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REVIEW ARTICLE
Comparison of the prevalence of bacterial enteropathogens, potentially zoonotic bacteria and bacterial resistance to antimicrobials in organic and conventional poultry, swine and beef production: a systematic review and meta-analysis

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SUMMARY

The prevalences of zoonotic and potentially zoonotic bacteria or bacteria resistant to antimicrobials in organic and conventional poultry, swine and beef production were compared using systematic review and meta-analysis methodology. Thirty-eight articles were included in the review. The prevalence of *Campylobacter* was higher in organic broiler chickens at slaughter, but no difference in prevalence was observed in retail chicken. *Campylobacter* isolates from conventional retail chicken were more likely to be ciprofloxacin-resistant (odds ratio 9·62, 95% confidence interval 5·67–16·35). Bacteria isolated from conventional animal production exhibited a higher prevalence of resistance to antimicrobials; however, the recovery of some resistant strains was also identified in organic animal production, where there is an apparent reduced antimicrobial selection pressure. Limited or inconsistent research was identified in studies examining the prevalence of zoonotic and potentially zoonotic bacteria in other food-animal species. There is a need for further research of sufficient quality in this area.

Key words: Antimicrobial resistance, food safety, organic, systematic review.

INTRODUCTION

Foodborne disease has a significant public health impact worldwide [1]. The monthly prevalence of acute gastroenteritis has been estimated to be 3·4% in Ireland, 6·4% in Australia and 7·6% in Canada and USA [1]. *Campylobacter* and *Salmonella* spp. account for more than 90% of all reported cases of bacterial foodborne illness worldwide [2]. Apparently healthy poultry, swine and beef carry such organisms, and contaminated products of animal origin are important sources of foodborne infections for humans [3–5]. While the bacterial food safety of conventional food-animal production has been extensively studied and reviewed by experts [4–7], both primary research and reviews on this aspect in organic food-animal production are scarce, perhaps due to the relatively recent growth in popularity of organic food-animal production [8, 9].

Organic production is an agricultural system that emphasizes animal welfare and ecosystem sustainability while minimizing the use of off-farm inputs.

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Prophylactic use of antimicrobials and growth hormones is prohibited, although antimicrobials can be used to treat ill animals when all other options fail [10, 11]. Organic animals generally require daily outdoor access and must receive organic feed, but these and other requirements can differ by jurisdiction [10, 11].

Consumers often purchase organic products because they believe them to be healthier or safer than conventional products [12, 13]. As consumer interest and demand for organic products increases, a better understanding of the microbial food safety of organic production is necessary. Systematic reviews are a transparent and replicable method for summarizing or synthesizing available evidence on a topic, yet are under-utilized in agri-food public health [14]. This methodology was used to compare the prevalence of bacterial enteropathogens, potentially zoonotic bacteria and bacteria resistant to antimicrobials in organic and conventional poultry, swine and beef production. The quantity, consistency and methodological soundness of all published primary research in this area was identified and evaluated. When appropriate, meta-analysis was applied on selected data subsets to generate pooled prevalence estimates.

METHODS

Literature search

An initial literature search was conducted in January 2007 and was updated in December 2007 and July 2008. No language or other restrictions were imposed. The databases used in the initial search were Agricola (1970–2007), Biological Sciences (1982–2007), BioMed Central (1997–2007), CAB Abstracts (1973–2007), Current Contents (1999–2007), Environmental Sciences and Pollution Management (1967–2007), Food Science and Technology Abstracts (1969–2007), Medline (1949–2007) and Scopus (1969–2007). The updated searches were restricted to Agricola, CAB Abstracts and Medline. Reference lists from all relevant articles ($n=50$) and from selected chapters of the *Handbook of Organic Food Safety and Quality* [15] were hand-searched to identify any additional relevant citations that were potentially missed by the online searches.

The searches were conducted using combinations of terms covering three main question components: (1) organic production, (2) food-animal populations and (3) bacterial and antimicrobial resistance (AMR) outcomes. To increase search specificity for the updated searches, only bacterial terms were used as the outcome because all studies describing AMR were also captured with these terms, and for the most updated search the search algorithm was shortened by removing redundant terms. Citations were managed and de-duplicated using Procite 5.0 (Thomson ResearchSoft, USA) and web-based software (SRS 4.0, TrialStat! Corporation, Canada) was used to manage the systematic review.

Relevance screening, quality assessment and data extraction

Relevance screening, quality assessment and data extraction was conducted using standardized web-based forms. Each form was pre-tested by all reviewers on a sample of citations ($n=50$ abstracts for relevance screening, $n=5$ articles for quality assessment, $n=2$ articles for data extraction). Forms were utilized when kappa agreements exceeded 0·8 for all reviewing pairs. Relevance screening was conducted in two stages. The first stage consisted of a form with one question that was used to rapidly identify all citations that described organic food-animal production, while the second stage consisted of a form used to classify abstracts by food-animal species, outcome and sampling point (i.e. farm, processing or retail). Non-primary research studies that did not clearly define 'organic animal production' or that did not measure the prevalence of bacterial enteropathogens, potentially zoonotic bacteria or bacterial resistance to antimicrobials were excluded.

Upon completion of relevance screening, relevant articles were procured and assessed for methodological soundness using the following five criteria: (1) comparison groups sampled from the same target population, (2) sample size explicitly justified, (3) formal systematic or random sampling method used to select the primary sampling units, (4) methods to measure the outcome described in sufficient detail to allow reproducibility of the study, and (5) statistical methods sufficiently described and appropriately used. Experimental studies were evaluated using two additional criteria: random allocation of treatment and reported use of blinding. These criteria were used for description purposes and not as exclusion criteria. Two forms, one web-based and one spreadsheet, were used to extract study design parameters (e.g. sampling and laboratory procedures) and outcome data [e.g. odds ratios (OR)] from each relevant study.

International and local collaborators assisted with translation of articles published in non-English (foreign) languages. Foreign-language articles confirmed as relevant were fully translated and reviewed using the same methods as articles published in English. Ten different reviewers conducted relevance screening for this review, while three and two of these reviewers also conducted quality assessment and data extraction, respectively. Two reviewers independently reviewed each abstract or article and disagreements were resolved by group consensus. A copy of the forms used and the list of search terms and combinations are available from the corresponding author upon request.

Statistical analysis

Data were stratified by food-animal species, outcome, sampling point, unit of analysis (e.g. flock *vs*. individual bird) and diagnostic test [e.g. enzyme-linked immunosorbent assay (ELISA) *vs*. culture]. Meta-analysis was conducted within each stratum if estimates were available from more than two studies. Crude OR and standard errors were calculated for each study, as none of the included studies provided adjusted estimates of effect, and pooled OR and forest plots were calculated for each analysis using the Mantel–Haenszel method [16]. Heterogeneity was evaluated using Cochran's Q statistic and I^2 (the percentage of total variation across studies due to heterogeneity) [17]. Heterogeneity was considered acceptable and pooled OR were reported when P values for the Q statistic were $>0{\cdot}1$ [16]. Pooled OR were considered statistically significant if 95% confidence intervals (CI) excluded the null. Publication bias was investigated using Begg's rank correlation test and Egger's regression test [16]. All analyses were performed in Stata 10 (Stata Corporation, USA).

RESULTS

Description of studies

The results of the systematic review process are summarized in Figure 1. Ninety-one citations were considered relevant based on the title and abstract; however, after assessment of the full article, 41 studies were excluded for reasons listed in Figure 1. In 12 studies, bacterial or AMR outcomes were investigated only in organic production (i.e. no conventional comparison group was used) and were not assessed for methodological soundness due to their descriptive nature. These studies are summarized in the Appendix (available in the online version of the paper) and are not discussed further.

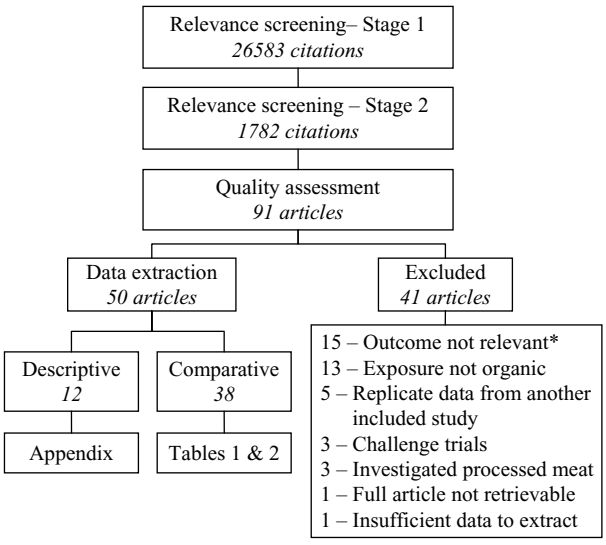

Fig. 1. Systematic review process flow-chart. * These studies did not measure the prevalence of bacterial enteropathogens, potentially zoonotic bacteria or bacterial resistance to antimicrobials in food animals, foods of animal origin or people.

In 37 studies, specific bacterial and AMR outcomes were compared between organic and conventional poultry, swine or beef production. In one additional study, organic food consumption was investigated as a risk factor in human cases of *Campylobacter*. Thirty-six (94·7%) of the 38 comparative studies were observational studies and two were experimental trials. Thirty-five (97·2%) of the 36 observational studies followed the principles of a cross-sectional design, five of which used longitudinal sampling, and a case-case design was used in the *Campylobacter* risk-factor study. Studies were published from 1991 to 2008, and 78·9% (30/38) were published between 2005 and 2008.

Sixteen studies published in seven foreign languages (Croatian, Danish, French, German, Italian, Spanish, Swedish) were investigated as potentially relevant, and four of these studies were included in the review [18–21].

Bacterial prevalence and mean count data

Bacterial prevalence or count data were reported in 34 studies (Table 1). Five studies investigated the

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Table 1. *Summary of 34 studies comparing the prevalence or mean counts of bacterial enteropathogens and potentially zoonotic bacteria between organic and conventional poultry, swine and beef production*

Food animal species/ reference	Study country	Sample period (month/year)	Sample point	Bacteria investigated	Sample type(s)/ pooling (yes/no)	Laboratory procedure	No. of units/samples: organic (conventional)	Significantly higher prevalence or mean counts (P value and/or OR)
Broiler chickens								
[31]	USA	04–05	Farm	*Salmonella*	Faeces/no	Culture	18 (14) farms, 512 (419) samples	Conventional: ($P<0{\cdot}0001$)
[43]	Denmark	10/98–02/01	Farm	VRE	Cloacal swabs/no	Culture	12 (17) farms, 22 (86) flocks	Conventional: ($P<0{\cdot}0001$)
[42]	UK	01/02–02/03	Farm	VRE	Faeces, farm environmental samples/yes	Culture	7 (26) farms	n.s.*
[24]	Belgium	Unknown	Farm, slaughter	*Campylobacter*, *Salmonella*	Hatching papers, faeces (*Campylobacter*), Hatching papers, overshoes (*Salmonella*)/ yes, caecum, duodenum/yes	Culture	9 (11) farms (one flock per farm)	Organic†: *Campylobacter* – caecum ($P=0{\cdot}024$), duodenum ($P=0{\cdot}036$)
[26]	Denmark	98–00	Slaughter	*Campylobacter*	Carcase swab/no	Culture	12 (18) farms, 22 (79) flocks	Organic†: ($P<0{\cdot}001$)
[22]	USA	08/00–11/02	Slaughter	*Campylobacter*	Intestinal tract/no	Culture	5 (10) farms, 355 (345) samples	Organic: ($P<0{\cdot}05$)
[25]	Belgium	10/03–04/04	Slaughter	*Campylobacter*, *Salmonella*	Caecum/no	Culture	3 (3) farms, 4 (4) flocks, 40 (40) samples	n.s.
[23]	USA	Unknown	Slaughter	*Campylobacter*, *Salmonella*	Viscera/no	Culture (*Campylobacter*), assay (*Salmonella*)	5 farms‡, 176 (250) samples	n.s.
[41]	Denmark	Unknown	Slaughter	*Clostridium perfringens*, coliforms, enterococci	Crop, ileum, caecum, rectum/yes	Culture	1 (1) farms, 10 (10) samples	Conventional§: coliforms – crop ($P=0{\cdot}006$), enterococci – crop ($P=0{\cdot}001$), ileum ($P=0{\cdot}002$), caecum ($P=0{\cdot}007$)
[32]	Italy	Unknown	Slaughter	*Salmonella*, enterobacteria, enterococci, staphylococci	Ileum, caecum/yes	Culture	4 (4) farms, 2 (2) samples (*Salmonella*), 4 (4) samples (other bacteria)	Conventional§: staphylococci ($P<0{\cdot}05$)
[28]	USA	02/03–05/03	Retail	*Campylobacter*	Chicken/no	Culture	2 (2) brands, 45 (45) samples	n.s.*
[29]	USA	01/04–06/06	Retail	*Campylobacter*	Chicken/no	Culture	3 (2) brands, 238 (170) samples	n.s.
[30]	UK	Unknown	Retail	*Campylobacter*	Chicken/no	Culture	30 (30) samples	n.s.*
[27]	USA	09/02–08/03	Retail	*Campylobacter*, *Salmonella*	Chicken/no	Culture	4 (6) brands, 198 (61) samples	n.s.*
[46]	Spain	05	Retail	*Enterobacteriaceae*	Chicken/no	Culture	30 (30) samples	Organic§: ($P<0{\cdot}0001$)
[45]	Spain	05	Retail	*Enterococcus*	Chicken/no	Culture	30 (30) samples	Organic§: ($P=0{\cdot}0002$)
[44]	USA	06/02–05/03	Retail	*Enterococcus faecium*	Chicken/no	Culture	26 (160) samples	n.s.*
[33]	USA	Unknown	Retail	*Salmonella*	Chicken/no	Culture	1 (2) brands, 12 (24) samples	n.s.

Table 1 (*cont.*)

Food animal species/ reference	Study country	Sample period (month/year)	Sample point	Bacteria investigated	Sample type(s)/ pooling (yes/no)	Laboratory procedure	No. of units/samples: organic (conventional)	Significantly higher prevalence or mean counts (P value and/or OR)
Broiler turkeys								
[22]	USA	08/00–11/02	Slaughter	*Campylobacter*	Intestinal tract/no	Culture	5 (10) farms, 230 (360) samples	n.s.
Laying hens								
[18]	Germany	03–04	Farm	*Salmonella*	Faeces/yes	Culture	88 (95) flocks, 124 (118) samples	n.s.*
[34]	Austria	10/04–10/05	Farm	*Salmonella*	Boot socks, dust (organic), faeces, dust (conventional)/ yes	Culture	69 (96) farms (one flock per farm), 7 samples per farm	n.s.*
[35]	France	10/04–09/05	Farm	*Salmonella*	Boot swabs, dust (organic), faeces, dust (conventional)/ yes	Culture	72 (230) farms (one flock per farm), 7 samples per farm	n.s.*
[20]	Italy	Unknown	Farm	*Salmonella*, enterobacteria	Eggs/no	Unknown	1 farm‡, 120 total samples	n.s.*
[21]	Italy	Unknown	Farm	*Salmonella*, *Staphylococcus*, coliforms	Whole eggs, egg shells, egg yolk/yes	Unknown	5 (4) flocks, 20 (30) egg, 54 (50) egg shell, 55 (50) egg yolk samples	Conventional§: coliforms – whole eggs ($P<0{\cdot}001$)
Swine								
[19]	Germany	03/01–04/02	Farm	*Salmonella*	Serum/no	ELISA	17 (78) farms, 144 (1009) farrowing farm, 372 (2270) finishing farm samples	n.s.*
[37]	USA	Unknown	Farm	*Salmonella*	Serum/no	ELISA	324 (292) samples	Organic: ($P<0{\cdot}001$)
[42]	UK	01/02–02/03	Farm	VRE	Faeces, farm environmental samples/yes	Culture	5 (9) farms	n.s.*
[49]	USA	10/02–10/04	Farm, slaughter	*Campylobacter coli*	Faeces, carcase swab/no	Culture	10 (11) farms, 141 (105) nursery farm, 292 (370) finishing farm, 341 (416) carcase samples	Organic: nursery farm ($P<0{\cdot}001$)
[38]	Denmark	Unknown	Farm, slaughter	*Salmonella*	Faeces, caecum, meat juice/no	Culture (faeces, caecum), ELISA (meat juice)	11 (12) farms, 1609 total faecal, 1556 total caecal samples	Conventional: faeces ($P<0{\cdot}0001$), caecum ($P<0{\cdot}01$)
[36]	USA	10/02–10/04	Farm, slaughter	*Salmonella*	Faeces, carcase swab/no	Culture	10 (10) farms, 414 (475) faecal, 362 (381) carcase samples	Organic: faeces ($P<0{\cdot}05$, OR 4·23)
[39]	Denmark	07/98–09/98	Slaughter	*Salmonella*	Meat juice/no	ELISA	21 (13 564) herds	n.s.
[40]	Denmark	01/05–01/06	Slaughter	*Salmonella*	Meat juice/no	ELISA	11 (11) herds	n.s.
[48]	Germany	Unknown	Slaughter	*Yersinia enterocolitica*	Tonsils, caecum, lymph nodes/no	PCR	3 (6) farms, 200 (210) samples	Conventional: tonsil ($P=0{\cdot}025$), lymph nodes ($P=0{\cdot}049$)
[47]	Spain	05	Retail	*Escherichia coli*	Pork/no	Culture	3 (14) brands, 54 (67) samples	Organic§: ($P=0{\cdot}023$)
Beef cattle								
[50]	Australia	Unknown	Farm, slaughter, retail	Class 1 and class 2 integron-containing bacteria, *intI1, intI2*	Pen faeces, hide, prechill carcase, intestinal faeces, ground beef/no	Culture (bacteria), PCR (integrase genes)	1 (1) farms, 10 (10) pen faeces, 14 (15) hide, carcase, and intestinal faeces, 36 total ground beef samples	n.s.*

Table 1 (*cont.*)

Food animal species/ reference	Study country	Sample period (month/year)	Sample point	Bacteria investigated	Sample type(s)/ pooling (yes/no)	Laboratory procedure	No. of units/samples: organic (conventional)	Significantly higher prevalence or mean counts (*P* value and/or OR)
[51]	USA	01/03–02/03	Retail	Coliforms, *Escherichia coli*, *E. coli* O157, *Salmonella*, VRE	Ground beef/no	Culture	73 (77) samples	n.s.*

CI, Confidence interval; ELISA, enzyme-linked immunosorbent assay; *intI1*, class 1 integrase; *intI2*, class 2 integrase; n.s., no significant difference reported; OR, odds ratio; PCR, polymerase chain reaction; VRE, vancomycin-resistant enterococci.

* No statistical comparisons conducted.

† Flock-level analysis.

‡ Experimental study where the same farm(s) housed both the organic and conventional production groups.

§ Comparison of mean counts of bacteria.

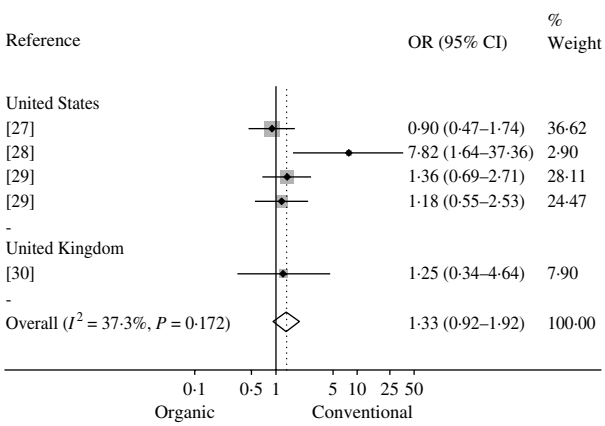

Fig. 2. Mantel–Haenszel meta-analysis of the prevalence of *Campylobacter* in organic and conventional retail chicken. Studies are stratified by country and estimates of effect are presented as odds ratios (OR) with 95% confidence intervals (CI). The *P* value refers to the *Q* statistic test for heterogeneity. Reference 29 was included twice since it provided estimates for two sampling periods (2004 and 2006).

prevalence of *Campylobacter* spp. in organic and conventional broiler chickens at slaughter in the USA [22, 23], Belgium [24, 25] and Denmark [26]. In three of these studies, a higher prevalence of *Campylobacter* was reported in organic broiler chickens [22, 24, 26], while *Campylobacter* was not isolated in one study and no difference in prevalence was observed in another study [23, 25]. Meta-analysis was precluded as only graphical data were reported in one study [24] and level of analysis differed between the other two studies (flock *vs*. individual bird) [22, 26]. The prevalence of *Campylobacter* spp. in retail chicken was investigated in four studies conducted in the USA [27–29] and UK [30], and a meta-analysis of their prevalence estimates is shown in Figure 2. A low to moderate amount of heterogeneity ($I^2 = 37{\cdot}3\%$, $P = 0{\cdot}172$) and a non-significant pooled OR of 1·33 (95% CI 0·92–1·92) were observed, indicating no significant difference in prevalence between organic and conventional retail chicken.

In four studies researchers investigated *Salmonella* spp. in broiler chickens on farms or at slaughter in the USA [23, 31], Belgium [25] and Italy [32], and found very few or no positive samples in both organic and conventional populations. However, a higher prevalence of *Salmonella* spp. was reported in organic retail chicken in two other studies [27, 33]. Insufficient data were available for meta-analysis. The prevalence of *Salmonella* spp. was investigated in laying hens in five studies conducted in Italy [20, 21], Austria [34],

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France [35] and Germany [18], but *Salmonella* spp. were identified in only three studies [18, 34, 35] reporting a higher prevalence in conventional laying hen flocks. Meta-analysis of estimates from these studies revealed significant heterogeneity ($I^2 = 79{\cdot}5\%$, $P = 0{\cdot}008$) and a pooled estimate is not reported.

Conflicting results were reported in six studies that examined the prevalence of *Salmonella* spp. in swine on farms and at slaughter in the USA [36, 37], Denmark [38–40] and Germany [19]. Meta-analysis was not conducted in these data subsets due to different diagnostic testing procedures and levels of analysis between these studies (see Table 1).

Several other bacterial outcomes were identified in this review [20, 21, 32, 41–51] (see Table 1); however, these outcomes were investigated in only a small number of studies, precluding the use of meta-analysis.

Antimicrobial resistance data

Bacterial AMR or multidrug resistance (MDR) data were extracted from 18 studies (Table 2). In seven studies researchers investigated AMR in *Campylobacter* spp. isolated from broilers chickens at slaughter or retail chicken [22, 26–30, 52]. A meta-analysis was conducted on five estimates from four studies [27–30] that examined the prevalence of ciprofloxacin-resistant *Campylobacter* spp. in organic and conventional retail chicken (Figure 3). No evidence of heterogeneity was observed ($I^2 = 0{\cdot}0\%$, $P = 0{\cdot}416$) and a pooled OR of 9·62 (95% CI 5·67–16·35) was obtained, indicating a higher prevalence of ciprofloxacin-resistant *Campylobacter* spp. in conventional chicken. Bacterial AMR was also investigated in 11 other studies [31, 34, 36, 44–47, 49, 51, 53, 54] (see Table 2). However, a meta-analysis was not attempted due to the limited data available.

In 10 studies researchers compared MDR or pan-susceptibility (i.e. susceptibility to all antimicrobials tested) in bacterial isolates from organic and conventional broiler chickens or turkeys in the USA [22, 31], retail chicken in the USA [27] or Spain [45, 46], swine in the USA [36, 49, 53], retail pork in Spain [47], or retail ground beef in the USA [51]. In seven studies bacterial isolates from conventional production were found to be significantly more likely to be resistant to multiple antimicrobials [22, 31, 45–47, 49, 53], and in one study bacterial isolates from organic production were found to be significantly more likely to be pan-susceptible [36]. No significant difference was found in the other two studies [27, 51].

Human risk-factor study

UK researchers examined different exposures in household case clusters of *Campylobacter jejuni* and found that cases who had at least one other family member ill within a week of their onset of symptoms were more likely to have eaten organic meats in the winter (OR 6·86, 95% CI 1·49–31·69) compared to cases who did not have other family members ill within a week of their onset of symptoms [55].

Assessment of study methodological soundness

The number of studies that met each of the criteria for methodological soundness is shown in Table 3. Only one study met all five criteria [55], and one of the eight studies that did not use statistical methods to compare organic and conventional production met the other four criteria [35]. Of the two experimental trials [20, 23], only one used a random allocation of treatment and neither reported the use of blinding.

DISCUSSION

The largest body of evidence pertained to the investigation of *Campylobacter* prevalence in organic and conventional broiler chickens at slaughter and retail. While it was previously thought that access to outdoor runs in organic production might result in a higher prevalence of *Campylobacter* in organic broiler chickens [56], a recent molecular and behavioural longitudinal study of 64 free-range broiler chicken farms in the UK has disputed this claim [57]. In this review, a higher prevalence of *Campylobacter* was reported in organic broiler chickens at slaughter in three studies [22, 24, 26]. Slower-growing breeds are used in organic production, and differences in average broiler age at slaughter (81 days in organic *vs*. 40 days in conventional) might, at least partially, explain these findings, as slaughter age is a recognized risk factor for the prevalence of *Campylobacter* in broiler chicken flocks [58]. No discernible differences were noticed among potential sources of heterogeneity, including study country, study quality parameters, sample size and sampling procedures, between these studies and two others [22, 23] in which *Campylobacter* was either not recovered or no differences between organic and conventional production were observed. In contrast to the findings at slaughter, no difference in the prevalence of *Campylobacter* was observed in organic and conventional retail chicken. Processing and

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Table 2. *Summary of 18 studies comparing the bacterial resistance to antimicrobials between organic and conventional poultry, swine and beef production*

Food animal species/ reference	Study country	Sample period (month/ year)	Sample point	Bacterium	Break-point reference	AMR panel	MDR def'n	No. of units/ samples: organic (conventional)	Significantly higher AMR or MDR (*P* value, RR and/or OR with 95% CI)
Broiler chickens									
[31]	USA	04–05	Farm	*Salmonella*	NARMS	AMP, AMX, CEF, CHL, CRO, GEN, KAN, STR, SLX, TET	>2	18 (14) farms, 162 (188) isolates	Conventional: MDR–2005 ($P<0{\cdot}0001$)
[26]	Denmark	98–00	Slaughter	*Campylobacter*	Unknown	AMP, ENR, ERY, STR, TET	>1	12 (18) farms, 22 (79) flocks, 62 total isolates	n.s.*
[22]	USA	08/00–11/ 02	Slaughter	*Campylobacter*	CLSI & NARMS†	AMP, CLI, CIP, ERY, GEN, KAN, NAL, NOR, TET	>2	5 (10) farms, 165 (167) isolates	Conventional: CIP, NAL, NOR, TET ($P<0{\cdot}05$) Organic: ERY ($P<0{\cdot}05$)
[28]	USA	02/03–05/ 03	Retail	*Campylobacter*	CLSI	CIP	n.a.	2 (2) brands, 33 (43) isolates	Conventional: CIP ($P<0{\cdot}01$, OR 25·2, CI 6·8, 111·4)
[52]	USA	02/03–06/ 04	Retail	*Campylobacter*	CLSI	ROX, As(III), As(V)	n.a.	3 (6) brands, 89 (162) isolates	Conventional‡: ROX ($P<0{\cdot}0001$)
[30]	UK	Unknown	Retail	*Campylobacter*	CLSI & BSAC	CIP, ERY, NAL	n.a.	120 (125) isolates	n.s.
[29]	USA	01/04–06/ 06	Retail	*Campylobacter*	CLSI	CIP	n.a.	3 (2) brands, 188 (141) isolates	Conventional: CIP–2004 ($P<0{\cdot}001$, RR 7·8, 95% CI 2·4–25·5), 2006 ($P<0{\cdot}001$, RR 5·0, 95% CI 2·5–10·3)
[27]	USA	09/02–08/ 03	Retail	*Campylobacter, Salmonella*	CLSI	CHL, CIP, ERY, TET (*Camp.*), AMP, AMK, AMX, APR, CEF, CFT, CHL, CIP, CRO, FLO, GEN, KAN, NAL, STR, SUL, TET, SXT (*Salm.*)	>1	4 (6) brands, 150 (45) *Campylobacter*, 121 (27) *Salmonella* isolates	Conventional: *Campylobacter* – CIP ($P<0{\cdot}05$)
[46]	Spain	05	Retail	Enterobacteriaceae	CLSI	AMP, CEF, CHL, CIP, DOX, GEN, NIT, SLX	>1	60 (60) isolates	Conventional: AMP ($P=0{\cdot}0001$), CHL ($P=0{\cdot}0004$), CIP ($P=0{\cdot}0034$), DOX ($P=0{\cdot}0013$), GEN ($P=0{\cdot}0295$), SLX ($P=0{\cdot}0442$), MDR ($P=0{\cdot}0197$)
[45]	Spain	05	Retail	*Enterococcus*	CLSI	AMP, CHL, CIP, DOX, ERY, GEN, NIT, VAN	>1	60 (60) isolates	Conventional: AMP ($P=0{\cdot}0067$), CHL ($P=0{\cdot}0154$), CIP ($P=0{\cdot}0024$), DOX ($P=0{\cdot}0277$), ERY ($P=0{\cdot}0028$), VAN ($P=0{\cdot}0241$), MDR ($P=0{\cdot}0021$)
[44]	USA	06/02–05/ 03	Retail	*Enterococcus faecium*	CLSI	Q/D	n.a.	23 (77) isolates	n.s.*
Broiler turkeys									
[22]	USA	08/00–11/ 02	Slaughter	*Campylobacter*	CLSI & NARMS†	AMP, CLI, CIP, ERY, GEN, KAN, NAL, NOR, TET	>2	5 (10) farms, 161 (201) isolates	Conventional: AMP, CLI, CIP, ERY, KAN, NAL, NOR, TET, MDR ($P<0{\cdot}05$)

Table 2 (*cont.*)

Food animal species/ reference	Study country	Sample period (month/ year)	Sample point	Bacterium	Break-point reference	AMR panel	MDR def'n	No. of units/ samples: organic (conventional)	Significantly higher AMR or MDR (*P* value, RR and/or OR with 95% CI)
Laying hens									
[34]	Austria	10/04–10/ 05	Farm	*Salmonella*	CLSI	AMP, AMX, APR, CEF, CFT, CHL, COL, CIP, FLO, GEN, NAL, NEO, SPE, STR, SUL, TET, SXT	n.a.	69 (96) farms, 1 (33) isolates	n.s.*
Swine									
[53]	USA	02–03	Farm	*Escherichia coli*	CLSI	AMP, AMX, CEF, CFT, CRO, CHL, CIP, GEN, KAN, NAL, STR, SUL, SXT, TET	>1	35 (60) farms, 498 (883) isolates	Conventional: AMP ($P=0{\cdot}002$, OR 2·02, 95% CI 1·29–3·18), CHL ($P=0{\cdot}003$, OR 2·71, 95% CI 1·39–5·3), STR ($P=0{\cdot}018$, OR 1·52, 95% CI 1·07–2·15), SUL ($P=0{\cdot}008$, OR 1·63, 95% CI 1·14–2·34), TET ($P=0{\cdot}006$, OR 2·38, 95% CI 1·29–4·42), MDR (OR 1·95, 95% CI 1·28–2·58)
[54]	New Zealand	03/01–10/ 01	Farm	*Escherichia coli, Enterococcus*	CLSI	AMP, CIP, GEN, NEO, STR, STX, TET (*E. coli*), AMP, ERY, GEN, STR, TET, VAN, VIR (*Enterococcus*)	n.a.	1 (3) farms, 79 (296) *E. coli*, 80 (273) *Enterococcus* isolates	n.s.*
[49]	USA	10/02–10/ 04	Farm, slaughter	*Campylobacter coli*	CLSI	CHL, CIP, ERY, GEN, NAL, TET	>2	10 (11) farms, 826 (633) isolates	Conventional: TET ($P<0{\cdot}05$), ERY ($P<0{\cdot}05$), MDR ($P=0{\cdot}005$)
[36]	USA	10/02–10/ 04	Farm, slaughter	*Salmonella*	CLSI	AMP, AMK, AMX, CRO, CEF, CHL, CIP, GEN, KAN, STR, SUL, TET	>1	10 (10) farms, 503 (200) isolates	Farm – conventional: AMP ($P<0{\cdot}001$, OR 3·95, 95% CI 1·9, 8·2), AMX ($P<0{\cdot}001$, OR 16·1, 95% CI 2·1, 125), CHL ($P<0{\cdot}01$, OR 2·84, 95% CI 1·32, 6·14), KAN ($P<0{\cdot}001$, OR 23·22, 95% CI 3·0, 177·2), STR ($P<0{\cdot}0001$, OR 10·92, 95% CI 5·4, 21·9), SUL ($P<0{\cdot}0001$, OR 4·3, 95% CI 2·39, 7·84), Organic: PAN ($P=0{\cdot}005$) Slaughter – conventional: AMP ($P<0{\cdot}0001$, OR 9·66, 95% CI 2·79–33·37), CHL ($P<0{\cdot}0001$, OR 56·1, 95% CI 3·4–940·5), KAN ($P<0{\cdot}0001$, OR 40·8, 95% CI 2·41–688·9), STR ($P<0{\cdot}001$, OR 2·85, 95% CI 1·59–5·1), SUL ($P<0{\cdot}001$, OR 3, 95% CI 1·65–5·46), TET ($P<0{\cdot}0001$, OR 25·4, 95% CI 7·54–85·6) Organic: PAN ($P<0{\cdot}0001$)

Table 2 (*cont.*)

Food animal species/ reference	Study country	Sample period (month/ year)	Sample point	Bacterium	Break-point reference	AMR panel	MDR def'n	No. of units/ samples: organic (conventional)	Significantly higher AMR or MDR (*P* value, RR and/or OR with 95% CI)
[47]	Spain	05	Retail	*Escherichia coli*	CLSI	AMP, CEF, CHL, DOX, ENR, GEN, NIT, SLX, STR	>1	3 (14) brands, 90 (90) isolates	Conventional: AMP ($P<0{\cdot}0001$), CEF ($P=0{\cdot}0046$), DOX ($P<0{\cdot}0001$), SLX ($P<0{\cdot}0001$), MDR ($P<0{\cdot}0001$)
Beef cattle									
[51]	USA	01/03–02/ 03	Retail	Non-specific bacteria	CLSI	AMP, AMX, CFT, CRO, CHL, CIP, GEN, KAN, STR, SXT, TET	>1	73 (77) isolates	Conventional: CFT ($P=0{\cdot}02$), CHL ($P=0{\cdot}01$)

AMP, Ampicillin; AMR, antimicrobial resistance; AMX, amoxicillin; APR, apramycin; BSAC, British Society for Antimicrobial Chemotherapy; CEF, cephalothin; CFT, ceftiofur; CHL, chloramphenicol; CI, confidence interval; CIP, ciprofloxacin; CLI, clindamycin; CLSI, Clinical Laboratory Standards Institute; CRO, ceftriaxone; COL, colistin; DOX, doxycycline; ENR, enrofloxacin; ERY, erythromycin; FLO, florfenicol; GEN, gentamycin; KAN, kanamycin; MDR, multidrug resistance; n.a., not applicable (no multidrug resistance data provided); n.s., no significant difference reported; NAL, naladixic acid; NARMS, National Antimicrobial Resistance Monitoring System; NEO, neomycin; NIT, nitrofurantoin; NOR, norfloxacin; OR, odds ratio; PAN, pansusceptible (susceptible to all antimicrobials tested); Q/D, quinupristin/dalfopristin; RR, relative risk; SPE, spectinomycin; STR, streptomycin; SUL, sulfamethoxazole; SLX, sulfisoxazole; TET, tetracycline; SXT, trimethoprim-sulfamethoxazole; VAN, vancomycin; VIR, virginiamycin.

* No statistical comparisons conducted.

† CLSI break-points were used for norfloxacin only.

‡ Comparison of minimum inhibitory concentrations (MICs).

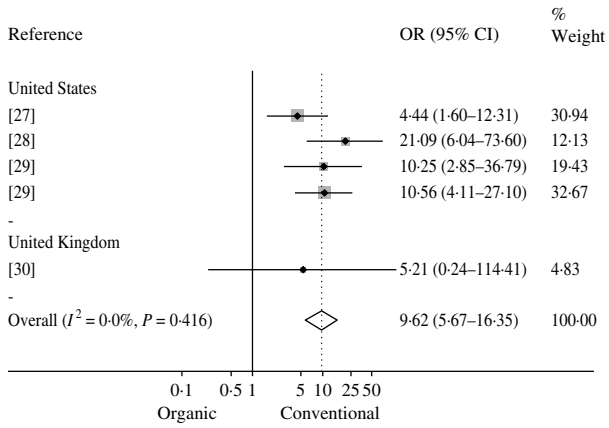

Fig. 3. Mantel–Haenszel meta-analysis of the prevalence of ciprofloxacin-resistant *Campylobacter* isolated from organic and conventional retail chicken. Studies are stratified by country and estimates of effect are presented as odds ratios (OR) with 95% confidence intervals (CI). The *P* value refers to the *Q* statistic test for heterogeneity. Reference 29 was included twice since it provided estimates for two sampling periods (2004 and 2006).

post-processing conditions can affect the prevalence of *Campylobacter* on broiler chicken carcases [59], and it is possible that any difference in prevalence between organic and conventional chickens on farms or at slaughter is altered by these conditions.

The consumption of organic meats was an identified significant risk factor for household case clusters of *C. jejuni* in one study [55]. However, this study compared cases of *C. jejuni* to other cases, and should be verified by future case-controls studies before the consumption of organic meats is considered a risk factor for this illness.

A higher prevalence of *Salmonella* in organic retail chicken, reported in only two studies, must be interpreted with caution and clearly indicates that more research in this area is necessary. In laying hens, a higher prevalence of *Salmonella* was reported in conventional flocks, although significant heterogeneity was observed. All three studies [18, 34, 35] were conducted in Europe, where organic requirements are similar, and the final sample size for each study was comparable. However, observed heterogeneity might be due to random sampling being utilized in only one study [35], sample size being justified in only two studies [34, 35] or differences in sampling methodology. It should also be noted that each study reported a much higher average flock size on conventional farms, a significant risk factor for *Salmonella* prevalence in laying hens [60]. Therefore, it is possible that the higher prevalence observed in

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Table 3. *Summary of the methodological assessment of 38 comparative studies*

Criterion	Categories	No. (%) of studies meeting each criterion
Comparison groups sampled from same target population	Yes/no	29 (76·3)
Sample size justified	Yes/no	7 (18·4)
Random sampling method used to select primary sampling units	Random/all available subjects/ convenience/not stated*	10 (26·3)*
Methods to determine outcome sufficiently defined to allow study reproducibility	Yes/no	36 (94·7)
Appropriate statistical methods	Yes/no/no analysis conducted†	8 (26·7)†

* Random (or systematic) sampling and selection of all available subjects in a database were considered 'yes', while convenience sampling or failure to state the sampling method were considered 'no'.
† Reasons for selecting 'no' included failure to adjust for longitudinal or hierarchical clustering or failure to adequately specify statistical methods; $n = 30$ for this criterion since eight studies did not conduct a statistical comparison on data of interest.

conventional flocks in each study may be confounding a true relationship between flock size and prevalence, as no analysis was conducted in these studies to control for this factor.

Differences were observed among the studies investigating the prevalence of *Salmonella* on organic and conventional swine farms. Geographic location is the most likely source of this heterogeneity as a higher on-farm prevalence of *Salmonella* in organic pigs was reported in two USA studies [36, 37] while a higher on-farm prevalence in conventional pigs was reported in two studies in Germany and Denmark [19, 38]. These differences might be due to different management and antimicrobial use practices in conventional and organic production in the USA and Europe [61]. Variation in sampling and diagnostic procedures can also lead to differences in the prevalence of *Salmonella* in swine due to differences in test sensitivity and specificity [62]. These factors may also explain some of the observed difference, as an ELISA test was used in two studies (each with a different cut-off value) [19, 37] and culture in two studies [36, 38]. Study design may be another contributory factor, as sample size was justified in only one study [19], random sampling was conducted in only one study [38], and in the three studies where a statistical analysis was conducted [36–38] methods were used that did not account for hierarchical clustering.

Several studies investigated AMR or MDR in commensal bacteria in broiler chickens, retail chicken, laying hens, swine, pork or ground beef. Commensal bacteria such as *Enterococcus* spp. can serve as a reservoir for the transfer of resistance genes from bacteria in foods of animal origin to bacteria in humans [63], and resistant enterococci such as vancomycin-resistant enterococci (VRE) are a recognized cause of nosocomial infections in humans [64]. Resistance to quinupristin/dalfopristin, one of the treatment options for infections caused by VRE, is of increasing concern given the use of virginiamycin (an analog of quinupristin/dalfopristin) in food-animal production [63]. In two studies in this review, higher prevalence of resistance to either virginiamycin or quinupristin/dalfopristin was reported in *Enterococcus* spp. isolates from conventional rather than organic swine in New Zealand [54] and retail chicken in the USA [44], respectively. However, while the USA study reported *E. faecium*, the New Zealand study only reported unspeciated enterococci, which could have included *E. faecalis*, a species known to carry natural resistance to this antimicrobial [65].

The prevalence of ciprofloxacin-resistant *Campylobacter* spp. was found to be higher in conventional compared to organic retail chicken (OR 9·62). Ciprofloxacin is a fluoroquinolone that is often used to treat severe human cases of *Campylobacter* infection [66]. The World Health Organization has placed fluoroquinolones in the 'critically important antimicrobial' category in terms of importance to human medicine [67]. Until 2005, fluoroquinolones were approved for use in the USA commercial broiler chicken industry for the flock-level treatment of infection [68, 69]. In the UK, therapeutic use of fluoroquinolones has been approved for use in poultry since 1993 [70]. As a result of increasing evidence that such practices were contributing to fluoroquinolone-resistant *Campylobacter* infections in humans, the U.S. Food and Drug Administration proposed a ban on all use of

fluoroquinolones in the broiler industry in 2000, which was enacted in 2005 [66, 68]. It should be noted that the USA studies included in this review were conducted before or during this time, and if the ban indeed results in reduced levels of resistance in *Campylobacter* isolates from conventional production, the observed difference would be expected to diminish over time.

In eight studies, significantly higher MDR was reported in bacterial isolates from conventional than organic broiler chickens, retail chicken, swine and pork, which suggests that antimicrobial use practices on conventional farms are more selective of MDR than those on organic farms. However, other management factors that differ between organic and conventional production, such as flock/herd size, type of feed and use of biosecurity practices may also contribute to this difference in selection pressure. Some bacterial isolates from organic production showed resistance to certain antimicrobials, such as ampicillin, erythromycin, streptomycin and tetracycline, in the apparent absence of selection pressure from growth-promoting or prophylactic antimicrobial use. However, some therapeutic use of antimicrobials is permitted in organic production in Europe [10], while in the USA, animals that are treated with antimicrobials cannot be marketed as organic [11]. These environments may provide sufficient selection pressure to retain some level of resistance, albeit at lower prevalence, in bacterial populations. Moreover, it is known that once resistance determinants are acquired by bacterial populations, they may in some instances be retained at the same or reduced prevalence for considerable periods of time, particularly if the encoding resistance genes are linked to other genes for which selection pressure remains [71, 72]. Resistance to chloramphenicol, for example, remains in faecal *Escherichia coli* populations in pigs and cattle decades after the drug was banned from food-animal production in North America [72, 73]. Co-selection by other antimicrobials still used in food-animal production is believed to be responsible [72, 73]. Similarly, research in Europe has shown that although there was a decline in the prevalence of resistance in bacterial isolates to certain antimicrobials after the discontinued use of antimicrobials for growth promotion in food animals [71, 74], there was some persistence of resistance observed [75, 76]. AMR surveillance programmes should target bacterial isolates from organic animal production to better understand AMR in this environment.

This review used a rigorous search strategy to identify all potentially relevant published literature and used a search verification strategy of hand-searching the reference lists of all relevant articles captured by the review. For the meta-analyses presented in this review, both Begg's and Egger's tests revealed no statistical evidence of publication bias. However, these tests suffer from a lack of power in meta-analyses that include only a small number of studies, and the existence of publication bias cannot be ruled out [16]. Moreover, no attempts were made to identify government or organizational research reports (e.g. AMR surveillance reports) that may not have been indexed in the included databases, nor were attempts made to identify unpublished studies, as unpublished literature is often incomplete and previous attempts to gather additional information from authors have proven to be very time consuming and largely unsuccessful [77]. Therefore, it is possible that unpublished studies have been conducted that are not included in this review.

The impact of language bias was reduced by utilizing translators to assist in interpreting relevant foreign-language articles, and this provided a more complete assessment of the currently available research in two European countries (Germany and Italy). The studies provided enough additional data to allow a meta-analysis of the prevalence of *Salmonella* in laying hens, demonstrating the advantage of including foreign-language studies in systematic reviews and meta-analyses, especially when data are scarce. Although article translation requires significant time and resources, the use of international collaborative reviewers might be an efficient way to overcome these obstacles.

Most studies included in this review were observational cross-sectional studies, which can only provide evidence for an association and cannot establish causation due to a lack of temporal evidence. In addition, cross-sectional studies are subject to selection, misclassification and confounding bias, which can lead to distorted results and conclusions [78]. Furthermore, many of the studies were based on a small sample size, and their purpose is most suitable for generating rather than testing hypotheses. They are a valuable source of initial evidence on new and emerging topics but do not provide high-quality evidence for systematic reviews or meta-analyses.

The assessment of study methodological soundness revealed that most studies did not explicitly justify their sample size, and to a lesser extent, state whether

a formal random sampling method was used to select the primary sampling units. In addition, many of the studies sampled animals or food products from multiple herds, farms or retail locations and used statistical methods that did not account for the hierarchical structure of the data. The lack of adjustment for clustering might have resulted in underestimated standard errors, increasing the chance of rejecting the null hypothesis when it is true [78].

Within most strata there were too few studies with sufficient data for a meta-analysis or significant heterogeneity was identified. The exceptions were the prevalence of *Campylobacter* and ciprofloxacin-resistant *Campylobacter* in organic and conventional retail chicken. However, these estimates should be interpreted with the aforementioned caveats in mind, as calculations were based on crude OR calculated *post hoc* from a small number of studies, and without adjustment for potential confounders or clustering. The fixed-effect Mantel–Hanszel method was chosen over the random-effects model since no significant heterogeneity was identified, and a sensitivity analysis showed little difference between results from both methods. Meta-regression is one approach that can be used to investigate potential sources of between-study heterogeneity [16]; however, the use of this method in this review was prohibited due to the small number of studies. Once more primary research of sufficient quality is generated, the precision of the pooled estimates presented in this paper can be improved and potential sources of heterogeneity such as study country, study quality parameters and laboratory procedures can be further investigated in meta-regression models.

CONCLUSION

Meta-analysis revealed no difference in the prevalence of *Campylobacter* in organic and conventional retail chicken, while *Campylobacter* isolates from conventional retail chicken were significantly more likely to be ciprofloxacin-resistant. Limited or inconsistent research was identified in studies examining the prevalence of bacterial enteropathogens and potentially zoonotic bacteria in other food-animal species. Bacterial isolates from conventional broiler chicken, turkey and swine production exhibited more AMR and MDR than isolates from organic production; however, the presence of some resistant strains in organic animal production was also identified. There is a need for future research of sufficient quality in this area, so that more accurate pooled estimates can be generated and potential sources of heterogeneity can be investigated using more advanced techniques such as meta-regression.

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NOTE

Supplementary material accompanies this paper on the Journal's website (http://journals.cambridge.org/hyg).

DECLARATION OF INTEREST

None.

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