Virulence of *Escherichia coli* B2 Isolates from Meat and Animals in a Murine Model of Ascending Urinary Tract Infection (UTI): Evidence that UTI Is a Zoonosis

Lotte Jakobsen,* Anette M. Hammerum, and Niels Frimodt-Møller

National Center for Antimicrobials and Infection Control, Statens Serum Institut, Artilverivej 5, DK-2300 Copenhagen S, Denmark

Received 11 February 2010/Returned for modification 18 March 2010/Accepted 21 May 2010

In vivo evidence of a connection between urinary tract infections (UTI) and foods is lacking. The virulence of 13 *Escherichia coli* B2 isolates from healthy animals and fresh meat was investigated in a murine model of ascending UTI. All isolates produced positive bladder cultures (10^2 to 10^7 CFU), and nine isolates produced positive kidney cultures (10^2 to 10^5 CFU).

Urinary tract infections (UTI) are one of the most common human bacterial infections. They are responsible for severe morbidity and loss of productivity, and the overall costs associated with UTI amount to $1 billion in the United States alone (12). In approximately 80% of cases, UTI is caused by *Escherichia coli* (12). These *E. coli* isolates belong to the pathogenic group of extraintestinal *E. coli* (ExPEC), stemming mainly from the host’s own fecal flora, and in the majority of cases belong to phylogroup B2 (14, 15). It has been suggested that one exterior reservoir of uropathogenic *E. coli* (UPEC) in the human intestine could be retail food or animals related to retail food (10). Many studies have investigated this hypothesis by using molecular techniques, including typing, virulence gene detection, and genome sequencing (8–10). Mostly, researches have focused on comparing *E. coli* from retail food and UTI patients or avian-pathogenic *E. coli* (APEC) with ExPEC in *vitro*, and their molecular characterizations have indicated a connection between UTI and food (7, 8). Despite many in vitro studies, the zoonotic risk of animal and meat isolates remains unclear due to a lack of *in vivo* studies. In this study, we set out to investigate the virulence of *E. coli* B2 isolates from healthy pigs, broiler chickens, pork, and broiler chicken meat in a murine model of ascending UTI, which is representative of UTI in humans (2, 4, 11).

A total of 13 *E. coli* strains from healthy Danish broiler chickens (fecal, n = 4), Danish broiler chicken meat (n = 2), imported broiler chicken meat (n = 2), healthy Danish pigs (fecal, n = 2), Danish pork (n = 2), and imported pork (n = 1), previously published (6), were used for this study. The isolates belonged to phylogroup B2 and carried two or more of the virulence genes *ituA*, *kpsM II*, *papA*, *papC*, *focG*, *sfaS*, and *hlyA*, as described previously (5). All isolates were used for positive for type 1 fimbriae by use of a modification of a phenotypic test described earlier (3). The clinical UTI *E. coli* isolate C175-94 was used as a positive control and an *E. coli* O rough:H− isolate as a negative control in the murine experiments (4). The virulence of the strains was tested in a murine model of ascending UTI (Danish Ministry of Justice animal ethics committee approval no. 2004/561-835) described by Hvidberg et al. (4). In short, mouse bladders were emptied by gently pressing the abdomen, and 50 microliters (5 × 10^6 CFU of each bacterial suspension was slowly inoculated tranurethrally in 4 to 6 outbred female albino CFW1 mice (26 to 30 g: Harlan Netherlands, Horst, Netherlands) by use of plastic catheters. The mice, which were housed 4 to 6 to a cage, were given free access to chow and 5% glucose-containing water. Seventy-two hours after inoculation, urine was collected from each mouse. The mice were then euthanized by cervical dislocation, and bladder and kidneys were removed and stored in Eppendorf tubes. The urine samples were processed the same day by spotting (20 μl) of a series of 10-fold dilutions (10^9 to 10^−6) in duplicate on bromthymol blue agar plates (SSI Diagnostika, Hillerød, Denmark). The bladder and kidneys were stored in 0.9% saline solution and kept at −80°C until processing. They were then incubated at room temperature for 1 h and subsequently homogenized using a TissueLyser (Qiagen, Ballerup, Denmark). Plates for bacterial counting were processed as described above. The detection limit was 25 CFU/sample.

Figure 1 shows the bacterial counts in urine, bladder, and kidneys of mice 72 h after inoculation with 13 B2 isolates from animals and meat and control strains. The negative-control strain failed to produce median bacterial counts above the detection limit (Fig. 1o). A positive culture for a given strain was defined as median bacterial counts above the limit of detection. The positive control and all 13 isolates produced positive bladder cultures, ranging from 10^2 to 10^7 CFU per bladder (Fig. 1a to n); 11 of these isolates (Fig. 1a and d to m) also produced positive urine cultures (10^3 to 10^7 CFU per ml urine). Further, nine isolates produced positive kidney cultures (10^2 to 10^5 CFU per 2 kidneys) (Fig. 1a, d, e, g, and i to m).

In recent years, the possible role of food-borne *E. coli* in causing UTI has been debated increasingly. Similarities among animal, meat, and UTI *E. coli* pheno- and genotypes have been illustrated (1, 7–10, 13, 16). However, the question of whether *E. coli* from animals and from fresh meat available at retail shop counters can cause UTI in humans and thus constitute a real zoonotic risk has remained unanswered. So far, emphasis has been placed on APEC. In support of the argument for

* Corresponding author. Mailing address: Statens Serum Institut, Building 47/201, Artilverivej 5, DK-2300 Copenhagen S, Denmark. Phone: (45) 3268 3423. Fax: (45) 3268 3231. E-mail: lj@ssi.dk.

1 Corresponding author. Mailing address: Statens Serum Institut, Building 47/201, Artilverivej 5, DK-2300 Copenhagen S, Denmark. Phone: (45) 3268 3423. Fax: (45) 3268 3231. E-mail: lj@ssi.dk.

Published ahead of print on 2 June 2010.
FIG. 1. Bacterial counts in urine, bladder, and kidneys of mice killed 72 h after inoculation with the different *E. coli* B2 strains from healthy production animals or meat. Each point represents the results from one mouse. The number of urine samples was smaller than the number of bladder and kidney samples in one case due to unsuccessful urine sample collection. The solid horizontal line represents the median bacterial count. The dotted line indicates the detection limit (25 CFU/sample). Danish b.c. meat, Danish broiler chicken meat; Imp. b.c. meat, imported broiler chicken meat; Pos. control, positive control; Neg. control, negative control.
similarities between APEC and ExPEC, researchers have shown that human ExPEC isolates (including UTI isolate CFT073) are virulent in chicken infection models (1, 9, 16) and that transfer of an APEC plasmid into a commensal avian E. coli strain significantly enhances the virulence of the commensal strain in murine kidneys but not in urine or bladder (13). Czirok et al. showed lethality of APEC after intraperitoneal inoculation in mice (1). However, the relevance of this murine model for human UTI is questionable. Recently, a study by Zhao et al. showed similar tendencies of expression of virulence and antibiotic resistance genes for APEC and a UPEC isolate in a murine model of UTI (16). However, in this model, the mice were reinoculated every 2 days to maintain infection levels, which brings the true virulence of the APEC isolate in the UTI model into question. No studies have included fecal levels, which brings the true virulence of the APEC isolate in this model for human UTI into question. Recently, a study by Czirok et al. showed lethality of APEC after intraperitoneal infection. APEC strain significantly enhances the virulence of the commensal strain, phylogroups, and sources investigated. Strengths include use of the in vivo model of ascending UTI, which is representative of human UTI. Our results therefore provide important circumstantial evidence that UTI is a zoonosis (8–10). More studies are needed to confirm this, e.g., studies determining a direct link between E. coli-contaminated food products and a subsequent E. coli UTI in humans after intake of the meat products. Further, it is important to investigate more potential reservoirs of UPEC (e.g., other food sources and animals but also environmental samples). Limitations of the study include the limited number of strains, phylogroups, and sources investigated. Strengths include use of the in vivo model of ascending UTI, which is representative of human UTI. In conclusion, we demonstrated that all 13 tested E. coli B2 isolates from healthy animals and fresh meat caused positive bladder cultures and that 9 out of 13 caused renal infection in the murine model of ascending UTI. This is the first report of solid in vivo studies providing important circumstantial evidence that UTI is a zoonosis.

This study was supported by grant no. 2101-05-001 from the Danish Research Council. This work is part of the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) and the Marie Curie program Training Risk Assessment in Nonhuman Antibiotic Usage (TRAINAU).

We thank Frederikke R. Petersen, Leila Borggild, Dorte Truchten, Jytte M. Andersen, Frank Hansen, and Karin S. Pedersen for excellent technical assistance and Flemming Schuetz for the kind gift of the negative-control strain E. coli D1923.

REFERENCES


