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Prevalence and Antibiotic Resistance Profile of Intestinal Bacteria Isolated from Captive Adult Houbara Bustards (*Chlamydotis macqueenii*) Exposed to Natural Weather Conditions in Saudi Arabia

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Abstract: Defining enteric bacterial flora of clinically healthy capitive houbara bustard and their resistance to antibiotics are an important step in understanding the epidemiology of bacterial diseases. These diseases may affect their population in captivity and so the environment after their release. The study amied to identify the intestinal bacterial flora associated with houbara bustard in captivity and their resistance to currently used antibiotics in Saudi Arabia. Samples from capitive houbara and their environment were cultured for enteric bacteria, tested for resistance to antibiotics and screend for integrons by polymerase chain reaction. A culture based method recoverd a total of 118 bacterial isolates and bacteriological identification revealed that the most encountered species were *Escherichia coli* 66 (55.9%), followed by *Enterococcus* sp. 19 (16.1%). The *Salmonella* sp. Was only detected in 6 (5.1%) samples. All tested samples were negative for *Campylobacter* sp. The antimicrobial susceptibility tests showed that 18 (15.2%) strains showed multidrug resistance phenotypes against various combinations of antimicrobials tested. Positive screening test for extended spectrum beta-lactams was obtaind in eleven strains. Integron detection by PCR identified class 1 and class 2 integrons in nine and two isolates respectively. These results suggest that captive bustard colonized with commensal or potentially pathogenic enteric bacteria and multi-resistance may be likely to contribute to disseminate these multiresistant bacteria to wildlife and local environment. This study provides researchers, wildlife managers and animal ethics committees with information to assist the captive measures and control.

Key words: Capitive Bustards · Drug Resistance · Enteric Bacteria · PCR. Prevalence · Saudi Arabia

INTRODUCTION

The houbara bustard is a traditional game birds an ultimate quarry for Arab Falconers occupying arid disert environments. It is listed among the globally threatened species by Bird Life International on the IUCN Red list as Vulnerable [1]. In the Arabian Peninsula, the species can be considered as critical endangered [2]. Because of houbara bustard population declines in Arabia, a captive-breeding programme was initiated at the National Wildlife Research Center (NWRC) in Taif, Saudi Arabia, during 1986, aim to protect and reintroduce this species to its former habitat [2]. Most researches of these programs concerned ecological, biomedical and captive breeding. However, little attention has been directed to their normal bacterial flora as well as analysis of antimicrobial resistance.

A variety of potentially pathogenic and zoonotic bacteria have been isolated from wild birds [3-6] in particular from houbara bustards [7]. Dissemination of microbial drug resistance observed in the antibiotic era is clearly related to selective pressure generated by the use of antibiotics in human, veterinary and agriculture practice [8]. Furthermore, antibiotic resistant bacteria had

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been reported also in wild animals living in remote areas where antibiotic exposure has been absent or minimal [9, 10]. A growing concern over the possibility of disease and antibiotic resistance transmission when captive raised animals are released into wild populations [1, 11]. The objective of this study was to determine the prevalence of antimicrobial resistance in cloacal microbiota from a captive human-raised Asian houbara bustard *Chlamydotis macqueenii* population. In addition, examination of post-mortem cases, fresh eggs and live food were presented. Resistant bacterial isolates were screened for integrons by polymerase chain reaction (PCR).

MATERIALS AND METHODS

Bustards and Sampling: Sixty-nine female captive-born 9 (4-17) year-old *C. macqueenii* houbaras, exclusively dedicated to the captive breeding programme of the National Wildlife Research Center (NWRC), Taif, Saudi Arabia and accommodated in open-air cages for sampling. Houbara bustards were mainly fed on antibiotics-free dry pellets and had an *adlibitum* access to bottle water. Few additional live food consisting of alfalfa leaves and mealworms *Tenebrio molitor* produced at the NWRC were also given on a daily basis. The duration of study was one year long.

These sixty-nine individuals belong to an adult flock where every houbara bustard is individually monitored from hatching to death, where the use of antibiotics is reduced to a minimum. However, since its creation in 1986, the NWRC houbara flock from time to time faced bacterial infectious outbreaks successfully treated with massive antibiotherapy. This included doxycycline (tetracyclines group) which was used 20 years ago for treating the whole adult flock, enrofloxacine (fluoroquinolones group) was used ten years ago to control an outbreak in the neonate flock and six years ago in the juvenile flock as well as a combination of lincomycine (lincosamides group) and spectinomycine (aminoglycosides group) used one year ago for treating the whole juvenile flock. However, during the last 12 years, enrofloxacine, was the main systemic antibiotic used, gentamycine (aminoglycosides group), oxytetracycline (tetracyclines group) and choramphenicol (amphenicols group) being used only as topical antibiotics.

None of the 69 sampled adult individuals received an antibiotherapy during the last two years; after neonatal age, 83% of them were never treated with antibiotics, 16% received one single antimicrobial treatment and 1% received two antimicrobial treatments.

Houbara bustards and staff in charge of the NWRC flock were subjected to strict sanitary procedures in order to keep sanitary risks under control. However, the private use of antibiotics by the NWRC staff was not under control. The NWRC houbara facilities were also exposed to microflora brought through well water use, which was also out of control.

The samples included cloacal swabs from clinically healthy individual bird and dead ones. Fresh laid eggs, foods, drinking water and sand from the cages were also collected. the Few rodents (gerbils *Gerbillus cheesmanii* and brown rats *Meriones rex*) trapped in the houbara captive breeding facilities were euthanized and intestinal swabs were collected. The procedure for sample collection from various wildlife species was described earlier [12].

Bacteriological Analysis: All swabs and samples were processed initially onto specified media and incubated at appreciated condition (aerobic and microaerophilic). Purified colonies were frozen at-80°C in brain hear infusion broth (BHI, Scharlau) supplemented with 20% glycerol for future analysis. Identification of bacterial isolates was based on morphology, Gram staining, biochemical characteristics [13] and commercial API systems (bioMérieux, Marcy l'Etoile, France). Samples were cultured aerobically onto blood agar (BA), Hektoen enteric agar (HE, Scharlau), Eosine-methylene blue agar (EMB, Scharlau), Salmonella-Shigella (S-S, Hi-Media) agar and subsequently inoculated into Rappaport medium (Hi-Media). To culture Salmonella, subcultures from Rappaport medium were done onto Salmonella-Shigella agar after incubation at 42°C for 3-4 days. To isolate Enterococci, swabs and samples were transferred to Enterococcosal broth (BBL), which was incubated for 24 hours at 37°C. A swab was used to transfer broth from positive cultures to Enterococcosel agar (BBL) for isolation of enterococci. Plates were incubated overnight at 37°C. To culture for thermophilic Campylobacter spp., swabs were plated for isolation on Campylobacter blood-free agar containing charcoal cefoperazone deoxycholate agar (CCDA, Oxoid) selective supplement. Inoculated plates were incubated at 42°C under microaerophilic condition using Camp-Pack kit (Oxoid, BR0060A) for 48 hours to detect thermophilic *Campylobacter* as described [14].

To culture Lactobacillus spp., Anaerobic MRS (LAB) with adjusted pH 4.2 was incubated at 30°C for 96 hours in an anaerobic Gas-Pack system and gas generating kit (anaerobic system, BR 0038B)(Oxoid).

Antibiotic Susceptibility Testing: The antimicrobial sensitivity phenotypes of recovered bacteria were determined on cation-adjusted Mueller-Hinton agar (Hi-Media) using a Kirby-Bauer disk diffusion assay according to the standards and interpretive criteria described by Clinical and Laboratory Standards Institute [15]. All bacterial strains were analyzed to the various antimicrobial agents studied. Salmonella strains were tested for polymyxin B and doxycycine, as well as Enterococcus strains to vancomycine. The antibiotic tested included: amoxicillin, ampicillin, cephalothin, chloramphenicol, ciprofloxacin, gentamycin, tetracycline, cefadroxil, cefodizime, cefuroxime, cefotaxime, erythromycine, amoxicillin-glavulenic acid, trimethoprim-sulfamethoxazole, cefoxtine, amikacin, aztreonam, piperacillin and imipenem. The quality control was performed to check the quality of medium and potency of antibiotic disks before use against some sensitive ATCC reference strains including *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853 (MicroTrol Discs, BD Diagnostics, France).

Phenotypic Detection of Extended-spectrum Beta-lactamase(ESBLS): The first step of extended-spectrum beta-lactamase detection criteria was resistance or reduced susceptibility to cefotaxime and/or ceftazidime. Phenotypic confirmation of extended-spectrum beta-lactamase production was performed by double disk diffusion synergy test (DDST) [16].

PCR Detection of Integrons: Template DNA from resistant bacteria was prepared from freshly cultured bacterial isolates by suspending 3-5 colonies in 50 ìl of distalled water and then heating at 95 °C for 5 minutes and immediately chilling at 4 °C. Primers 5'-CS and 3'-CS, which amplify the region between the 5' conserved segment and 3' conserved segment of class 1 integrons, were used as previously described [17]. On the other hand, for the detection of class 2 integrons, PCR was performed with primer hep74 and hep51, specific to the conserved regions of class 2 integrons, as described previously [18].

RESULTS

Bacteriological examination of different samples from houbara are presented in Table 1. A total of 118 bacterial isolates were detected and bacteriological identification revealed that the most encountered species were *Escherichia coli* 66 (55.9%), followed by Enterococcus sp. 19 (16.1%), Lactobacillus sp. 9 (7.6%), Salmonella sp. 6 (5.1%), *Shigella flexnari* 5 (4.2%), *Proteus mirabilis* 3 (2.5%), two isolates (1.7%) of *Enterobacter cloacae*, *Citrobacter freundii*, *Klebsiella oxytoca* and single isolates (0.8%) of *Klebsiella pneumonae*, *Citrobacter youngae, Proteus vulgaris* and *Shigella sonni*. All tested samples were negative for Campylobacter sp. The isolates from cloacal swabs collected included *E. coli*, *Enterobacter cloacae*, *Citrobacter youngae*, *Shigella flexnari*, *Shigella sonni*, *Enterobacter cloacae*, *Salmonella* sp., *Klebsiella oxytoca* and Lactobacillus sp. Campylobacter was not isolated from any samples. The isolates from postmortem cases included *E. coli*, Salmonella sp., *Shigella flexnari*, *Enterobacter cloacae* and *Proteus vulgaris*.

The content of fresh houbara eggs did not reveal any positive bacterial isolation. However, *E. coli* was the only bacterium recovered from the egg shells. No Salmonella spp. was isolated from either the egg contents or the egg shells.

Bacteriological examination of the mealworms body, sand from the cages and intestinal contents of wild rodents revealed positive *E. coli* culture from two mealworms body and two rodents. In addition, *Proteus mirabilis* and *E. coli* isolates were cultured from two sand samples.

In the disk diffusion assay, of 118 bacterial strains tested, 37 (31.3%) showed resistance to two or more of antimicrobial agents (Table 2). The most common resistant species were *E. coli* 18 (15.5%), Salmonella spp. 5 (4.5%), Enterococcus spp. 3 (2.5%), two isolates (1.7%) of *Shigella flexinari*, *Enterobacter cloacae*, *Citrobacter freundii* and *Klebsiella oxytoca* and one isolate (0.8%) of *Proteus mirabilis*, *Citrobacter youngae* (0.8) and *Klebsiella pneumoniae* (Table 2). Furthermore, A reduced susceptibility was observed frequently to ampicillin (28.8%), cephalothin (19.5%), tetracycline (17.8%) and 11% of extended spectrum beta-lactam drugs and erythromycin (11%). A relatively low resistance was observed to fluoroquinolones, sulphonamide and chloramphenicol. All isolates exhibited reduced or even rare resistance to amikacin, imipenem, gentamicin and piperacillin (Table 3).

Table 1: Prevalence of bacterial species from different examined samples of houbara bustard and their environment in NWRC

Bacterial species (N)	Cloacal swabs (n=90)N %	PM swabs (n=5)N %	Surface of fresh eggs (n=8)N %	Mealworms (body) (n=4)N %	Sand from cages (n=8)N %	Gut contents of rats (n=4)N %
Escherichia coli (66)	52 (57.8)	5 (100)	2 (25.0)	2 (50.0)	2 (25.0)	3 (75.0)
Enterococci spp. (19)	16 (17.8)	3 (60.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Lactobacillus spp. (9)	9 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Salmonella spp. (6)	4 (4.4)	2 (40.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Shigella flexnari (5)	4 (4.4)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
Shigella sonni (1)	0 (0.0)	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.00
Proteus mirabilis (3)	1 (1.1)	1 (20.0)	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)
Proteus vulgaris (1)	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Enterobacter cloacae (2)	1 (1.1)	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Citrobacter freudii (2)	2 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Citrobacter youngae (1)	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Klebsiella oxytoca (2)	1 (1.1)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)
Klebsiella pneumonae (1)	0 (0.0)	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total (n= 118)	93 (78.8)	13 (11.0)	3 (2.5)	3 (2.5)	3 (2.5)	3 (2.5)

Table 2: Incidence of antimicrobial resistant (AR) bacteria cultured from houbara bustard and their environment in NWRC, Taif, Saudi Arabia *

Bacteria	Total No. (%) of AR isolates*	No. (%) of MDR isolates**
Escherichia coli (66)	18(15.5)	6 (5.1)
Enterococci spp. (19)	3 (2.5)	1 (0.8)
Lactobacillus spp. (9)	0 (0.0)	0 (0.0)
Salmonella spp. (6)	5 (4.2)	1 (0.8)
Shigella flexnari (5)	2 (1.7)	2 (1.7)
Shigella sonni (1)	0 (0.0)	0 (0.0)
Proteus mirabilis (3)	1 (0.8)	1 (0.8)
Enterobacter cloacae (2)	2 (1.7)	2 (1.7)
Citrobacter freudii (2)	2 (1.7)	2 (1.7)
Citrobacter youngae (1)	1 (0.8)	0 (0.0)
Klebsiella oxytoca (2)	2 (1.7)	1 (0.8)
Klebsiella pneumoniae (1)	1 (1.7)	1 (0.8)
Total (n=118)	37 (31.3%)	18 (15.2%)

* AR, an* AR, antimicrobial resistance to two or more antibiotics.

** MDR, antimicrobial resistance to four or more antibiotics.

Table 3: Frequency of resistance to antimicrobial agents among bacterial population of houbara bustard and their environment on NWRC

					Beta-lactams							Aminoglycosides	
Bacterial species	AMP	CEF	CFR	FOX	CAZ	ATM	CXM	CTX	PRL	IPM	AMC	AMK	GEN
Escherichia coli (66)	21	11	4	3	3	2	3	2	2	1	8	1	1
Enterococci spp. (19)	0	9	0	4	3	3	0	0	0	1	0	0	0
Lactobacillus spp. (9)	0	0	0	0	0	0	0	2	0	0	0	0	0
Salmonella spp. (6)	2	1	0	0	0	0	0	0	0	0	0	1	0
Shigella flexnari (5)	2	2	1	1	0	0	0	0	0	0	1	0	0
Shigella sonni (1)	1	0	0	0	0	0	0	0	0	0	0	0	0
Proteus mirabilis (3)	0	0	0	1	0	0	0	1	0	0	0	0	0
Enterobacter cloacae (2)	2	0	1	1	0	2	1	1	0	0	0	0	0
Citrobacter freudii (2)	2	0	1	1	0	1	1	1	0	0	2	0	0
Citrobacter youngae (1)	1	0	1	1	1	0	1	0	0	0	1	0	0
Klebsiella oxytoca (2)	2	0	1	0	1	2	1	0	0	0	0	0	0
Klebsiella pneumoniae (1)	1	0	1	1	1	1	1	1	0	1	1	0	1
Total (n=118)	34 (28.8)	23 (19.5)	10 (8.5)	13 (11.0)	9 (7.6)	11 (9.3)	8 (6.8)	8 (6.8)	2 (1.7)	3 (2.5)	13 (11)	2 (1.7)	2 (1.7)

Table 3: Continue

Bacterial species (No)	Fluoroquinolones CIP	Sulphonamides SXT	Tetracyclines TET	Phenicoles CHL	Macrolides ERY
Escherichia coli (66)	2	7	9	1	10
Enterococci spp. (19)	2	NT*	5	0	0
Lactobacillus spp. (9)	0	0	3	1	0
Salmonella spp. (6)	1	0	2	1	2
Shigella flexnari (5)	0	0	1	0	1
Shigella sonni (1)	0	0	0	0	0
Proteus mirabilis (3)	1	1	0	0	0
Enterobacter cloacae (2)	1	0	1	0	0
Citrobacter freudii (2)	1	1	0	1	0
Citrobacter youngae (1)	0	0	0	0	0
Klebsiella oxytoca (2)	0	0	1	0	0
Klebsiella pneumoniae (1)	1	0	0	1	0
Total (n=118)	9 (7.6)	9 (7.6)	22 (18.6)	5 (4.2)	13 (11.0)

AMC: Amoxycilline-Glavulinic acid; AMK: Amikacin; AMP: Ampicillin; ATM: Aztreonam; CAZ: Ceftazidime; CEF: Cephalocine; CFR: Cefadroxile; CHL: Chloramphenicol; CIP: Ciprofloxacin; CXM: ceforuxime; CTX: Cefotaxime; ERY: Erythromycin; FOX: Cefoxtin; GEN: Gentamicin; IPM: Imipenem; PRL: Piperacillin; SXT: Sulfamethoxyzole-Trimethoprim; TET: Tetracycline.

*NT: Not tested.

Table 4: Resistance phenotypes and prevalence of integrons in bacterial isolates recovered from houbara bustard and their environment

Isolate code	Bacteria	MDR phenotypes pattern	Integrons
EC-27	*Escherichia coli*	SXT, CEF, CIP, TET	Class 2
CF-1	*Citrobacter freundii*	AMC, AMP, CHL, CTX, FOX	-
KP-1	*Klebsiella pneumoniae*	AMC, AMP, ATM, CAZ, CHL, CIP, CFR, CXM, GEN, IPM	-
SF-18	*Shigella flexnari*	AMP, CEF, CFR, CHL, CTX, CXM, FOX	Class 1
EK-1	*Enterobacter cloacae*	AMP, ATM, CFR, CIP, TET	-
EK-6	*Enterobacter cloacae*	AMP, ATM, CEF, CTX, CXM, FOX, SXT	Class 1
CF-2	*Citrobacter freundii*	AMC, AMP, ATM, CFR, CIP, CXM, SXT	-
SA-30	*Salmonella* spp.	AMP, AMK, CEF, DC, ERY, PB, TET	-
SA-113	*Salmonella* spp.	AMP, CHL, CIP, DC, ERY, PB, TET	Class 1
EC-48	*Escherichia coli*	AMC, AMP, AMK, ATM, CAZ, CEF, CIP, GEN, FOX, IPM, TET	Class 1
EC-37	*Escherichia coli*	AMP, ATM, CAZ, CEF, FOX, IPM, PRL, SXT	Class 1
PM-30	*Proteus mirabilis*	FOX, CIP, CTX, SXT	Class 1
KO-31	*Klebsiella oxytoca*	AMP, ATM, CAZ, TET	-
EN-118	*Enterococcus* spp.	ATM, CAZ, CEF, CIP, FOX, TET, VAN	Class 2
SF-44	*Shigella flexnari*	AMC, AMP, CEF, ERY, TET	-
EC-1	*Escherichia coli*	AMC, AMP, CDZ, CTX, CXM, ERY, SXT, TET	Class 1
EC-43	*Escherichia coli*	AMC, AMP, CEF, PRL, SXT, TET	Class 1
EC-36	*Escherichia coli*	AMC, ATM, CAZ, CHL, CIP, FOX, IPM, SXT	Class 1

AMC: Amoxycilline-Glavulinic acid; AMK: Amikacin; AMP: Ampicillin; ATM: Aztreonam; CAZ: Ceftazidime; CEF: Cephalocine; CFR: Cefadroxile; CHL: Chloramphenicol; CIP: Ciprofloxacin; CXM: ceforuxime; CTX: Cefotaxime; ERY: Erythromycin; FOX: Cefoxtin; GEN: Gentamicin; IPM: Imipenem; PRL: Piperacillin; SXT: Sulfamethoxyzole-Trimethoprim; TET: Tetracycline; DC: Doxycycline; PB: Polymyxin B.; VAN: Vancomycin.

In addition, Enterococcus spp. with one isolate was vancomycine-resistant Enterococcus (VRE) and two exhibited intermediate resistance. Though Salmonella spp. was sensitive to most of antimicrobials, all isolates exhibited resistance to polymyxin B and doxycycline and only one Salmonella spp. isolate was multidrug resistant. Multidrug resistance was expressed by 15.2% of the isolates (Table 4). The results of double disk diffusion synergy test (DDST) revealed that the eleven (9.3%) Gram-negative isolates exhibited marked degree of resistance to more than three beta-lactam drugs tested and four (3.4%) isolates were found to beta-lactamases producing organisms.

PCR screening detected class 1 integrons in nine bacterial isolates, five *E. coli* isolates, one isolates of *Shigella flexinari*, Salmonella sp., *E. cloacae* and *P. mirabilis* (Table 4). Class 2 integrons were detected in two isolates, including one isolates of *E. coli* and Enterococcus spp. (Table 4).

DISCUSSION

The results of the present study indicate presence of multidrug resistant bacterial species associated with captive houbara bustards in Saudi Arabia. Furthermore, many of the bacterial species are documented potentially pathogenic at least for human. Bailey *et al*. [19] found that the majority of gram-negative bacteria cultured from bustards maintained in captivity in the United Arab Emirates were *E. coli* and Enterococcus of 80% of the samples collected [5]. Other bacteria isolated from the gut include Lactobacillus, Citrobacter, Enterobacter, Proteus and Klebsiella. Proteus spp., Enterobacter spp., *E. coli*, Klebsiella spp. and Enterococcus spp., which are considered as a part of the normal intestinal bacterial flora of captive bustards and they were also isolated from the food items used to feed the captive bustards [5]. Other studies indicate that omnivorous diets may predispose animals increase prevalence of certain bacteria, including Enterococcus spp. [20, 21].

Concerning the data, from the gut, the wide range of bacteria encountered suggests that houbara bustards harbor many organisms, some of which can be isolated from cases of bacterial disease. The present study showed that *E. coli* and Enterococcus spp. were the most common organism of houbara bustard faeces. Houbara bustards can also carry Salmonella and Shigella in their digestive tract. This poses a potential hazard to man and animals. Salmonella is environmentally persistent pathogens and individually infected birds may harbor it commensally in their intestinal tract and thus act as a reservoir for this organism, shedding it into environment for weeks or months [3]. Similar finding reported isolation of *Salmonella* in the gut samples from captive houbara bustards [22].

From the surface of the shell of fresh houbara eggs only *E. coli* was isolated. Egg's antimicrobial defenses against horizontal contamination consist of physical defense through the egg shell and chemical defense through an alkaline pH of the albumen and the presence of ovotransferrin which reduces the availability of iron ions that micro-organisms need for their growth [23]. Certain avian pathogens like Salmonella spp. can be also transmitted vertically into the egg. However, in the present study none of the egg content samples allowed any bacterial development.

Bacteria from wild birds have received less attention with regard to resistance relative those from human and domestic animals [21]. Despite enrofloxacine (a fluoroquinolone which is close to ciprofloxacine) was the most antibiotic used for treating clinical infection in the NWRC houbara flock, resistance against ciprofloxacine was low. The most common resistance phenotypes were against: ampicillin, tetracycline, beta-lactam drugs and erythromycin. However, low or even rare degree of resistance was found to sulphonamides, gentamicin, imipenem and piperacillin. Similar resistance phenotypes have been previously recorded for strains of Gram-negative bacteria isolated from wild animals in Portugal, free-living Canada geese in Georgia and north Carolina (USA), black-headed gulls in the Czech Republic, Zoo animals and pet birds in Japan [24, 25, 26] as well as captive houbara bustards in United Arab Emirates [19]. Furthermore, Livermore *et al*. [15] and Nakamura *et al*. [27] examined *E. coli* isolates from bird species, also found resistance to tetracycline more often than other resistances. The previous author reported resistances to ampicillin, extended spectrum beta-lactam drugs, erythromycin and sulphonamides which is in agreement with our results. In addition, Salmonella showed high resistance rate to polypeptide-related antibiotic (Polymyxin B) and the tetracycline drug doxycycine. In contrast, Dobbin *et al*. [3] found that none of Salmonella isolates were resistant to polymyxin B. The doxycycline has been widely used to control *Chlamydia Psittacae* presence in NWRC houbara bustards 20 years ago.

Integrons play a major role in the spread of antibiotic resistance genes in bacteria [18]. PCR screening detected class I integrons in nine bacterial isolates and class 2 integrons in two bacterial isolates.

The Results in Our Study Are Surprising: NWRC Houbara bustards were in little exposure to antibiotics since they exposed for years to the microflora brought by the human staff in charge of the live food production and of the houbara flock as well as to the microflora brought by the well water. The presence of antibiotic resistant bacteria has recently been reported also in humans and in wild animals living in remote areas where antibiotic exposure has been absent or minimal. In a recent publication, resistance among enteric bacteria from bank voles and wood mice, which presumably had no direct contact with antibiotics, humans, or antibiotic exposed animals, were shown to be highly prevalent [10]. Similar investigation carried out early [27], who found resistant *E. coli* in several Japanese avian species that have little or no human contact. Meanwhile, low rate of resistance was found in investigation carried out on captive houbara bustard [22]. In contrast, Osterblad *et al*.

[28] found very little resistance among faecal bacteria from moose, deer and voles in remote parts of Finland. In addition, Sherley *et al*. [29] found that resistance was rare although widely scattered among Enterobacteriaceae from Australian mammals.

Special interest is that Gram-negative bacteria from different sources with high level of resistance to most prescribed antimicrobials in Saudi Arabia had been reported in many several analyses [30-34]. The extended-spectrum β-lactamases (ESBL) with growing epidemiologic importance have been reported in Enterobacteriaceae from wild animals (birds of prey, foxes) [25]. In the last study, eleven (9.3%) bacterial strains exhibited resistance phenotypes of two or more beta-lactam drugs, four (3.4%) of them were beta-lactamases producing organisms. In addition, one isolate with vancomycin-resistant enterococci (VRE) was reported.

Recently, data from Arabian Gulf region showed high occurrence of ESBL-producing isolates, with rates as high as 31.7% in Kuwait, 41% in United Arab Emirates and 55% in Saudi Arabia [32, 35, 36] and gradual increase resistance to vancomycin [37, 38].

There are a number of factors that have been associated with increased development of antimicrobial resistance as well as potential pathogens including genetic changes among bacterial strains, changes in host populations; population health and ecology as well as captivity of free-living wild animals that can also represent a source of severe stress for them [3, 39]. Previous investigations have identified specific genes that confer innate resistance (intrinsic factor) against aminoglycoside, cephalosporin and other antimicrobials [40].

COCLUSION

The finding of a high prevalence of antimicrobial resistant bacteria in captive houbara bustard with little exposure to the antibiotics might be attributed to a primitive selection of resistance, introduction of resistant strains from the human staff, well water, feeds given. Furthermore, movementof the other captive mammals and wild rodents between different cages at NRWC, followed by the local dissemination of antibiotic resistance determinants (plasmid, integrons and transposons), resistance gene flow and maintenance of resistance. This unexpected finding raises a question on the mechanisms responsible for spreading and maintenance of antibiotic resistance in houbara bustard captive colony.

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