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Phenotypic Study of the Antibiotic Resistance *Escherichia coli* from Crow (*Corvus Corax*) in Djelfa (Algeria)

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ABSTRACT

This study was performed on a collection of fecal simple from the crow into the detection of *Escherichia coli* strains resistant to antibiotics. 27 strains of *E. coli* were isolated from crow in Moudjbara at Djelfa cities, the activity of the antibiotics against the isolates was determined by the agar diffusion test. The highest-level resistance are recorded for tetracycline (96.30%). Low-levels resistance were obtened with a ceftazidime and aztreonam (25.92%), cefotaxime (22.22%), sulfamethoxazole (11.11%), ciprofloxacin (7.40%), amoxicillin-clavulanic acid, acid nalidixic and imipenem (3.70%). In contrast, all isolates were sensitive to tobramycin and gentamycin. Finally, the results show that crows can be a reservoir of antibiotic resistance *E. coli*, and potentially transmit it through the world.

Keywords: *Escherichia coli*; Antimicrobial Resistance; Crow; Djelfa; Algeria

Introduction

The Crow (*Corvus corax*) is the largest passerine bird in Europe much of northern Asia and northern America, India, Palestine, Egypt, the Canary Islands, Australia, Tasmania, China, Madagascar, tropical and southern Africa and the Hawaiian Islands [1]. It is a common species in North Africa and is widely distributed in non-desert areas of the northern hemisphere. It uses a wide variety of habitats and builds its nests preferentially on cliffs, sometimes on trees or, more rarely, on human constructions [2]. The Crow (*Corvus Corax*) is one of the most widespread species in Algeria where it has been the subject of very few studies [3]. His distribution in Algeria would have recently progressed towards the south, in the Sahara (Biskra, Djelfa, Laghouat, Aïn Sefra) [4]. Wild birds are vectors and reservoirs for the maintenance and spread of infections, interactions between humans and wild birds are evident, they reside in human habitats, migrate between waste collection areas. Cattle, pig and poultry farms, and deposit their droppings in the ground and in water, thus allow the transmission of these zoonoses to humans and animals [5]. In addition, the emergence and spread of multi-resistant bacteria in environments poses a global risk to human and animal health [6]. Wild birds can serve as reservoirs of antibiotic-resistant bacteria, including *E. coli*, and contribute to the global spread of *E. coli* in natural ecosystems [7]. A study of Belmahdi et al. on the sparrow in Algeria showed the presence of strains of *E. coli* carrying CTX-M-14 type resistance genes [8]. Studies on the resistance to antibiotics of strains isolated from the crow have already been published. Among these studies is the study by (Yuko Aruj et al. [9]) on the crow in Ueno Zoo [9], studies on the Russian Rook (Corvus frugilegus) wintering in the Czech Republic and on the Commonly Wintering Rooks throughout Europe [10,11], on the Migratory and Resident Crows (Corvus frugilegus) in Austria [12],

(Halová, et al. [13]) on the American crow [13], on the house crow (Corvus splendens) in Bangladesh [14], on Corvus brachyrhynchos and *Corvus corax* roosting in Canada [15]. For this, we proposed this study analyses of the antibiotic resistance in *E. coli* isolates from Crow. To our knowledge this is the first study concerning the antibiotic resistance *E. coli* in this type of bird in Algeria.

Material and Methods

Sampling and Strains Isolation

A total of 57 fecal samples were collected from crows, from Moudjbara in Djelfa cities (Algeria), between February and April 2019. Fecal samples and intestinal swabs were inoculated in nutrient broth (BHIB) at 37°C for 24 h for enrichment. After that, enriched culture was seeded on MacConkey agar plates (Pasteur Institute of Algeria) and incubated for 24 h at 37°C. Isolates with typical *E. coli* morphology were selected (one isolate per sample), identified by classical biochemical methods.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was performed on Mueller–Hinton agar by standard disk diffusion procedure as described by the Antibiogram Committee of the French Society for Microbiology (CA-SFM) (www.sfm-microbiologie.org/). Eighteen antibiotics were tested including, amoxicillin–clavulanic acid (AMC), cefoxitin (FOX), cefotaxime (CTX), ceftazidime (CAZ), aztreonam (ATM), imipenem (IMP), tobramycin (TOB), gentamicin (GN), nalidixic acid (NAL), ciprofloxacin (CIP), gentamicin (GEN: 10 µg), tetracycline (TET: 30µg) and co-methoprim (COT: 25µg) (Bioanalyse). The diameter of the zone of inhibition around the disc was measured. The results were then interpreted according to the AC-FSM breakpoints [16]. *E. coli* isolates were finally classified as resistant (R), susceptible (S), or intermediate (I) for each of the antimicrobials tested.

Phenotypic ESBL Detection

A screening test for extended spectrum β-lactamases (ESBL) production was carried out on Mueller–Hinton agar using the double disc synergy test (DDST) by placing disks of CAZ (30 µg) and CTX (30 µg) at a distance of 20 mm center to center from an amoxicillin-clavulanic acid disk (30 µg). An extension of the edge of the inhibition zone of the third generation cephalosporins disks (CAZ and/or CTX) in proximity to the AMC disk indicates a positive ESBL production [17].

Results

Bacterial Isolation

Among the 57 fecal samples, 27 cefotaxime-resistant *E. coli* isolates were recovered, and no strain positive ESBL test was detected. One *E. coli* isolate per fecal sample was selected for further studies, making a collection of 27 isolates (Table 1).

Table 1: The resistance phenotype of the 27 E. *coli* strains isolates from Crow.

Isolate code	Origin	Resistance phenotype	Isolate code	Origin	Resistance phenotype
CM2	Moudjbara (Djelfa)	CTX CAZ AMC NA CIP TET COT	CM16	Moudjbara (Djelfa)	TET
CM3	Moudjbara (Djelfa)	Sensitive	CM17	Moudjbara (Djelfa)	ATM
CM4	Moudjbara (Djelfa)	TET	CM18	Moudjbara (Djelfa)	Sensitive
CM5	Moudjbara (Djelfa)	CIP TET	CM19	Moudjbara (Djelfa)	CTX CAZ ATM TET
CM6	Moudjbara (Djelfa)	Sensitive	CM20	Moudjbara (Djelfa)	CTX CAZ ATM TET
CM7	Moudjbara (Djelfa)	TET	CM21	Moudjbara (Djelfa)	TET COT
CM8	Moudjbara (Djelfa)	Sensitive	CM 22	Moudjbara (Djelfa)	Sensitive
CM9	Moudjbara (Djelfa)	TET	CM23	Moudjbara (Djelfa)	CTX CAZ ATM TET
CM10	Moudjbara (Djelfa)	TET	CM24	Moudjbara (Djelfa)	CTX CAZ ATM TET
CM11	Moudjbara (Djelfa)	TET	CM25	Moudjbara (Djelfa)	Sensitive
CM12	Moudjbara (Djelfa)	Sensitive	CM26	Moudjbara (Djelfa)	Sensitive
CM13	Moudjbara (Djelfa)	TET	CM27	Moudjbara (Djelfa)	CTX CAZ ATM TET
CM14	Moudjbara (Djelfa)	TET	CM28	Moudjbara (Djelfa)	TET COT
CM15	Moudjbara (Djelfa)	Sensitive	/////	//////////////	/////////

Note: AMC: amoxicillin-clavulanic acid, ATM: Aztreonam, CAZ: ceftazidime, COT: Co-methoprim, CIP: ciprofloxacin, CTX: cefotaxime, TET: tetracyclin, TOB: tobramycin, NAL: nalidixic acid.

Antimicrobial Resistance Phenotype

The results of antibiotic susceptibility testing for the 27 *E. coli* isolates revealed that most isolates demonstrated high-level resistance to tetracycline (> 96%). The rates of resistance to aztreonam, ceftazidim and cefotaxime were 25,92%, 25,92%, and 22,22 % respectively. But, most isolates demonstrated low-level resistance to co-methoxazol (11,11%), cipofloxacin (7,40%), amoxicillin–clavulanic acid, nalidixic acid and imipenem (3,7%) from the all. However, all isolates remained susceptible to gentamicin and tobramicin.

Discussion

Although wild birds are not normally exposed to the use ofantimicrobial markers, they can acquire antibiotic resistant bacteria from the environment [18]. It has also been suggested that Rooks could disseminate these bacteria over long distances and pose a risk of environmental contamination [11]. One study investigated the presence of faecal bacteria with plasmid-mediated quinolone resistance (PMQR) genes in Rooks (Corvus frugilegus, medium-sized corvid birds) wintering in continental Europe during the winter of 2010-2011, quinolones are commonly used in antimicrobial therapy in human and veterinary medicine worldwide, plasmid-mediated quinolone resistance (PMQR) was first identified in 1998 [19]. The presence of these genes (PMQR) was revealed by in strain of *E. coli* isolated from winter roosting sites of American crows (*Corvus brachyrhynchos*) and common ravens (*Corvus corax*) in Canada [14]. In our case, lows-levels resistance quinilones were obtained with cipofloxacin (7,40%) and acid, nalidixic acid (3,7%). detected the CTX-M-1, 3 and 15 type genes in *E. coli* isolated from the Migratory and Resident Population of Rooks (Corvus frugilegus) [12]. In addition to CTX-M-1 and 15 other CTX-M; CTX-M-8,14, 24, 25, 28 and 55 and also type SHV-12 and TEM-55 have been identified in *E. coli* isolated from rooks wintering in the Czech Republic, France, Germany, Italy, Poland, Serbia, Spain, and Switzerland [20]. The presence of these genes signifies the resistance of these strains to β-lactam, especially 3rd generation cephalosporin. In our case we are limited to the phenotypic study which reveals average resistance rates to 3rd generation cephalosporin such as ceftazidime (25.92%) and cefotaxime (22.22%). Gentamicin and tobramicin (aminoside) remain the most active antibiotics on the isolated strains. Contrary to what (Loncaric, et al. [20]) found. Because they isolated strains resistant to tobramycin [12]. Crows near settlements and areas with high livestock density have been colonized with antibiotic resistant strains that were likely selected by antibiotic practice in humans and domestic animals. Antibiotic-resistant isolates of *Escherichia coli* have been found in various corvids, including pies (Corvus corone, C. frugilegus, C. macrorhynchos, Pica pica, and Pyrrhocorax pyrrhocorax) [21]. Tetracycline proves its therapeutic failure either in our case or other studies with high resistance rates [12,14]. From this work and the studies cited above, we can conclude that the Crow can be a reservoir of the antibiotic resistant strains. It can be also a source of propagation of these resistant strains in its environment or through the world in since it is recognized as a migratory bird.

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