Do Human Extraintestinal *Escherichia coli* Infections Resistant to Expanded-Spectrum Cephalosporins Originate From Food-Producing Animals? A Systematic Review

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To find out whether food-producing animals (FPAs) are a source of extraintestinal expanded-spectrum cephalosporin-resistant *Escherichia coli* (ESCR-EC) infections in humans, Medline, Embase, and the Cochrane Database of Systematic Reviews were systematically reviewed. Thirty-four original, peer-reviewed publications were identified for inclusion. Six molecular epidemiology studies supported the transfer of resistance via whole bacterium transmission (WBT), which was best characterized among poultry in the Netherlands. Thirteen molecular epidemiology studies supported transmission of resistance via mobile genetic elements, which demonstrated greater diversity of geography and host FPA. Seventeen molecular epidemiology studies did not support WBT and two did not support mobile genetic element-mediated transmission. Four observational epidemiology studies were consistent with zoonotic transmission. Overall, there is evidence that a proportion of human extraintestinal ESCR-EC infections originate from FPAs. Poultry, in particular, is probably a source, but the quantitative and geographical extent of the problem is unclear and requires further investigation.

*Keywords.* zoonosis; ESBL; *E. coli*; ST131; urinary tract; poultry.

The global spread, rapidly rising incidence, and increased mortality of expanded-spectrum cephalosporin-resistant *Escherichia coli* (ESCR-EC) infections over the past decade have made it one of the biggest threats to human health worldwide [1, 2]. In many regions, this rising incidence has coincided with a shift in the epidemiology of human infection, from healthcare associated to community acquired [1]. Discovering the origins of this shift may reveal new targets for public health intervention [3].

From as early as 1969, it has been speculated that food-producing animals (FPAs) may be a potential source of antimicrobial-resistant *E. coli* in humans [4, 5]. The use of large volumes of penicillins and cephalosporins in FPAs has been proposed as a contributing factor to the current ESCR-EC pandemic in humans [6]. Numerous studies have suggested that the use of antibiotics in FPAs directly correlates to the emergence of ESCR-EC within animal populations [7–11]. However, whether ESCR-EC from animals represents a source for human infections has been controversial [12–14].

It has been hypothesized that widespread transmission of resistant bacteria between FPAs and humans may occur via the ingestion of contaminated meat [15]. ESCR Enterobacteriaceae, residing in the gut of FPAs, may directly contaminate meat products during slaughter, or may be released into the environment and indirectly contaminate produce via an intermediary mechanism, such as soil or water. Following ingestion, resistant strains may colonize the intestinal tract and...
subsequently proceed to cause infections at extraintestinal sites, such as the urinary tract [16, 17].

Within this transmission paradigm, human ESCR-EC infections may occur via two mechanisms. First, the entire resistance harboring bacterium may be directly transmitted from animal to human. In this mechanism of whole bacterium transmission (WBT), a bacterial clone from the FPA propagates through the food production process, is ingested, and goes on to cause human extraintestinal infection with minimal genetic changes. Alternatively, the genes mediating ESCR may be transferred from Enterobacteriaceae of animal origin to a human pathogenic E. coli, via plasmids or other mobile genetic elements (MGEs). This may occur at any stage of the transmission paradigm, including within the human gastrointestinal tract. We termed this process MGE-mediated transmission.

In the event that FPAs are a significant source of human ESCR-EC infections, strategies incorporating the veterinary, agricultural, and retail industries may be effective at reducing the escalating global burden of ESCR-EC [18]. To address this knowledge gap, we performed a systematic review of all available published evidence that supported, or did not support, the hypothesis that FPAs are a source of extraintestinal ESCR-EC infection in humans.

METHODS

Protocol
A systematic review of published literature was undertaken, following the Preferred Reporting Items for Systemic Reviews and Meta-analyses guidelines [19]. Our initial aim was to undertake a systematic review and meta-analysis to quantify the contribution of FPA to human ESCR-EC infections, however, evaluation of the published studies indicated that only 1 study was suitable for meta-analysis.

Data Sources and Search Strategy
We developed search strategies for 3 electronic databases: Medline, Embase, and the Cochrane Database of Systematic Reviews. The most recent search was conducted in October 2013. Language of publications reviewed was not restricted. Terms included Escherichia coli, technical (eg, bovine) and non-technical (eg, cow) animal descriptors, food descriptors (eg, beef), and antimicrobial resistance terminology (eg, cephalosporin resistance, extended-spectrum β-lactamase [ESBL]). Full details are included in the Supplementary Data. The bibliographies of all included publications and relevant review articles on this topic were searched for further publications.

Study Selection
Original research studies that presented a comparative analysis addressing the research question were included. We applied no exclusion criteria or limitations based on the methodology used for this comparison. Studies that reported only on animal or human data, and did not address the research question of the relationship between these 2 groups, were excluded, as were those that analyzed only enteropathogenic E. coli. Studies that reported only on transmission of resistance after physical contact between FPAs and humans (eg, farm or abattoir workers) were excluded, due to the limited generalizability of this phenomenon.

Data Extraction
Two authors (B. L., B. A. R.) independently reviewed all abstracts identified by the search strategy. The full text of all publications potentially meeting inclusion criteria, selected by either researcher, was obtained for review. After full independent review, any disagreement about the relevance of an article to the research question was settled by a discussion. Data were extracted from the publications by 1 author (B. L.).

RESULTS

In total, 2301 abstracts were reviewed and 34 full articles were identified with relevant data (Figure 1). The annual frequency of publications has increased markedly over the last decade (see Supplementary Data).

Molecular Evidence Supporting Transmission Between Animals and Humans

Whole Bacterium Transmission
Six published articles provide data to support the hypothesis of WBT of ESCR-EC between poultry and extraintestinal human sites (Table 1). Publications originate from 3 regions: the Netherlands, Spain, and North America.

Three Dutch studies illustrating WBT of ESCR-EC between poultry and extraintestinal human infections were identified [15, 20, 21]. All 3 collected geographically and temporally matched isolates between 2006 and 2010, found a similar distribution of ESBL genes, and, using multilocus sequence typing (MLST), found that animal- and human-sourced bacteria clustered in identical clonal complexes. An extended chromosomal analysis, involving pulsed-field gel electrophoresis (PFGE) and multivariate discriminant function analysis, of the retail chicken, human rectal, and blood culture isolates found extensive similarity between E. coli from these sources [15, 20, 21]. Collectively, clonally related isolates were identified at all levels of the transmission paradigm, including poultry flocks, retail chicken meat, and human commensal and extraintestinal clinical sources.

The findings in all 3 studies were limited to poultry despite substantial sampling of non-poultry retail meats [21]. Significantly fewer ESCR-EC isolates were identified in non-poultry meat types [21].
Two Spanish studies provide limited evidence, both based on PFGE, to suggest that some WBT of ESCR-EC between poultry and humans may occur in this country [22, 23] (Table 1). One study was conducted retrospectively sampling E. coli sequence type 131 (ST131) isolates selected from much larger previous samplings over a 17-year period [23].

One supportive study from North America identified genetic relatedness of isolates from FPAs and humans, albeit using molecular techniques that have a limited resolution [24] (Table 1). No distinction was made between freshly slaughtered chicken carcass samples and retail poultry products in this study.

**Mobile Genetic Element-Mediated Transmission**

Thirteen studies provide data to support the hypothesis of MGE-mediated transmission of ESCR between animal bacteria and E. coli causing human extraintestinal infections (Table 2).

The geographical setting, host animals, and host bacteria that appear to mediate MGE-mediated transmission are diverse. Studies originated from Western Europe (n = 6), North America (n = 3), and Asia (n = 2). Two additional studies were conducted across multiple continents. The animal hosts included poultry, pigs, and cattle. Whereas all MGEs were identified among E. coli in humans, the host Enterobacteriaceae for the MGE in animals included Salmonella species, Klebsiella pneumoniae, and E. coli.

The genes encoding ESCR that are described appear to vary by geographical location. ESBL genes, especially the bla_{CTX-M} family, predominated in Europe, whereas bla_{CMY-2} was predominant in North America, and both were prevalent in Asia.

**Molecular Studies Not Demonstrating Transmission Between Animals and Humans**

**Whole Bacterium Transmission**

Seventeen studies did not demonstrate direct WBT of ESCR-EC in North America, Europe, and Asia. These comprised 8 studies that found evidence to support MGE-mediated transmission and have already been discussed (Table 2) [25–32], and 9 further studies (Table 3).

Of the 9 studies, 3 suggest that FPAs do not appear to play the predominant role in the spread of a single clone, E. coli ST131, among humans [33–35]. Two studies did not isolate any ST131 from FPAs [33, 35]. One extensive study found that PFGE-defined pulsotypes of ST131 were predominantly source specific, although some pulsotypes contained isolates from both human and FPA sources [34].

The remaining 6 studies compared a variety of isolates from animal and human sources, and did not find any significant evidence of transmission [14, 36–40]. One of these studies contained isolates from 3 European countries, including the Netherlands [40].

**MGE-Mediated Transmission**

Two studies did not demonstrate MGE-mediated transmission of ESCR genes between E. coli from FPAs and human sources.
<table>
<thead>
<tr>
<th>Location, Sampling Dates</th>
<th>Nature of Sample Selection</th>
<th>Source and No. of ESCR Escherichia coli Isolates</th>
<th>Methods of Comparison, No. of Human ESCR-EC Compared Using This Method</th>
<th>Whole Bacterium Comparison</th>
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<tbody>
<tr>
<td>The Netherlands, 2006–2010 [15]</td>
<td>Healthy poultry, n = 35</td>
<td>Retail chicken, n = 81/98</td>
<td>ESBL genotype frequency, n = 208</td>
<td>51 human isolates carried bla&lt;sub&gt;CTX-M-1&lt;/sub&gt; and 14 carried bla&lt;sub&gt;TEM-52&lt;/sub&gt;. These were the 2 most common ESBL genes identified in poultry</td>
</tr>
<tr>
<td>The Netherlands, 2008–2009 [20, 21]</td>
<td>Retail chicken, n = 68/89</td>
<td>Rectal swabs (commensal), n = 45</td>
<td>ESBL genotype frequency, n = 55</td>
<td>22 human rectal swab and 5 bloodstream ESCR E. coli contained bla&lt;sub&gt;CTX-M-1&lt;/sub&gt; genes, the most common ESBL genes in FPA</td>
</tr>
<tr>
<td>Spain, 1993–2010 [23]</td>
<td>Highly selected&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Retail chicken, n = 4/100</td>
<td>PFGE, all human and FPA isolates included</td>
<td>3 human bla&lt;sub&gt;CTX-M-1&lt;/sub&gt; isolates (2007–2010) showed &gt;90.9% similarity to 4 ESCR retail chicken isolates and 2 poultry ESCR isolates</td>
</tr>
<tr>
<td>Spain, 2003 [22]</td>
<td>Poultry, n = 57</td>
<td>Extraintestinal, n = 80</td>
<td>Serotyping</td>
<td>55 human isolates shared serotypes identified in poultry and pig isolates, namely O2:HNM (24 isolates), O2:H6 (14 isolates), and O25 (17 isolates)</td>
</tr>
<tr>
<td>USA, 2002–2004 [24]</td>
<td>Slaughtered and retail poultry, n = 100/220</td>
<td>Rectal swabs (commensal) and extraintestinal, n = 14</td>
<td>Phylogenetic group and virulence genotype, n = 931&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Antimicrobial resistant human isolates were statistically more similar to poultry isolates than to susceptible human isolates (P &lt; .001)</td>
</tr>
</tbody>
</table>

Abbreviations: AFLP, amplified fragment-length polymorphisms; CC, clonal complex; ESBL, extended spectrum β-lactamase; ESCR-EC, expanded-spectrum cephalosporin-resistant Escherichia coli; FPA, food-producing animal; MLST, multilocus sequence typing; PFGE, pulsed-field gel electrophoresis; ST, sequence type.

<sup>a</sup> In some studies, multiple isolates were taken per sample, so the number of isolates may not reflect the number of positive samples.

<sup>b</sup> Multifactorial discriminant function analysis based on phylogenetic group, ESBL genotype, plasmid replicon type, and virulence genes (Wilks λ = 0.08).

<sup>c</sup> Selected O25b: H4 ST131 bla<sub>CTX-M-9</sub> Ibe<sub>A</sub> strains from 6 previous studies.

<sup>d</sup> Chicken (n = 2) and turkey (n = 1), selected from a combined pool of 1494 poultry and 408 turkey isolates collected between 1993 and 2009.

<sup>e</sup> Fifteen non-Ibe<sub>A</sub> bla<sub>CTX-M-9</sub>–non-9–positive strains were also compared and found not to be similar.

<sup>f</sup> Based on phylogenetic group and virulence genotype of isolates. Non-ESCR isolates were included in analysis, and no subgroup analysis on ESCR isolates was performed.
<table>
<thead>
<tr>
<th>Location, Sampling Dates</th>
<th>Nature of Sample Selection</th>
<th>Source and No. of ESCR Escherichia coli Isolates</th>
<th>Methods Used to Exclude Whole Bacterium Transmission</th>
<th>Comparison of MGEs Across Sources</th>
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<tbody>
<tr>
<td>United Kingdom, 2006–2010 [30]</td>
<td>Selected bla&lt;sub&gt;CTX-M-14&lt;/sub&gt; isolates only</td>
<td>Animal: Cattle, n = 9; Meat: Poultry, n = 9</td>
<td>bla&lt;sub&gt;CTX-M-14&lt;/sub&gt; PFGE and virulence genotype</td>
<td>4 human isolates carried bla&lt;sub&gt;CTX-M-14&lt;/sub&gt; on IncK, pCT-like plasmid, highly related to plasmids from 9 cattle and 8 poultry isolates</td>
</tr>
<tr>
<td>Sweden, 2010 [25]</td>
<td>Selected bla&lt;sub&gt;CMY-2&lt;/sub&gt; isolates only</td>
<td>Animal: Broilers, n = 2</td>
<td>bla&lt;sub&gt;CMY-2&lt;/sub&gt; PFGE and MLST</td>
<td>19 human clinical isolates carried bla&lt;sub&gt;CMY-2&lt;/sub&gt; on IncFII plasmids similar to those previously reported in human E. coli infections [65–67]</td>
</tr>
<tr>
<td>Spain, 2006–2007 [27]</td>
<td>Highly selected, all bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;, ST410, and retrospective</td>
<td>Animal: Turkey, n = 2</td>
<td>bla&lt;sub&gt;CTX-M-15&lt;/sub&gt; PFGE&lt;sup&gt;a&lt;/sup&gt;</td>
<td>All 7 isolates carried bla&lt;sub&gt;CTX-M-15&lt;/sub&gt; on IncFII plasmids similar to those previously reported in human E. coli infections [65–67]</td>
</tr>
<tr>
<td>Portugal, 2006–2007 [56]</td>
<td>Any ESCR-EC from animals but no human isolates</td>
<td>Animal: Pigs, n = 22</td>
<td>bla&lt;sub&gt;TEM-52&lt;/sub&gt; No human isolates for direct comparison</td>
<td>8 pig isolates carried bla&lt;sub&gt;TEM-52&lt;/sub&gt; on 90kb IncI1 plasmid identified as ST3. Previously identified in human clinical isolates in Portugal</td>
</tr>
<tr>
<td>Belgium, 2001–2007 [29]</td>
<td>Highly selected, all plasmids previously analyzed</td>
<td>Animal: Pigs, n = 2; Non-EC, n = 6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>bla&lt;sub&gt;TEM-52&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-2&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;</td>
<td>Conjugative resistance transfer, size, PBRT, RFLP, southern blot hybridization</td>
</tr>
<tr>
<td>International&lt;sup&gt;c&lt;/sup&gt;, 1995–2006 [53]</td>
<td>Highly selected, only bla&lt;sub&gt;TEM-52&lt;/sub&gt; isolates over 10-y period</td>
<td>Animal: Poultry, n = 2; Non-EC, n = 5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>bla&lt;sub&gt;TEM-52&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-2&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-15&lt;/sub&gt;</td>
<td>PBRT, plasmid size, ESBL gene location relative to Tn3 transposon, RFLP with EcoRI</td>
</tr>
<tr>
<td>Spain, 2006–2007 [26]</td>
<td>Any ESCR-EC</td>
<td>Animal: Mixed, n = 55&lt;sup&gt;d&lt;/sup&gt;</td>
<td>bla&lt;sub&gt;SHV-12&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-9&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-1&lt;/sub&gt; PFGE</td>
<td>Distribution of genes mediating ESCR between meat and human isolates</td>
</tr>
<tr>
<td>USA, 2006–2007 [26]</td>
<td>Any ESCR-EC</td>
<td>Animal: Mixed, n = 34&lt;sup&gt;e&lt;/sup&gt;</td>
<td>bla&lt;sub&gt;SHV-12&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-9&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-1&lt;/sub&gt;, bla&lt;sub&gt;CTX-M-2&lt;/sub&gt; PFGE&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Distribution of genes mediating ESCR between meat and human isolates</td>
</tr>
</tbody>
</table>
Table 2 continued.

<table>
<thead>
<tr>
<th>Location, Sampling Dates</th>
<th>Nature of Sample Selection</th>
<th>Source and No. of ESCR Escherichia coli Isolates</th>
<th>Methods Used to Exclude Whole Bacterium Transmission</th>
<th>Comparison of MGEs Across Sources</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Animal</td>
<td>Meat</td>
<td>Human</td>
</tr>
<tr>
<td>Canada, 1999–2006 [28, 46]</td>
<td>Retrospectively selected human bla&lt;sub&gt;CMY-2&lt;/sub&gt; isolates</td>
<td>Cattle, n = 26</td>
<td>. . .</td>
<td>Clinical, n = 22 (69)</td>
</tr>
<tr>
<td>USA, 1998–2000 [31]</td>
<td>Any ESCR-EC from multiple sampling sites</td>
<td>Cow or pig, n = 59 Non-EC, n = 4</td>
<td>. . .</td>
<td>Urine, n = 5 Blood, n = 1</td>
</tr>
<tr>
<td>Hong Kong, 2002–2010 [54]</td>
<td>Retrospectively collected bla&lt;sub&gt;CTX-M-14&lt;/sub&gt; isolates</td>
<td>Pigs, n = 14 Chickens, n = 16 Cattle, n = 8 Pigs, n = 20</td>
<td>. . .</td>
<td>Clinical (UTI), n = 37 Commensal (fecal), n = 65</td>
</tr>
<tr>
<td>Taiwan, 2001–2002 [32]</td>
<td>Any ESCR-EC from multiple sampling sites</td>
<td>Poultry stool, n = 1 Pig stool, n = 7</td>
<td>Ground chicken, n = 14 Ground pork, n = 1</td>
<td>UTI, n = 11</td>
</tr>
</tbody>
</table>

Abbreviations: EC, <i>E. coli</i>; ESBL, extended-spectrum beta-lactamase; ESCR, expanded-spectrum cephalosporin resistant; FPA, food-producing animal; MGE, mobile genetic element; PBRT, PCR-based replicon typing; PFGE, pulsed-field gel electrophoresis; pMLST, plasmid multilocus sequence typing; RAPD, randomly amplified polymorphic DNA; RFLP, restriction fragment-length polymorphism analysis; UTI, urinary tract infection.

<sup>a</sup>Identified 81.2% similarity between clinical (n = 3) and both meat isolates.

<sup>b</sup> <i>Klebsiella pneumoniae</i> (n = 1) and <i>Salmonella</i> spp (n = 4) isolates from healthy poultry feces.

<sup>c</sup>Isolates collected from Denmark, France, Netherlands, Belgium, Spain, Korea, and Canada.

<sup>d</sup>Salmonella spp from poultry.

<sup>e</sup>Poultry (n = 15), pork (n = 3), beef (n = 1).

<sup>f</sup>Exact number of isolates not mentioned, sourced from poultry (n = 31), pork (n = 2), and beef (n = 1) samples.

<sup>g</sup>One clonal bla<sub>CTX-M-1</sub>–producing isolate in chicken meat and human sources was identified by PFGE; however, this was in the context of no other bla<sub>CTX-M</sub> being identified in poultry and the single isolate being a perfect match, so the authors reasoned that this may have been a contaminant.

<sup>h</sup>Salmonella spp from bovine (n = 3) and porcine (n = 1) sources.

<sup>i</sup>Eight FPA and 2 human isolates underwent plasmid analysis.
Table 3. Molecular Evidence That Does Not Support Whole Bacterium Transmission

<table>
<thead>
<tr>
<th>Location, Sampling Dates</th>
<th>Nature of Sample Selection</th>
<th>Source and Number of ESCR Escherichia coli Isolates</th>
<th>Whole Bacterium Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internationalb, 2005–2009 [40]</td>
<td>Any ESCR-EC</td>
<td>Poultry, n = 133 Pigs, n = 4 Cattle, n = 35 Calves, n = 7 Turkey, n = 1</td>
<td>Virulence genotype based on microarray MLST and virulence genotype</td>
</tr>
<tr>
<td>UK, 2006–2009 [14]</td>
<td>Highly selected</td>
<td>Poultry: Healthy, n = 26 Sick, n = 3</td>
<td>ESBL genotype Virulence genotype</td>
</tr>
<tr>
<td>Spain, 2005 [36]</td>
<td>Sampled environmental waste</td>
<td>Poultry waste, n = 9</td>
<td>Phylogenetic group</td>
</tr>
<tr>
<td>Japan, 2010 [37]</td>
<td>No human isolates</td>
<td>. . . Chicken, n = 52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ESBL genotype Serotype</td>
</tr>
</tbody>
</table>

<sup>a</sup>None, compared with published studies [72, 73]

<sup>b</sup>Virulence genotype based on microarray MLST and virulence genotype

<sup>c</sup>Virulence genotype based on microarray

<sup>d</sup>Virulence genotype based on microarray MLST and virulence genotype

<sup>e</sup>Virulence genotype based on microarray MLST and virulence genotype

<sup>f</sup>Virulence genotype based on microarray
Resistance plasmids of the same replicon type were identified in FPAs and humans; however, higher-resolution comparison by plasmid-MLST and PFGE found them to be heterogeneous.

**Observational Epidemiological Studies**

Four observational epidemiological studies fulfilled our inclusion criteria (Table 5). One ecological study and 2 case-control studies supported the hypothesis of FPAs as a source of ESCR-EC infections in humans [6, 43, 44]. One other case-control study found that urinary tract infections caused by the ST131 strain of ESCR-EC was negatively associated with frequent consumption of poultry, as compared to infections caused by non-ST131 ESCR-EC [45].

**DISCUSSION**

Through the use of a systematic review, we present scientific data related to the hypothesis that a proportion of human ESCR-EC infections may originate from an FPA source. Specifically, poultry in the Netherlands have been implicated as a likely source of human infections. A variety of FPA and geographical regions have also been investigated, but the evidence is mixed. Whole bacterium and MGE-mediated mechanisms of transmission may both play a role.

At a population level, WBT is a phenomenon that is most well characterized in the Netherlands, with 3 geographically and temporally matched molecular epidemiological studies being conducted there [15, 20, 21]. Unlike in other regions, the evidence in the Netherlands is consistent across studies, which used widespread and representative sampling methodologies and a range of well-validated molecular epidemiological methods. These studies also consistently demonstrated an unusually high proportion of human clinical extraintestinal infections that could have resulted from a poultry source.

One recent study does not support these findings, but the authors of that study highlight some limitations in their data, when compared to previous work [40]. Perhaps the most significant of these is that genetic relatedness was evaluated by comparing similarity of virulence genotypes, which is a less stable method than MLST and PFGE, because virulence genes are acquired by horizontal transfer rather than spontaneous mutations to the genetic backbone.

In the future, whole genome sequencing may be the most methodologically sound and cost-effective way of conducting molecular epidemiological analysis. In addition to providing the highest possible discriminatory power, this method also results in a permanent data output that can be made easily accessible for comparison between researchers.

Outside the Netherlands, 3 studies supported WBT, however, all have limitations that precluded stronger conclusions being
drawn in these locations. Specifically, 2 Spanish studies found only a very small number of related isolates [22, 23]. One of these studies analyzed a highly selected population of bacteria that had been collected over 17 years [23]. The North American study relied on molecular techniques with limited discriminatory power, did not distinguish between ESCR-EC and other forms of resistance, and lacked samples from human infection [24].

A considerable body of work conducted outside of the Netherlands has not demonstrated WBT. Seventeen publications, mostly from Spain, North America, the United Kingdom, and China, found evidence that did not support WBT using contemporary molecular epidemiological techniques [14, 25–28, 30–40, 46]. Supporting the validity of these negative findings, 8 of the studies identified MGE-mediated transmission instead of WBT [25–32] (Table 2).

It is not clear whether the concentration of evidence supporting WBT in the Netherlands represents a truly geographically defined phenomenon or relates to the limited breadth and methodology of research in other parts of the world. If a geographical finding is real, the relatively high rates of antimicrobial use in animal husbandry in the Netherlands may be a contributing factor [15]. Other potential factors, including differences in farming practice, the food production and supply chain, and human population dynamics, may warrant further investigation.

One area of concordance among the molecular epidemiological evidence of WBT is the identification of poultry as the most likely FPA source of ESCR-EC in humans. In several studies, retail meat samples from beef, pork, and other FPAs either did not harbor ESCR-EC [21, 37], or harbored strains clonally unrelated to human infections (Tables 2 and 3). Certain multilocus sequence types (ST10, ST155, and ST177), which have been found in poultry and poultry products, appear to be most frequently associated with a zoonotic risk [15, 20, 21]. Conversely, ST131, a common cause of human ESCR-EC infections, seems less likely to originate from poultry or other FPA sources [34].

This apparent host specificity to poultry, and identification of ST10 as a high-risk strain, is consistent with existing studies investigating zoonotic transmission of non-ESCR-EC [47–50]. It is also consistent with all 4 observational epidemiological studies (Table 5), which collectively suggest that poultry is the primary FPA host and that non-ST131 ESCR-EC strains may be more likely to mediate this zoonotic potential [6, 43–45]. The consistent identification of poultry as a source, rather than other FPAs, further supports the hypothesis of transmission.

Genomic data offer some explanation for this finding. Studies have demonstrated that human extraintestinal pathogenic E. coli and avian pathogenic E. coli share numerous virulence factors [51, 52]. Therefore, resistant strains that are able to infect
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<th>Location, Dates</th>
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<th>Method of Association and Results</th>
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<td>Europe*, 2005–2008 [6]</td>
<td>Ecological Prevalence</td>
<td>Prevalence of AMR among <em>Escherichia coli</em> from poultry, pigs, and cattle, using surveillance data&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Prevalence of AMR in human bloodstream <em>E. coli</em> infections, using surveillance data&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Correlation of ESCR between FPAs and human sources in 11 European countries</td>
<td>Statistical correlation between poultry and human ESCR across 11 European countries. $r = 0.76$ ($P &lt; .05$, Spearman correlation coefficient)</td>
</tr>
<tr>
<td>Germany, 2011–2012 [43]</td>
<td>Case-control None</td>
<td>Cases = hospitalized patients colonized with CA-ESCR <em>E. coli</em> Controls = patients colonized with CA non-ESCR <em>E. coli</em></td>
<td>Verbal questionnaire including dietary habits in the past 12 mo; multivariate analysis</td>
<td>Frequently eating pork (≥3 meals per week) was independently associated with ESCR <em>E. coli</em> colonization (OR = 3.5; 95% CI, 1.8–6.6). Frequent consumption of poultry, beef, veal, and fish were not associated</td>
<td></td>
</tr>
<tr>
<td>USA, 2003–2004 [44]</td>
<td>Case-control None</td>
<td>Cases = women with antimicrobial-resistant <em>E. coli</em> UTI Controls = women with fully susceptible <em>E. coli</em> UTI</td>
<td>Verbal questionnaire including dietary habits in the past 6 mo prior to UTI; adjusted OR</td>
<td>Women with UTI caused by ampicillin or cephalosporin-resistant <em>E. coli</em> reported more frequent consumption of pork (adjusted OR = 4.0; 95% CI, 1.1–15.5). Women with multidrug-resistant <em>E. coli</em> UTI more likely to report chicken exposure (adjusted OR = 3.7; 95% CI, 1.1–12.4)</td>
<td></td>
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<tr>
<td>Paris, 2008–2009 [45]</td>
<td>Case-control None</td>
<td>Cases = inpatients with clinical sample positive for <em>bla&lt;sub&gt;CTX-M&lt;/sub&gt;</em> positive ST131 <em>E. coli</em> Controls = inpatients with clinical sample positive for <em>bla&lt;sub&gt;CTX-M&lt;/sub&gt;</em> positive non-ST131 <em>E. coli</em></td>
<td>Evaluated consumption of poultry, beef, and raw meat; exposure to livestock using unreported method; multivariate analysis</td>
<td>Regular consumption of poultry products was negatively associated with ST131 ESCR <em>E. coli</em> infections (OR = 0.2; 95% CI, 0.1–0.6); regular consumption of beef, consumption of raw meat, and exposure to livestock were not statistically associated</td>
<td></td>
</tr>
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</table>

Abbreviations: AMR, antimicrobial resistant; CA, community acquired (identified <72 hours after admission); CI, confidence interval; ESCR, expanded-spectrum cephalosporin resistant; FPA, food-producing animal; OR, odds ratio; UTI, urinary tract infection.

* Eleven European countries: Austria, Denmark, Finland, France, Germany, Italy, the Netherlands, Norway, Spain, Sweden, and Switzerland.

<sup>a</sup> The Data from Animals originates from European Food Safety Authority.

<sup>b</sup> The Data from Humans originates from European Antimicrobial Resistance Surveillance.

<sup>c</sup> Consecutive inpatients across 10 hospitals in France with clinical *E. coli* sample positive for *bla<sub>CTX-M</sub>*.
avian sources are also more likely to possess the cellular machinery required to infect humans.

The dynamics of MGE-mediated transmission of ESCR, between FPAs and humans, appear quite different to that of WBT. Studies that support MGE-mediated transmission are characterized by an extraordinary degree of diversity. Positive findings originate from many regions, collectively encompassing all major forms of livestock, across different species of Enterobacteriaceae with different ESCR resistance genes, plasmid types, and MGEs [27–32, 46, 53–57].

Data also suggest some differences between ESCR gene classes. MGE-mediated transmission of ESBL genes may be primarily mediated by whole plasmids [27, 29, 30, 53–55, 58], whereas \( \text{bla}_{CMY-2} \) may move within a smaller MGE owing to its close relationship with ISEcp1 [31], as described previously [10, 59].

The wide range of host Enterobacteriaceae and host FPA observed in this form of transmission reflect a more flexible and ubiquitous phenomenon, where transmission of resistance can occur independently of host bacterium specificity. There are, however, some limitations with the studies that support MGE-mediated transmission. Only a relatively small number of isolates underwent comprehensive plasmid sequence analysis, perhaps owing to high labor-intensity of this work. Most studies are based on lower-resolution measures of plasmid similarity, such as plasmid replicon types. Two recent European studies have demonstrated a poor correlation between plasmid replicon typing and higher-resolution techniques [41, 42]. However, the interpretation of these 2 studies is unclear, because only a small number of isolates underwent high-resolution comparison. Thus, the lack of support may reflect an insufficient sample size rather than a true absence of relatedness.

The strengths of our systematic review include a comprehensive search strategy, a structured approach to selecting articles, and integration of data from multiple fields, including molecular and observational epidemiology.

Our review has a number of limitations. First, many molecular epidemiology studies included in the review used inherently biased sampling methodologies, and only analyzed a small number of isolates. Selection bias, and the associated heterogeneity of sampling methodologies, limits the conclusions that can be drawn regarding the extent of the problem. Only 1 study was able to provide meaningful quantitative assessment of the burden of FPA-associated ESCR-EC within a population [15]. On the other hand, underpowered studies that did not find evidence of transmission can be difficult to interpret, because the absence of evidence may be due to an insufficient sample size rather than a true absence of relatedness.

Second, molecular epidemiological techniques have inherent limitations, with considerable variability in the discriminatory power afforded by the technique utilized [60]. Underlying this limitation is the fact that genetic relatedness exists on a spectrum. Dichotomous conclusions about “transmission” between 2 sources frequently require an element of subjective judgment. The emerging use of whole-genome sequencing in molecular epidemiology of Enterobacteriaceae has already confirmed the inherent limitations of older techniques [61, 62], and will certainly shape future research on this topic. Furthermore, genetic similarity also does not necessarily prove the origin of transmission. For example, an external source that contaminates FPAs and humans would also result in genetic similarity. The potential for transmission mechanisms external to the food chain deserves further research.

Research bias and publication bias may have influenced our results. Most research effort has focused on a limited number of geographical areas, almost all within the developed world. The impact of publication bias is harder to ascertain, but likely present. We identified a considerable number of negative studies in this area, although approximately half of these results were presented in publications with other positive findings.

Owing to the specificity of the research question, we have also limited the review to evaluate human infections caused by ESCR-EC. Extraintestinal and intestinal human infection with other species of ESCR Enterobacteriaceae of zoonotic origin, such as Salmonella species, also appears to be a problem [31, 46].

Similarly, we have investigated only unidirectional transmission from animal to humans. Transmission in both directions is possible, and may be responsible for the original incursion of resistance into animal species [63]. A recent study from the Netherlands indicates that the dynamics of the secondary spread of FPA-associated resistant strains within human communities are complex, and will require further research [64].

In conclusion, there is evidence that a proportion of human extraintestinal ESCR-EC infections originate from FPAs. Poultry appears to be a more likely source than other FPAs based on current evidence. Transmission of whole ESCR-EC and mobile ESCR genetic elements from poultry to humans probably occurs, but the specific parameters surrounding this, including the magnitude and geographical extent of the problem, remain inadequately understood. A broader sampling methodology, including environmental and human commensal samples, and higher-resolution molecular comparisons are required. Such research would also lend insight into the specific mechanisms involved in transmission and potentially offer a means for public health intervention.

**Supplementary Data**

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.
Note

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