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Virulence and antibiotic resistance of *Escherichia coli* isolated from rooks

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Abstract

With regard to antibiotic resistance studies in various model animals in the urban environment, the presented study focused on the rook, many behavioural and ecological aspects of which are important from an epidemiological point of view. A total of 130 *Escherichia coli* strains isolated from rook faeces during a two-year period (2011–2012) were investigated for antibiotic resistance and virulence. Resistance to ampicillin (60%) and streptomycin (40%) were the most frequent, followed by resistance to fluoroquinolones (ciprofloxacin-22% and enrofloxacin-24%), tetracycline (18%), cotrimoxazol (17%) and florfenicol (14%). Ceftiofur resistance occured in 10.7 % of strains and cefquinom resistance in 1.5 % of strains. Twenty-five *E.coli* strains with a higher level of MICs of cephalosporins (over 2mg/L of ceftazidime and ceftriaxon) and fluoroquinolones were selected for detection of betalactamase genes (CTX-M, CMY), plasmid-mediated quinolone resistance *qnrS*, integrase 1, and for APEC (avian pathogenic *E.coli*) virulence factors (*iutA, cvaC, iss, tsh, ibeA, papC, kpsII*). Genes of CTX-M1, CMY-2, integrase 1, *papC, cvaC, iutA* were detected in one strain of *E.coli,* and *qnrS*, integrase 1, *iss*, *cvaC*, *tsh* were detected in another *E.coli*. DNA microarray revealed the absence of verotoxin and enterotoxin genes and pathogenicity islands. The results show that rooks can serve as a reservoir of antibiotic-resistant *E. coli* with avian pathogenic virulence factors for the human population, and potentially transmit such *E.coli* over long distances.

Keywords

Escherichia coli, ESBL, virulence, PCR, DNA microarray, rooks

INTRODUCTION AND OBJECTIVE

With regard to antibiotic resistance studies in various model animals in the urban environment, the presented study focused on the rook, many behavioural and ecological aspects of which are important from an epidemiological point of view. The rook (*Corvus frugilegus* Linnaeus, 1758) has a great range of distribution. Palaearctic distribution extends from the British Isles across Europe, to the central and northern parts of Asia. The present estimate of the number of breeding pairs of rooks in Slovakia each year varies between 700,000–1,000,000 [1, 2]. Rook populations live in huge flocks in European conditions and often several hundred to several thousand individuals meet together, either by nesting in colonies or at roosting places at point trees, as well as by searching for food in the fields. The next important point in its ecology is that rook populations in central Europe are partially migratory or stray during winter. Most rooks from western Slovakia survive the winter in France and Spain, but populations from eastern Slovakia migrate to southern Europe. Wintering rooks in Slovakia come from the western part of Russia, Belarus, the Baltic Republics and the Ukraine [3, 4]. The number of wintering rooks in Slovakia varies between 300,000–1 million individuals. Partial migration and spatial overlap of wintering rooks from different parts of the continent is a precondition for the maintenance and spread of resistant bacterial strains (or pathogens) in Europe [1, 5].

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Wild animals are potential reservoirs of zoonotic pathogens, and the detection of extended spectrum beta-lactamase producers and fluroquinolones among *Escherichia coli* has increased in recent years. Literak et al. [1] found that 13.7% *E. coli* isolates were antibiotic resistant in rooks wintering in the Czech Republic, and the dominant type of resistance was to tetracycline.

The objective of the presented work was to study antibiotic resistance and virulence factors of faecal *Escherichia coli* isolated from rooks breeding in the urban agglomerations of eastern Slovakia. Three similar nesting colonies of rooks in the city of Košice were monitored during two years of research with the aim of confirming the persistence of microbial resistance in autochthonous rook populations.

MATERIAL AND METHODS

The faeces of rooks were collected in the spring season during the course of two years (2011 and 2012) under the trees of three rook nesting colonies in town parks in Kosice (480 43′07′′ N, 210 15′25′′ E; 480 43′20′′ N, 210 15′49′′ E; 480 42′51′′ N, 210 16′05′′ E. The number of nests in the colonies ranged from 6–20, and were similar in both years. Faeces of rooks were collected once a month during the breeding season (early March – early May). Faeces were repeatedly collected at the sites studied during both years of research. In addition, one-time collection of rook faeces (April 2012) was carried out in two towns – Rimavska Sobota (480 23′06′′ N, 200 00′15′′ E) and Cierna nad Tisou (480 25′06′′ N, 220 05′22′′ E).

Faeces were restored in buffered peptone water (Oxoid, Basingstoke, UK) and then subcultured on MacConkey

agar (Oxoid). Bacterial diagnostics was performed using Malditof analysis. Bacterial extracts for mass spectrometry measurements were prepared as recommended by the manufacturer of the MS instrument [6].

For determination of minimal inhibitory concentration (MIC) according to CLSI guidelines M31-A3 [7], there were used: ampicillin (AMP), ampicillin and sulbactam (A+IB), ceftiofur (CFF), cefquinome (CFQ), gentamicin (GEN), streptomycin (STM), neomycin (NEO), nalidixic acid (NAL), enrofloxacin (ENR), chloramphenicol (CMP), florfenicol (FLO), tetracycline (TET) and cotrimoxazol (COT) [8]. ESBL genes for CTX-M [9], CMY-2 [10], *gnrS* [11] and integrase 1 [12] were determined by PCR.

Genes of virulence factors for APEC (avian pathogenic *E.coli*), e.g. *iutA*- receptor for aerobactin, *kpsII*-capsular polysialic acid virulence factor and *cvaC*-colicin V, were investigated by Johnston and Stell ([13] 2000), *iss*-increase serum survival by Foley et al. [14], *tsh*-temperature sensitive haemagglutinin by Dozois et al. [15], *ibeA*-invasive factor responsible for neonatal meningitis in humans by Germon et al. [16], and *papC*-P fimbriae by Le Bouguénec et al. [17].

An additional 50 virulence factors for VTEC/EPEC were also screened using tube DNA microarray (Identibac, Alere Technologies GmbH, Jena, Germany). The protocol for microarray comprised genomic DNA extraction, linear amplification and bioting labelling as well as hybridisation. All these steps were performed according to the manufacturer's instructions. Array spots were detected by the ArrayTube Scanner. Spot signals were measured with IconoClust software.

RESULTS

The occurrence of antibiotic resistance in the 130 *Escherichia coli* isolates recovered from rook faeces during the two–year period is shown in Fig. 1. Resistances to ampicillin (60%) and streptomycin (40%) were the most frequent, followed by resistance to fluoroquinolones (ciprofloxacin-22% and enrofloxacin-24%), tetracycline (18%), cotrimoxazol (17%), spectinomycin (10%), neomycin (4.6 %), florfenicol (14%) and chloramphenicol (3%). Ceftiofur (3rd generation) resistance occurred in 10.7 % of strains and cefquinom (4th generation) resistance in 1.5 % of strains. All strains were sensitive to ampicillin with sulbactam and gentamicin.

MIC 90 of enrofloxacin in 32 *Escherichia coli* resistant isolates were very high and reached 16 mg/L. Ceftiofur resistant *Escherichia coli* produced relatively lower levels of MIC90 for ceftazidime and ceftriaxon (phenotype indicators for ESBLs) 8 mg/L.

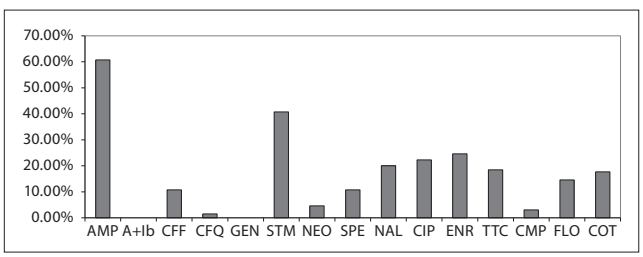

Figure 1. Antimicrobial resistance of faecal *E.coli* from rooks

The six virulence genes *iutA*, *iss, cvaC, ibeA, kpsII* and *tsh* were also detected, together with ESBLs genes in faecal *E.coli* from rooks (Tab. 1). Genes of CTX-M1, CMY-2, integrase 1 *papC*, *cvaC, iutA* were detected in one strain of *E.coli*. Fluoroquinolone resistant strain *E.coli*contained *qnrS*, integrase 1 and three virulence genes *iss, cva, tsh*. However, there was an absence of typical VTEC/EPEC virulence factors, e.g. verotoxins, enterotoxins, intimin and pathogenicity islands (data not shown). Tube DNA microarrays revealed only genes coding *lpfA*-long polar fimbriae and colicins M and H in *E.coli* from rooks.

Table 1. Betalactamase, quinolone and virulence genes in 25 *E. coli* strains.

Strains with CTX-M genes and virulence	**No. of strains**
CTX-M1, *cvaC, iutA*	2
CTX-M1, *iutA*	1
Strain with CTX-M, CMY-2 and virulence	
CTX-M1, CMY-2, *papC,cva C, iutA*	1
Strains with CMY-2 genes and virulence	
CMY-2, *iss, ibeA, iutA, kpsII tsh*	3
CMY-2, *cvaC, iss, iutA, tsh*	1
CMY-2, *cvaC, iutA*	3
CMY-2, *cvaC, iss*	1
CMY-2, *cvaC*	1
Virulence genes and integrase 1	
cvaC, iss, iutA, tsh, Int1	2
iss, iut, Int1	1
iss, tsh, Int1	1
iss, kps, Int1	1
iss, Int1	2
iut, Int1	1
Strain with *qnrS*, integrase 1 and virulence	
qnrS, cvaC, iss, tsh, Int1	1
Only virulence gene	
papC	1
iss	1
Strain with CMY-2 gene and integrase 1	
CMY-2, Int1	1
Total	**25**

DISCUSSION

The results obtained in the presented study indicate that rooks can serve as a reservoir of antibiotic-resistant *E. coli*. Hovewer, typical human strains of *E.coli* have much higher MIC levels of cephalosporins, usually 32–64 mg/L. ESBLs are widely detected in various human medical institutions, but they are not so frequently reported in the bacterial population circulating in animals. This could indicate that these enzymes are less prevalent in animals than in humans [18]. A third generation of cephalosporin, e.g. cefotaxime and ciprofloxacin, are critically important antimicrobials for human medicine. Resistance to third-generation cephalosporins was observed in indicator *E. coli* isolates from *Gallus gallus* and from meat derived from broilers at very low levels varying from 0.2 %-7 %. However, high levels of ciprofloxacin resistance (29 %) were described in *E.coli* isolates from *Gallus gallus* in an EFSA report from 2010

[19]. Those results are more or less similar to our data for ciprofloxacin resistance (22%) in *E.coli* from rooks. Moreover, plasmid coded quinolone resistance *qnrS* was detected in one strain of *E.coli* from rooks.

Avian pathogenic *E. coli* (APEC) are known to possess a large number of potential virulence factors. APEC strains show similarities with human extraintestinal pathogenic *E. coli* (ExPEC) strains [20]. Long polar fimbriae are related to type I fimbriae, but they are not specific to the VTEC strains [21]. Verotoxigenity is not usual for avian *E.coli*. Data in the presented study show the presence of the invasive factor responsible for neonatal meningitis *ibeA* with a combination of cephalomycinase (CMY-2) and cefotaximase (CTX-M1) genes in three strains of *E.coli*. Such strains from rooks may pose a zoonotic risk and are a concern for human health.

In contrast to recent studies of microbial resistance in rooks from other parts of Europe, the presented research repeatedly confirms the persistence of bacterial resistance of the autochthonous rook population [1, 5]. This is influenced by the long-term stability of nesting colonies in Kosice and the longevity of rooks (up to 20 years according to bird ringing data), as well as regular wintering by rooks from other countries of Europe where resistance has been confirmed [5]. The sources of infection of rooks with *E. coli* could be food and/or drinking water. Ecological studies and ornithological observation of rooks in the field have shown an omnivorous feeding pattern in agricultural, rural and urban habitats during winter [e.g. 22]. Rooks infected with pathogenic and antibiotic resistant bacteria from animal and human sources may disseminate these bacteria over long distances and pose a risk for environmental contamination [1]. This problem requires long-term monitoring and comprehensive solutions (e.g. source of resistance, animals as reservoirs, circulation in nature).

CONCLUSIONS

The results show that rooks can serve as a reservoir of antibiotic resistant *E. coli* with avian pathogenic virulence factors, and potentially transmit such *E. coli* over long distances. Our study of breeding (autochthonous) rook populations in Eastern Slovakia during the spring period, and similar studies of antibiotic resistant *E. coli* of wintering populations (migratory) in many countries throughout Europe, show that *C. frugilegus* can be a reservoir or vector of many resistant strains and genes of *E. coli*. Due to the large number of migratory birds moving throughout Europe every year, this fact represents a serious epidemiological problem that needs to be monitored.

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