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Fluoroquinolone-resistant extraintestinal *Escherichia coli* clinical isolates representing the O15:K52:H1 clonal group from humans and dogs in Australia

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ABSTRACT

Antimicrobial-resistant extraintestinal pathogenic *Escherichia coli* (ExPEC) impact both human and veterinary medicine. One ExPEC clonal group that has become increasingly multidrug-resistant is serotype O15:K52:H1. Accordingly, we sought O15:K52:H1 strains among fluoroquinolone-resistant (FQ^r) *E. coli* clinical isolates from humans ($n = 582$) and dogs ($n = 120$) in Australia. The phylogenetic group D isolates (267/702; 38%) were screened for O15:K52:H1-specific single-nucleotide polymorphisms (SNPs) in *fumC* and the O15 *rfb* variant. The 34 so-identified O15:K52:H1 isolates (33 human, 1 canine) underwent antimicrobial susceptibility profiling, virulence genotyping, and macrorestriction profiling. Although susceptibility profiles varied, the 34 isolates were closely related by pulsed-field gel electrophoresis and exhibited typical O15:K52:H1-associated virulence profiles (complete *pap* operon, F16 *papA* allele, *papG* allele II, *iha*, *fimH*, *sat*, *fyuA*, *iutA*, *kpsM*II, *ompT*). The canine isolate closely resembled human isolates. Thus, O15:K52:H1 strains contribute to the FQ^r ExPEC population in Australia and may potentially be transferred between humans and dogs.

1. Introduction

The uropathogenic clonal group *Escherichia coli* O15:K52:H1, which equates with the phylogenetic group D-associated sequence type ST31 clonal complex according to multilocus sequence typing (MLST) (http://mlst.ucc.ie./mlst/dbs/Ecoli) [1], has been associated with urinary tract infection (UTI) since its initial detection in an outbreak in South London, UK, in 1986 and 1987 [2,3]. It has been linked both to community-acquired cystitis and to more severe clinical syndromes such as pyelonephritis and septicaemia [2–4]. Over the last 25 years, this clonal group has continued to be detected and reported as an agent of UTI and urosepsis, albeit at low prevalence [4,5].

The London outbreak strain was first recognized because of its distinctive (and, at that time, concerning) multidrug-resistant (MDR) phenotype, AmpCSSuTTp, i.e., resistance to ampicillin, choramphenicol, streptomycin, sulfonamides, tetracycline, and trimethoprim. Subsequently, however, antimicrobial susceptibility patterns within the clonal group have varied [4,5]. Fluoroquinolone (FQ) resistance first appeared in this group in 1995 and has been increasing in prevalence since [6], adding to the clinical significance of this clonal group as a uropathogen [7].

The London outbreak strain demonstrated, in addition to multidrug resistance, a range of virulence markers,

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including *pap* (P fimbriae; particularly *papG* allele II and the F16 *papA* allele) and *iutA* (aerobactin system), combined with the absence of traditional group B2-associated urovirulence factors such as *sfa* (S fimbriae), *hly* (hemolysin), and *cnf* (cytotoxic necrotising factor) [5,8]. Subsequent O15:K52:H1 isolates exhibited similar virulence genotypes [5], indicating that the O15:K52:H1-associated virulence profile has been conserved over time.

E. coli O15:K52:H1 was first reported outside of Europe in 2002, in a study that screened several archival collections (1984–1998) of *E. coli* isolates from humans and animals [5]. The only non-human-source clonal group member identified was a non-motile (i.e., H antigen-negative: O15:K52:H-) turkey isolate [5]. A subsequent survey of 10,000 archived *E. coli* isolates of animal origin, representing several countries over a 50-year time period (1951–2006), identified no O15:K52:H1 isolates [7]. To date, *E. coli* O15:K52:H1 has not been reported in Australia from either humans or animals, apart from a preliminary report by our group [9].

In that preliminary report, fluoroquinolone-resistant (FQ^r) *E. coli* were prospectively collected from extraintestinal infections in humans ($n = 582$) and companion animals ($n = 120$) over a 2-year period (2007–2009) in eastern Australia [9]. According to PCR-based detection of an O15:K52:H1-specific single nucleotide polymorphism (SNP) in *fumC*, and the O15 *rfb* variant, the O15:K52:H1 clonal group accounted for 33 (5.7%) human isolates, and one (0.8%) canine isolate [9]. Here we sought to further characterize the 34 Australian O15:K52:H1 isolates from that pilot study [9] for a range of molecular and phenotypic traits, both to assess the degree of relatedness between the human and canine isolates and to compare Australian isolates with international O15:K52:H1 clonal group members.

2. Materials and methods

2.1. Bacterial isolates

As reported elsewhere [9], the above-mentioned Eastern Australian collection of 702 FQ^r clinical extraintestinal *E. coli* isolates from humans ($n = 582$) and dogs ($n = 120$) (October 2007–October 2008) underwent determination of major *E. coli* phylogenetic group (A, B1, B2, D) by triplex PCR [10]. The 267 group D isolates (210 human, 57 canine) were screened for O15:K52:H1 status by PCR-based detection of an O15:K52:H1-specific SNP in *fumC* [1] and the O15 *rfb* variant [11].

The 34 O15:K52:H1 isolates thereby presumptively identified as O15:K52:H1 [9] were from urine ($n = 32$) or an unknown site of infection ($n = 2$), and were of human ($n = 33$) or canine ($n = 1$) origin. The single canine isolate was recovered in April 2008 from the urine of a female Rottweiler, as the predominant organism in a mixed culture that also contained an *Enterococcus* spp. These 34 isolates, which constituted the Australian study population, underwent lactose fermentation determination by plating on MacConkey's agar (all proved to be lactose-nonfermenting) and further characterization, as described below. Two historical O15:K52:H1 urosepsis isolates from Seattle, Washington, United States, isolated in 1985 [5] (both lactose fermenting), were studied in parallel for comparative purposes.

2.2. Susceptibility testing

Disk-diffusion susceptibility testing was performed using 12 antimicrobial agents, including enrofloxacin (Enr) and ciprofloxacin (Cip), to confirm FQ resistance, and 10 additional agents (ampicillin [Amp], amoxicillin-clavulanic acid [Amc], cefoxitin [Fox], ceftazidime [Caz], cephalothin [Kf], chloramphenicol [C], gentamicin [Gm], streptomycin [S], tetracycline [T], and trimethoprim-sulfamethoxazole [Sxt]), to assess co-resistance. Methods and interpretive criteria were as specified by the Clinical and Laboratory Standards Institute [12,13]. Isolates testing as intermediate or resistant were considered resistant. An isolate's resistance score was the number of antimicrobials agents (including FQs) to which the isolate was resistant. Isolates with a resistance score of ≥ 4 antimicrobial agents were considered MDR.

2.3. Pulsed-field gel electrophoresis (PFGE) profiling

The study isolates underwent comparative *Xba*I PFGE analysis according to a standardized protocol [14], with the addition of thiourea to prevent shearing [15]. Dice coefficient-based similarity dendrograms were constructed within BioNumerics (Bio-Rad) according to the unweighted pair group method with arithmetic mean. Isolates were considered to represent the same pulsotype if they exhibited $\geq 94\%$ profile similarity to the pulsotype's index isolate, which approximates to a ≤ 3-band difference [16].

2.4. Virulence genotyping

The study isolates were screened by PCR for 53 ExPEC-associated virulence genes and variants [17–19]. Virulence scores were the number of virulence genes detected for each isolate. Virulence genotype similarity between two isolates was calculated as the number of shared virulence genes over their combined total number of virulence genes.

3. Results

3.1. Susceptibility profiles

The 34 Australian *E. coli* O15:K52:H1 clonal group isolates displayed a median resistance score of four (range, 0–7). Most qualified as MDR (28 isolates; 82%), including the sole canine isolate, which was resistant to Amp, Amc, Enr/Cip, Kf, S, Sxt, and T. Whereas most of the isolates were resistant to Sxt (31; 91%), T (29; 85%), Amp (26; 76%), and S (23; 68%), only a minority were resistant to Gm (12; 35%), Kf (5; 15%), Amc (3; 9%), and C (1; 3%), and none were resistant to Fox or Caz. Overall, the isolates exhibited 14 different resistance profiles, the most frequent of which (27% of isolates) involved Amp, Enr/Cip, S, Sxt, and T (Table 1). Only one isolate exhibited the classic O15:K52:H1-associated