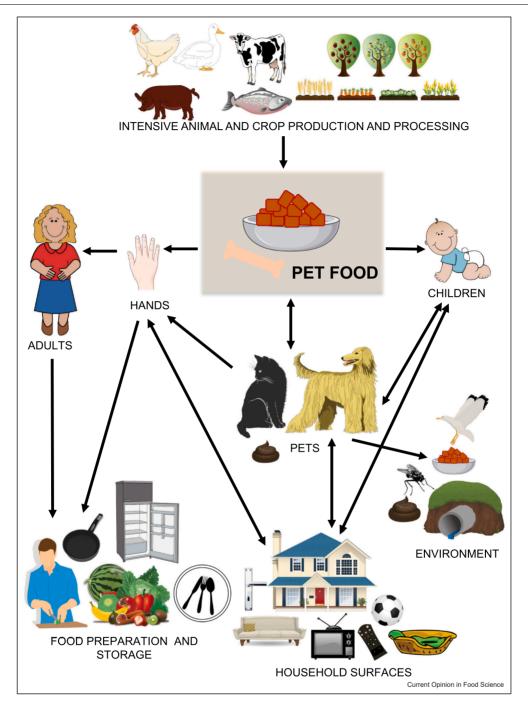
#### Introduction

The increase in pet ownership, especially of dogs and cats, coupled with the evolving societal perspective on pets as cherished family members, has driven a transformation in the pet care industry, generating a growing demand for high-quality pet food products [1]. Diverse animal and vegetable by-products that are not intended for human consumption have emerged as components of pet food. The traditional methods of producing conventional processed pet food (e.g. dry and canned food), involving grinding, cooking, extrusion, and/or dehydration, have long been renowned for their microbiological safety and nutritional suitability for pet consumption [1-3]. However, there has been a noticeable rise in the popularity of raw meat-based diets (RMBDs) for pets in developed countries, which can be considered more natural. These diets often comprise uncooked or minimally processed meat, bones, and organs, frequently frozen, therefore with a potential higher risk for pets and humans [1,4,5].

Microbial contamination during pet food production stems from various sources covering the raw animal- or vegetable-based ingredients and the entire manufacturing process (e.g. mixing, packaging, storage, distribution, or handling within processing facilities) due to lack of hygiene and cross-contamination [1]. To counter these risks, strict regulations in developed countries, including third-country imports, have been adopted to enforce hygiene standards, quality control, and manufacturing practices to ensure pet food safety and quality. In Europe, regulatory guidelines governing the utilization of animal by-products and their derivatives, specifically in the production of both processed and raw pet food, play a crucial role in upholding microbiological safety standards [6–8]. In the United States, the US Food and Drug Administration (FDA), in collaboration with state and federal authorities, is responsible for enforcing pet food laws and regulations [9].

Nevertheless, microbiological hazards have been documented within various types of pet food, but particularly in RMBDs, encompassing bacteria, viruses, and parasites. The most substantial body of evidence and consequent risk stems from bacteria, either those responsible for zoonotic disease or antimicrobial-resistant (AMR), often displaying resistance to diverse





Routes of pathogen and AMR bacteria spread between pet food, pets, humans, and the environment. Most images were depicted via Pixabay.

therapeutic drugs [1]. Transmission of such bacteria can occur through direct contact with pets or their feed and indirectly through contaminated household surfaces or hands during feed preparation (Figure 1) [1,10–12]. Hypothetically, environmental microbial spread beyond the household can also occur (e.g. through wastewater or via livestock or wild animals in contact with pet food or animal feces).

The extensive interaction between pets and humans (particularly vulnerable populations as in old people's home or when dogs as used as therapy) emphasizes the importance of thoroughly understanding pet food as a potential pathway for transmitting zoonotic pathogens and AMR bacteria to humans. Therefore, this review aims to consolidate recent evidence from the literature concerning the contamination of pet food, especially RMBDs, with diverse bacterial pathogens and/or AMR bacteria, and their transmission to both pets and humans. It also emphasizes the value of use whole-genome sequencing (WGS) as the reference typing method to substantiate such findings, as well as implementing focused strategies to mitigate those microbiological hazards in the pet food production sector.

#### Bacterial hazards associated with pet food Foodborne pathogens

Safety concerns related to pet food primarily focus on the potential contamination of raw ingredients with zoonotic pathogenic bacteria, especially in RMBDs [1,12-16]. Such contamination could result in the transmission of these pathogens to both pets and humans living with pets, posing an emerging public health issue. While most foodborne infections usually result in self-limiting gastroenteritis in generally healthy individuals, some are linked to low infectious doses and can lead to severe infections, as seen in some cases of salmonellosis, campylobacteriosis, Shiga toxin-producing *Escherichia coli* (STEC) infections. These infections as well as listeriosis pose a significantly greater risk to immunocompromised individuals, as well as to the young and elderly [17].

The first piece of evidence supporting pet food microbiological risks is the multitude of studies that have shown contamination levels exceeding established hygiene limits [5,12] and the presence of various zoonotic pathogens, including Salmonella, Campylobacter, and pathogenic *E. coli*, primarily or exclusively in commercially available raw pet food batches (Table 1) [1,4,5,12]. For example, in Europe, the frequency of Salmonella detection in raw pet food available to pet owners has varied, ranging from 7% in Portugal to 20% in Germany, and a substantial 71% in Italy [5,12,18]. In the United Kingdom, a recent report spanning 2020-2022 indicated that Salmonella detection in raw meat pet food has increased, with the highest number of 406 isolations in 2022. Approximately one-third of these cases involved serotypes of public health significance, such as Salmonella Typhimurium and Salmonella Infantis [19]. Additionally, in terms of European Union (EU) official controls, the presence of Salmonella (of diverse serotypes) in pet food has consistently been the most frequent cause of notification. For instance, since 2020, there have been > 20 notifications of RMBDs related to Salmonella contamination through the Rapid Alert System for Food and Feed [20]. Due to the health risk associated with exposure to zoonotic pathogens, the detection of *Salmonella*, whether through official sampling or consumer complaints, has increasingly resulted in extensive recalls of pet food brands, especially raw pet food batches [21–24], but also dry foods [25]. However, recent studies have also reported a frequent occurrence of other pathogens of major concern in raw pet food, such as STEC including serotypes associated with human disease worldwide, for example, O157:H7, O26:H11, O91:H10, O91:H14, O145:H28, O146:H21, and O146:H28 [12,26], as well as *Listeria monocytogenes* [4,12] and *Campylobacter* [4,12].

The second strand of evidence of pet food microbiological risks is provided by studies demonstrating clonal relationships between strains from pet food and pets or humans. In two comprehensive case investigations by the FDA, involving three households with animal illnesses, WGS analysis demonstrated that the pets likely acquired Salmonella after ingesting their respective raw pet foods (animal clinical isolates were closely related to one or more raw pet food bacterial isolates) [13]. A recent study, conducted in Chile, confirmed the genetic association through PFGE analysis, linking Salmonella isolates in raw pet food to fecal samples from dogs fed with RMBDs in the same household. Notably, no pathogens were detected in extruded food samples or feces from dogs fed with extruded food [4]. Moreover, some studies have revealed a significant difference in the excretion of zoonotic bacteria (e.g. Salmonella and Campylobacter) in feces between dogs fed with RMBDs and dogs fed with dry food, underscoring the microbiological risk posed by RMBDs not only to dogs but also to individuals handling RMBD and dog feces, as well as to the environment [3,27]. Additionally, a recent study in Portugal identified the presence of the epidemic S. Typhimurium monophasic variant ST34 clone with genetic similarities to human clinical isolates from various geographic regions in a batch of turkeybased RMBD [18]. Also, in the United Kingdom, Salmonella isolates of two serotypes (Salmonella Derby and S. Typhimurium) recovered from nonprocessed dog treats were also similar to published genomes from human clinical cases [28]. Considering the limited availability of public genomes from bacteria found in pet food, we cannot dismiss the potential for strains related to both pet food and humans to circulate among different food animal hosts across countries in a greater extent than currently recognized.

The third set of evidence is supported by several welldocumented outbreaks and investigations linking contaminated pet food products to human infections [29–32]. The largest documented human salmonellosis outbreak linked to *Salmonella*-contaminated pet foods occurred in 2019 across 34 US states, infecting more than 150 people and was related to the improper handling of pig ear treats for dogs, as confirmed by WGS [32]. A

Table 1								
Description of z	Description of zoonotic pathogen contamination by type		of pet food samples.					
Pathogen	% of positive samples (type of feed)	Sampling details ( purchase local)	uils (type of feed, num I)	Sampling details (type of feed, number, main ingredients, purchase local)	Country (year)	Strain typing-serotypes; sequence type <sup>a</sup>	Methods	Ref.
Salmonella	0%	35 Dry dog food	Single or multiple animal species (meat, fish, or insects)	Pet stores (international and local brands)	Poland (2022)	NA	Cultural	[54]
	%0	24 Dry dog food	Unknown	Commercial brands	Chile (NK)	NA	Cultural, PFGE	[4]
	0%	36 Dry dog food	Single or multiple animal species (meat, fish, or insects)	Commercial suppliers and supermarkets (27 international and 9 local brands)	Poland (NK)	NA	Cultural	[14]
	%0	41 Dry dog food	Single or multiple animal species (meat or fish)	Commercial or specialized stores (25 brands)	Portugal (2019–2020)	NA	Cultural, WGS	[18]
	7% (turkey)	14 Dog RMBDs (frozen)	Single or multiple animal species (meat or fish)	Commercial or specialized stores (2 international brands)	Portugal (2019–2020)	S: Typhimurium monophasic variant; ST34	Cultural, WGS	[18]
	16% (dried bull's penis 'pizzle sticks', bison ears, furry rabbit ears, and dried chicken treats)	84 Dried natural dog treats	Single animal species (meat)	Local pet shops and online retailers	The United Kingdom (2021)	S. Derby-ST40/ST682, Salmonella Dublin -ST10, S. intantis-ST32, Salmonella anatum-ST64, S. typhimurium monophasic variant-ST34	Cultural, WGS	[28]
	12% (deer, horse, chicken)	60 Dog RMBDs (frozen)	Single or multiple animal species (meat)	Local stores (domestic and imported brands)	Japan (2016–2017)	S. Infantis, S. Typhimurium, and S.Schwarzengrund	Cultural	[16]
	26% (chicken, beef)	42 Dog RMBDs	Single or multiple animal species (meat or fish)	Commercial (6 brands) and home prepared	Chile (NK)	И	Cultural, PFGE	[4]
	20% (chicken and beef from organic farms)	10 Dog and cat RMBDs (frozen)	Single or multiple animal species (meat or fish)	German internet top sellers (3 brands)	Germany (2017)	Ę	Cultural	[5]
	50%	17 RMBDs (frozen and freeze dried)	Single or multiple animal species (meat or fish)	Online and physical stores (12 brands)	Thailand (2019–2020)	Ę	Cultural	[15]
	71%	21 RMBDs (frozen)	Single or multiple animal species (meat)	Online stores (3 brands)	Italy (NK)	Ę	Cultural	[12]
	4% (lamb, turkey)	51 Dog RMBDs (frozen)	Single or multiple animal species (meat or fish)	Commercial or internet pet shops in 6 cities (8 suppliers)	Switzerland (2018)	S. Typhimurium monophasic variant; S. London	Cultural	[39]
STEC	41% (beef, poultry, horse, lamb, venison, rabbit. and fish)	59 RMBDs (frozen)	Single or multiple animal species (meat or fish)	Online and physical stores (10 suppliers)	Switzerland (2018–2020)	091:H14, 0146:H21, 076:H19, 0113:H21, 0146:H28, and 0168:H8; ST33. ST442. ST718. and ST738 <sup>b</sup>	Cultural, PCR, and WGS	[26]
	23%	21 RMBDs (frozen)	Single or multiple animal species (meat)	Online stores (3 brands)	Italy (NK)	0157:H7	Cultural	[12]

Table 1 (continued)	<i>(</i> , <i>L</i> )							
Pathogen	% of positive samples (type of feed)	Sampling details ( purchase local)	ls (type of feed, num )	Sampling details (type of feed, number, main ingredients, purchase local)	Country (year)	Strain typing-serotypes; sequence type <sup>a</sup>	Methods	Ref.
Campylobacter	%0	24 Dry dog food	Unknown	Commercial brands	Chile (NK)	NA	Cultural	[4]
	%0	42 Dog RMBDs	Single or multiple animal species (meat or fish)	Commercial (6 brands) and Chile (NK) home prepared	Chile (NK)	NA	Cultural	[4]
	%0	17 RMBDs (frozen and	Single or multiple animal species	Online and physical stores (12 brands)	Thailand (2019–2020)	NA	Cultural	[15]
	29%	(frozen) (frozen)	Single or multiple animal species (meat)	Online stores (3 brands)	Italy	0157:H7	Cultural	[12]
L. monocytogenes 0%	%0	36 Dry dog food	Single or multiple animal species (meat, fish, or	Commercial suppliers and supermarkets (27 international and 9 local brande)	Poland (NK)	AA	Cultural	[14]
	%0	24 Dry doa food	Unknown	Commercial brands	Chile	NA	Cultural	[4]
	19% (chicken, beef, salmon, or guanaco)	42 Dog RMBDs	Single or multiple animal species (meat or fish)	Commercial (6 brands) and home prepared	Chile	ΤΊ	Cultural	[4]
	20% (chicken)	17 RMBDs (frozen and	Single or multiple animal species	Online and physical stores (12 brands)	Thailand (2019–2020)	ИТ	Cultural	[15]
	%06	21 RMBDs (frozen)	Single or multiple animal species (meat)	Online stores (3 brands)	Italy	0157:H7	Cultural	[12]
Abbreviations: NA, <sup>a</sup> Strain typing (sei <sup>b</sup> Only the sequen	Abbreviations: NA, not applicable; NK, not known; NT, not typed. <sup>a</sup> Strain typing (serotype and/or sequence type as obtained by multi <sup>b</sup> Only the sequence types identified more than once are described.	iown; NT, not typ be as obtained by an once are desc	ed. / multilocus sequenc sribed.	yped. by multilocus sequence typing schemes) is included upon the availability of each study. scribed.	d upon the availat	oility of each study.		

recent well-documented familial human salmonellosis outbreak of monophasic Salmonella Typhimurium (ST34) involved dogs and their owner's two children, WGS confirmed a high relatedness between the strains [29]. There is also a recent outbreak of S. Reading in North America with some human cases being linked with raw pet food containing turkey [33]. And in a very recent Salmonella outbreak involving seven illnesses and one hospitalization in seven US states, authorities alerted for the link between dry dog food and ill people (most infants), with WGS showing the same strain in dog food and sick people [25]. They suggested that people in this outbreak got sick from touching recalled dog food, touching things such as dog bowls that contained the dog food, or touching the feces or saliva of dogs that were fed the dog food. These findings support the hypothesis that dogs can serve as asymptomatic carriers of Salmonella, which can be transmitted through direct contact, the handling of dogs' food and/or the environment (e.g. bowls) [29].

In addition to *Salmonella* investigations, a cluster of severe cases of STEC O157:H7 infections in humans identified in the United Kingdom, with one death, pointed to exposure to raw pet food (specifically tripe), raising concerns about potential risks to individuals, especially children, who come into contact with raw pet food [30-]. In this context, it seems very likely that conventionally heat-treated pet food is a safer alternative to raw diets, emphasizing the critical role of heat treatment in effectively mitigating microbiological hazards during production.

#### Antimicrobial resistance

Microbiological risks also include the identification of AMR strains and strains carrying acquired antimicrobial resistance genes in pets and their owners, with pet food emerging as a potential source [1,34–36]. While the quantity of studies is still limited, there is a growing body of evidence indicating that pet food can act as a vehicle of multidrug-resistant (MDR) bacteria. The identification of antibiotic-resistance genes or bacteria exhibiting clinical resistance to antimicrobials ranking as the most critically important in human medicine (e.g. ampicillin, third generation cephalosporins, colistin, linezolid, vancomycin) [37] in available studies is of utmost importance (Table 2).

Different studies, among the earliest and most current, assessing AMR bacteria in RMBDs have identified the presence of extended-spectrum beta-lactamases (ESBLs) in high proportions (>60%) in various *Enterobacterales* species, such as *E. coli* and *Salmonella* [1,18,38,39]. These data are of concern as ESBLs confer resistance to extended-spectrum cephalosporins such as ceftriaxone and cefotaxime, which are critically important to treat Gram-negative infections of humans [37], and also used therapeutically in veterinary species.

Resistance to colistin, a last-resort antibiotic used to treat severe MDR infections [37], has been detected in dog RMBD samples (4-14%) contaminated with E. coli in different European countries (Table 2). It has been associated with mcr-1 gene carried by isolates that also showed resistance to multiple antimicrobials, including some considered critically important in human medicine (e.g. ampicillin, gentamicin, and ciprofloxacin; Table 2) [18,39]. Among the most important therapeutic options to treat human Gram-positive bacterial infections are the antimicrobials vancomycin and linezolid [37]. Pet food, obtained in Europe and China, has also been linked to bacteria with genes conferring resistance to such lastresort antimicrobials (Table 2). For vancomycin resistance, a single vanA-carrying Enterococcus faecium was recovered in a wet food sample [40], while all samples with bacteria carrying linezolid-resistance genes (optrA-, poxtA-, and/or cfr-positive Enterococcus or Vagococcus lutrae) were raw based with diverse by-products originating (where known) from EU and Australia [41-44].

Other acquired genes conferring resistance to a variable number of antimicrobials have been also detected in different hosts (*Salmonella* and other Enterobacterales, *Enterococcus*), not only in RMBDs but also in dry/treats and wet dog foods obtained in Portugal, the United Kingdom, and Japan [16,18,28,40]. Resistance to other antimicrobials that can also have significant implications in human medicine (e.g. tigecycline) or be part of mobile genetic elements often containing multiple resistance genes cannot be underestimated due to coselection events (Table 2).

Remarkably, additional studies comparing strains identified in pet food samples with publicly available genomes, by using genomics and phylogenetic tools, have identified the same strain and/or plasmid carrying antimicrobial resistance genes across dog RMBDs and human clinical samples from different countries [18,42] or between raw-fed dogs and their respective food products [44]. For example, comparative genomics revealed that international RMBDs batches contained E. coli clones harboring the mcr-1 gene on IncX4 plasmids, which were identical to others circulating worldwide among diverse hosts (humans, pig, poultry) and the environment [18]. Also, the same (99% identity) optrA-carrying plasmid was identified in linezolid-resistant Enterococcus faecalis strains from raw dog food in Portugal and from hospitalized patients in Spain and China [42]. The same study described phylogenetic relationships between strains obtained from dog food marketed in Portugal with strains obtained from swine, chicken, and wastewaters in the United Kingdom, as well as from hospitalized patients in the Netherlands.

#### Hygiene perspective

In addition to the aforementioned, it is crucial to highlight the absence of information regarding food safety practices (e.g. handwashing, safe handling) when

amp	yin d		antimicrobial resistance genes they carry. Suppliers/brands of Country (year) Coun	ce genes they c Country (year)	<b>carry.</b> Country of	Host	Serotype/sequence	Associated AMR	Ref.
details (type of feed, (n composition)	of feed,	amples analyze	σ		origin of the raw meat		type	phenotype or genotype <sup>d</sup>	
14 (2/14) Dog Turkey, deer Commercial or RMBDs salmon, white fish, specialized stores vegetables, and/ (2 international or fruit brands)		Commercial or specialized store 2 international orands)	S	Portugal (2019–2020)	UK, Europe	E. coli	ST297, ST3997	AML, AMC, CHL, CIP, KAN, GEN, NAL, STR, SUL, TET, and/or TRP	[18]
Dog RMBDs	ced	Commercial or nternet pet sho n 6 cities (8 suppliers)	sd	Switzerland (2018)	Switzerland, Germany	E. coli	ST69, ST1431	AMP, CIP, KAN, NAL, STR, SXT, and/or TET	[39]
2% (1/55) Dog Meat and animal Commercial or pate subproducts (4% specialized stores poultry, 4% liver) in 4 cittes (25 brands)		Commercial or specialized sto n 4 cities (25 orands)	res	Portugal (2019–2020)	Germany	E. faecium	ST17	VAN, TEC, AMP, CIP, ERY, GEN	[40]
<ul> <li>41% Dog Beef, poultry, Commercial or (24/59) RMBDs lamb, horse, internet pet shops rabbit, in 6 cities (10 venison, fish suppliers)</li> </ul>	oultry, orse, , fish	Commercial or nternet pet shu n 6 cities (10 suppliers)	sdo	Switzerland (2018–2020)	Switzerland, Germany, Australia, EU	E. faecalis	ST16, ST86, ST207, ST256, ST369, ST376, ST474, ST476, ST593, ST631	aac(6')-aph(2''), ant (9)-la, aant(6)-la, aph(3')-lll, cat, dfrG, emr(A), erm (B), fexA, fexB, Isa (A), Isa(E), Inu(B)	[43]
Dog Beef, poultry, RMBDs venison, fish	eef, poultry, anison, fish					E. faecium	ST168, ST264, ST822, ST1846	aac(6)-aph(2"), ant(6)-la, aph(3)-lll, clpL, dtFG, erm (A), erm(B), fexA, fexB, Inu (B), Inu(G), Isa(E), cat (pC233), msr(C), tet(L) and /or tet(M)	)- <i>la,</i> , <i>erm</i> B, <i>lnu</i> t , and
Dog Beef, poultry, RMBDs horse, rabbit	eef, poultry, orse, rabbit					V. lutrae	μ	aac(6)'-aph(2"), ant(6)-la, aph(3)-lll, cat(pC221), dfrG, em(B), fexA, lnu(B), lsa(E), tet(L), tet(N), mef(A) and/or msr(D)	)-la, ), nu(B), nef(A)
64% (9/14) Dog Duck, chicken, Commercial or RMBDs turkey, goose, specialized stores salmon (2 international brands)		Commercial or specialized sto 2 international orands)	les.	Portugal (2019–2020)	UK, Europe	E. faecalis	ST40, ST674, ST1008, ST1009	aac(6)-aph(2'), ant (9)-la, ant(6)-la, aph (3')-III, fexA, fexB, cat, dfr(G), erm(B), Isa(A), Isa(E), Inu (B), tet(L) and/or tet(M)	[42]
Dog Beef, duck, deer, RMBDs salmon	eef, duck, deer, almon					E. faecium	ST25, ST80, ST264, ST1091, ST1263	aac(6)-aph(2"), ant(9)-la, ant(6)-la, aph(3)-lll, em (A), erm(B), msr(C), fexB, cat, Inu(B), Isa(E), Inu(G), tet(M), tet(L) and/or dfr(G)	)-la, erm exB, u(G), dfr(G)

Table 2 (continued)	(pə									
Acquired AMR genes <sup>a</sup>	% of positive samples ( <i>n</i> of samples)	AMR-carryinç details (type composition)	AMR-carrying sample's details (type of feed, composition)	Suppliers/brands of samples analyzed	Country (year)	Country of origin of the raw meat	Host	Serotype/sequence type <sup>b</sup>	Associated AMR phenotype or genotype <sup>d</sup>	Ref.
	49% (11/224)	Dog RMBDs	Beef, chicken wing, pork, egg, onion, caraway seeds, cucumber	Vegetables/raw meat products obtained at 28 supermarkets in Beiiing	China (NK)	NK	E. faecalis	ST16, ST256, ST309, ST474, ST476, ST632	CIP, CHL, ERY, FFC, GEN, LIN, MIN, and/or RIF	[44]
				5			Enterococcus casseliflavus	NT	CIP, CHL, DPC, ERY, FFC, MIN, and/or RIF	≻" ⊑
ESBLs	61% (31/51)	Dog RMBDs	Beef, poultry, horse, lamb, rabbit, venison, fish	Commercial or internet pet shops in 6 cities (8 suppliers)	Switzerland (2018)	Switzerland, Germany	Enterobacteriaceae	ST23, ST58, ST88, ST117, ST155, ST361, ST7085°	CTX-M variants, SHV-12	[39]
	78% (14/18)	Cat RMBDs	Chicken, beef, beef tripe, turkey, duck, lamb	Supermarkets, pet shops, garden centers (5 brands, Utrecht)	The Netherlands (NK)	X	Enterobacteriaceae	Ţ	CTX-M variants, OXA-1, CMY-2, SHV-12, TEM-1 variants	[38]
Miscellaneous	7% (1/14)	Dog RMBD	Turkey, lamb, vegetables	Commercial or specialized stores (2 international brands)	Portugal (2019–2020)	UK, Europe	Salmonella	S. Thyphimurium monophasic-ST34	aac(6')-laa, bla (TEM), tet(B), sul2, strA-strB	[18]
	78% (11/14)	Dog RMBDs	Ruminant/poultry- based; salmon oil, vegetables, and/ or fruit	Commercial or specialized stores (2 international brands)	Portugal (2019–2020)	UK, Europe	Enterobacteriaceae	T	Т	
	16% (13/84)	Dog treats	Chicken treat, bison ear, furry rabbit ear, dried bull's penis 'pizzle sticks'	Pet shops and online retailers (4 suppliers)	UK (2021)	¥	Salmonella	<ul> <li>S. Dublin -ST10, S. Infantis -ST32, S. Thyphimurium monophasic-ST34, S. Anatum -ST64, S. Derby -ST40, ST782</li> </ul>	AMP, TIG	[28]
	0.5% (3/60)	Dog RMBDs	Chicken, deer	50 domestic and 10 imported products (3 brands)	Japan (2016–2017)	Japan, Canada, USA, Mexico, New Zealand	Salmonella	S. Thyphimurium , S. Infantis, S. Schwarzengrund	floR, aadA1, tetB, dfrA12	[16]
	20% (11/55)	Dog dry food	Meat/fish/animal subproducts, cereals, oils/fats, potatoes, peas, vitamins	Commercial or specialized stores in 4 cities (25 brands)	Portugal (2019–2020)	¥Z	E. faecium	۲	ERY, STR, and/ or ΩD	[40]
		Dog treats Dog wet foods	Meat/animal subproducts (4% c cereals, fish Stew with chicken (8%) and vegetables (2%) Beef (>40%), meat broth, tripe, vegetables, rice	oducts (4% chicken, 4% cow), (8%) and it broth, tripe,	% cow),		E. faecalis	ŧ	ERY, TET, STR, and/ or CHL	2

Table 2 (continued)	ed)								
Acquired AMR genes <sup>a</sup>	% of positive samples ( <i>n</i> of samples)	% of AMR-carrying sample's positive details (type of feed, samples (n composition) of samples)	Suppliers/brands of Country (year) Country of samples analyzed origin of the raw meat	try (year) C o	Country of origin of the raw meat	Host	Serotype/sequence type <sup>b</sup>	Associated AMR phenotype or genotype <sup>d</sup>	Ref.
		Dog Dehydrated proteins of poultry/pork, dry food chicken meat/fat Dog Cereals, oils/fats, meat, and animal treats subproducts	is of poultry/pork, neat, and animal			Enterococcus gallinarum	ИТ	TET and/or STR	
Abbreviations: A erythromycin; FF sulfamethoxazol	MC, amoxicilli C, florfenicol; e; TEC, teicop	Abbreviations: AMC, amoxicillin + clavulanic acid; AML, amoxicillin; AMP, ampicillin; AMP, antimicrobial resistance genes; CHL, chloramphenicol; CIP, ciprofloxacin; DPC, daptomycin; ERV, erythromycin; FFC, florfenicol; GEN, gentamicin; KAN, kanamycin; LIN, linezolid; NAL, nalidixic acid; NK, not known; NT, not typed; RIF, rifampicin; STR, streptomycin; SUL, sulphonamides; SXT, sulfamethoxazole; TEC, teicoplanin; TET, tetracycline; TIG, tigecycline; TRP, trimethoprim; VAN, vancomycin.	xicillin; AMP, ampicillin; AMF /cin; LIN, linezolid; NAL, nalid gecycline; TRP, trimethoprim	8, antimicrob ixic acid; NM ; VAN, vanc	oial resistance ζ, not known; N omycin.	genes; CHL, chloram IT, not typed; RIF, rifar	phenicol; CIP, ciprofloxac mpicin; STR, streptomycin	in; DPC, daptomycin; ; SUL, sulphonamides	ERY, SXT,
<sup>a</sup> Only antimicro highlighted in th. <sup>b</sup> Strain typing ( <sup>c</sup> <sup>c</sup> Only the seque <sup>d</sup> The presentati	bial resistance s column; oth erotype and/c ince types ide	<sup>a</sup> Only antimicrobial resistance genes conferring resistance to last-resort antimicrobials considered of critical importance in human medicine according to WHO (indicated in brackets) are highlighted in this column; others are included as 'miscellaneous' (in this case, the total incidence of antimicrobial resistance is considered). <sup>b</sup> Strain typing (serotype and/or sequence type as obtained by multilocus sequence typing schemes) is included upon the availability of each study. <sup>c</sup> Only the sequence types identified more than once are described. <sup>d</sup> The presentation of results varies depending on the available data for each study, including either the antibiotic susceptibility phenotype (in capital letters) or the genotype (genes in italics)	to last-resort antimicrobials considered of critical importance in human medicine according to WHO (indicated in brackets) are sous' (in this case, the total incidence of antimicrobial resistance is considered). by multilocus sequence typing schemes) is included upon the availability of each study. scribed.	considered ncidence of g schemes) ding either	of critical impo antimicrobial r is included up the antibiotic s	ortance in human mei esistance is considere on the availability of € usceptibility phenotyr	dicine according to WHO ad). aach study. oe (in capital letters) or the	(indicated in bracket	s) are talics)

handling raw pet food by most manufacturers [31], including on labels found on the raw pet food samples. These findings emphasize the importance of implementing and adhering to appropriate hygiene measures and safe handling practices when dealing with pets, raw pet food, and within household environments (e.g. refrigerators/freezers, bowls, pet bedding, litter boxes, toys, floor, and any other surfaces that the food or pet may have contact with) (Figure 1) to mitigate the risk of MDR bacterial infections in humans. Children and infants, in particular, are at higher risk of exposure (e.g. hand-oral transfer) due to their immature juvenile behaviors and hygiene practices, including being more likely attracted to pet snacks with toy-like shapes [45]. Moreover, recent consumer surveys evaluating pet owners' food safety knowledge and pet food handling practices indicate the need for consumer education about handling pet food since, among other relevant findings, less than one-third of pet owners practiced proper hand hygiene, most pet owners lacked awareness of pet food recalls or outbreaks related to foodborne pathogens, or they lack an adequate understanding of antimicrobial resistance [4,10,31,46,47]. Pet owners should be educated about personal hygiene and proper handling of pet food (e.g. hand washing and separation of human and pet foods), and warnings and handling instructions should be included on product labels of RBMD packages.

## **Concluding remarks**

Documented microbiological hazards in pet food, particularly raw diets, primarily involve bacterial risks, with the most substantial threats evidence coming from Salmonella. Pet food contamination and the risk of pathogens and AMR bacteria transmission to both pets and humans, leading to significant public health concerns, are currently substantiated through WGS-based typing methods, primarily focusing on dog food but also in some reports including cat food. Although data should be interpreted considering the sampling strategies and testing methodologies employed, it is undeniable that pet food products, and RMBDs especially, are potential vehicles for the transmission of MDR zoonotic-related bacteria and antimicrobial resistance genes of highest priority. Supporting this, risk factor analysis in various studies has concluded that raw feeding or using offal as main feed is associated with carriage of pathogenic and/ or AMR bacteria in dogs [3,27,48-50].

Overall, any bacterial species, more or less pathogenic to humans, can contribute to the spread of clinically relevant antimicrobial resistance genes across different hosts and settings, ultimately posing a risk to humans. Raw pet food, in particular, could serve as an indicator for emerging antimicrobial resistance traits of foodborne origin once these products often incorporate ingredients

identified

from various sources, including animals linked to intensive farming and from third countries with different food safety policies. Indeed, MDR *Salmonella* and *E. coli* isolates have been detected in poultry, pork, and beef products intended to be used for pet diets [51]. This adds another dimension to the global challenge of antimicrobial resistance, underscoring the need for increased vigilance within One Health perspective.

Taking into account the current compelling body of evidence, it becomes crucial to adopt mitigation measures focused on human health, including awareness of the risks associated with feeding RMBDs to companion animals. We also anticipate that, among other measures like improved labeling, emerging food technology treatments (e.g. high-pressure processing, ozone) of raw pet food may be adopted by the pet food industry to control pathogens [52–54]. The escalating microbiological hazards of pet food are of particular relevance to stakeholders engaged in the One Health initiative and policymakers responsible for overseeing food safety regulations and safeguarding consumer health.

### **CRediT** authorship contribution statement

PA: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. CN: Conceptualization, Visualization, Writing – review & editing. LP: Writing – review & editing. ARF: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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## **Data Availability**

Data will be made available on request.

## **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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