

## Genetic Traits of Some Gastrointestinal Bacteria Isolated from Dorcas Gazelles Collection at Giza Zoo

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### Abstract

The Dorcas gazelles (*Gazella dorcas*) are strict herbivorous which play an important role in the ecological balance. They breed in zoos and susceptible to various bacterial affections such as *Escherichia coli*, *Salmonella* and *Klebsiella*, causing them to be a source of diseases for other animals and human. The present study aimed to investigate the gastrointestinal bacteria in the population of Dorcas gazelles at Giza Zoo during different seasons of the year and identifying their antibiotic sensitivity, virulence, and resistance traits with PCR. A total of 70 fecal swabs were collected and examined. The yielded *E. coli* was the most dominant bacteria followed by *Klebsiella* then *Salmonella* and others. The isolated bacteria showed high multiple resistance with various degrees to erythromycin, tetracycline, ceftazidime, clindamycin and cefaclor. The *fimH* and *invA* virulence genes were detected in the isolated *E. coli* and *Salmonella* isolates respectively. Moreover, the molecular examination of antibiotic resistant genes was confirmed the presence of *bla<sub>CTX-M</sub>* and *tetA* in all isolates of *E. coli* and *Salmonella*. To our knowledge, this is the first study which reports the fecal shedding of these zoonotic bacteria from Dorcas gazelles in Egypt. Further studies are required to evaluate the other bacterial burden infecting this vulnerable species.

**Keywords:** Dorcas gazelles- *E. Coli*-*Salmonella*-*Klebsiella*- Antibiotic-PCR

### Introduction

Dorcas gazelle (*Gazella dorcas*) is a small, thin antelope native to North Africa's deserts, dry, and semi-arid climates (Abáigar et al., 2018). They used to have the largest range

of any African gazelle, but it is now extinct in several of its former ranges (Frost, 2014). They are subjected to be threatened by poaching, habitats deterioration, hunting, and habitat loss.

Conservation efforts which save this group are not just facing the issue of maintaining rest populations but also natural populations which have estimated from wildlife (*Lerp et al., 2011*). According to IUCN RED LIST OF THREATENED SPECIES they are considered as vulnerable (*IUCN, 2017*).

Although, there is a little data on Dorcas gazelles, they are significant to the ecosystems in which they live. These gazelles, as browsers, help to prevent plants from being overgrown. They are also a source of food for carnivores. Between both the Red Sea and Israel, Dorcas gazelle and other ungulates are the primary method of seed dispersal for several Acacia genus species (*Halevy, 1974*). Non-domestic bovids are susceptible to almost all the diseases that affect domestic ruminants. Because of multidrug-resistant diseases, bacterial diseases are becoming more common in zoological species (*Miller and Fowler, 2014*).

This work aimed to isolate the gastrointestinal bacteria from Dorcas gazelles in Giza Zoo, study the effect of different seasons of the year on bacterial isolation. In addition to identifying their antibiotic sensitivity, some virulence and antibiotic resistance genes with PCR.

#### **Materials and methods**

##### **Ethical approval:**

All the procedures of the study were adapted according to the ethical and

humane principles of the Ethics and Animal Experimentation Committee of Suez Canal University (approval No 201847). All the laboratory work was conducted according to isolation, biosafety, and quality standards of AHRI, ARC, Dokki, Giza, Egypt.

##### **Sampling:**

A total of 70 fresh fecal samples were collected aseptically from 17 apparently healthy Dorcas gazelles by using sterile cotton swabs then, immersed in test tubes contained 10ml peptone water. Each sample was labeled and transferred in ice box to the bacteriological laboratory under complete aseptic conditions. The number of samples which collected in summer, spring and winter were (25, 13 and 32) respectively.

##### **Bacterial isolation and identification:**

The tubes of peptone water were incubated at 37° C for 18 hours before being plated onto Rappaport Vassiliadis broth (**Himedia**) and incubated at 41.5 ±1 °C for 24±3 hours for *Salmonella* enrichment. Then, a loopful was streaked onto Xylose Lysine Deoxycholate media (**Himedia**), Hektoen enteric agar (**LabM**) and *Salmonella-Shigella* media (**Himedia**) plates and incubated at 37° C for 18-24 hours. Purification was done for studying the cultural characters according to *Gelaw, et al. (2018)*. Biochemical identification of bacterial isolates was carried out according to *Bullock and Aslanzadeh (2013)*.

The isolation of *E. coli* was done according to **Mac Faddin (2000)**. However, the serological identification of the isolated *E. coli* was done according to the manual of the Reference Lab for Veterinary Quality Control on Poultry Production, Animal Health Research Institute (AHRI), Dokki, Giza, Egypt (**Ewing 1986**). Furthermore, all the recovered identified bacterial isolates were preserved in tryptone broth at 1% after adding glycerol. Then these bacterial strains were kept at  $-20^{\circ}\text{C}$  for further PCR analysis.

#### **Antimicrobial sensitivity testing:**

In this study, the sensitivity of the pure recovered isolates was tested against 10 different antimicrobial agents including amoxicillin-clavulanic acid, cephradine, gentamycin, ceftazidime, clindamycin, tetracycline, cefotaxime, levofloxacin, norfloxacin, and ampicillin+sulb) according to the Standard Kirby-Bauer disc diffusion method and then the results were interpreted according to **CLSI (2020)**.

#### **PCR analysis of virulence and antibiotic resistance genes of isolated bacteria:**

The DNA of bacterial isolates was extracted following the manufacturer's instructions for

QIAamp DNA Mini Kit (**Qiagen, Germany**). Oligonucleotide primers (**Metabion, Germany**) were listed in **table (1)**, and utilized in a  $25\mu\text{l}$  reaction volume with;  $12.5\mu\text{l}$  of Master Mix (**EmeraldAmp Max PCR Takara, Japan**),  $1\mu\text{l}$  of each primer of (20 pmol),  $5.5\mu\text{l}$  of Dnase free water, and  $5\mu\text{l}$  of DNA template were added. The reaction was performed in an Applied biosystem 2720 thermal cycler. A negative and Positive control of reference strains were included in all reactions, provided from AHRI, Dokki, Giza, Egypt.

Finally, PCR products were separated for the analysis step using gel electrophoresis. The gel was prepared using 1.5% agarose gel (**Appllichem, Germany, GmbH**) stained with ethidium bromide, in 1x TBE buffer at room temperature using gradients of 5V/cm. Then, for gel analysis,  $20\mu\text{l}$  of the products were loaded in each gel slot and a 100 bp DNA ladder (**Fermentas, Germany**) also was used to determine the DNA fragment sizes. After that, the gel was photographed by a gel documentation system (**Alpha Innotech, Biometra**) and the data was analyzed through computer software.

**Table (1):** Oligonucleotide primers sequences of target virulence and antibiotic resistance genes of *E. coli* and *Salmonella*.

Gene	Primer Sequence (5'-3')	Amplified product	Annealing temp.	Reference
<i>fimH</i>	TGCAGAACGGATAAGCCGTGG	508 bp	50°C	(Ghanbarpour and Salehi, 2010)
	GCAGTCACCTGCCCTCCGTA			
<i>invA</i>	GTGAAATTATCGCCACGTTCCGGCAA	284 bp	55°C	(Oliveira et al., 2003)
	TCATCGCACCGTCAAAGGAACC			
<i>bla<sub>CTX-M</sub></i>	ATGTGCAGYACCAAGTAARGTKATGGC	593 bp	54°C	(Archambault et al., 2006)
	TGGGTRAARTARGTSACCAGAAAYCAGCGG			
<i>tetA</i>	GGTTCACCTCGAACGACGTC	576 bp	50°C	(Randall et al., 2004)
	CTGTCCGACAAGTTGCATGA			

## Results

The bacteriological examination of fecal samples of Dorcas gazelles revealed that, the total bacterial isolation percentage was 65/70 (92%). The highest isolated bacteria were *E. coli*, *Klebsiella*, *Pseudomonas aeruginosa*, *Salmonella*, *Pseudomonas fluorescens* and finally *Achromobacter xylosoxidans*, and their prevalence rate were 38.5%, 22%, 9%, 7.7%, 6.2% and 3.1% respectively. Some other bacteria were recorded for the first time in Dorcas gazelles such as *Shigella*, *Mannheimia hemolytica*, *Vibrio metschnikovi*, *Pseudomonas putida*, *Moraxella atlanta*, *Sphingomonas paucimobilis*, *Borkholderia pseudomallei*, *Ewingella americana*, *Sphingobacterium spiritivorum* (1.5% for each) as showed in **table (2)**.

Regarding the bacterial isolation in different seasons, 50.8% of the yielded bacteria was isolated in winter. Moreover, 49.2% was isolated in summer. *E. coli* was the most common species isolated in winter (39%) while, *Klebsiella* was

the most common in summer (43.8%). Five *Salmonella* and one *Shigella* isolates were reported in summer only (15.6% and 3.1%). Additionally, various bacteria were recorded in winter only; *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Achromobacter xylosoxidans* (18.1%, 12%, 6.1%) respectively. *Mannheimia hemolytica*, *Vibrio metschnikovi*, *Pseudomonas putida*, *Moraxella atlanta*, *Sphingomonas paucimobilis*, *Borkholderia pseudomallei*, *Ewingella americana*, *Sphingobacterium spiritivorum* (3.1% for each).

Concerning the antimicrobial sensitivity test, the recovered bacteria showed multiple drug resistance with various degrees. *E. coli* was highly resistance to erythromycin and tetracycline (100%), cephradine and ceftazidime (92%), and clindamycin (84%). At the same time, *E. coli* was highly sensitive to norfloxacin (92%), azithromycin (68%) and ampicillin + sulbactam (60%).

*Salmonella* was 100% resistance to erythromycin, clindamycin,

cephradin, tetracycline, and cefaclor (CEC). Meanwhile, it was completely sensitive to norfloxacin (100%), gentamycin (80%) and ampicillin + sulbactam (80%). Moreover, *klebsiella* was (100%) resistant to erythromycin and tetracycline, and sensitive to imipenem (71.5%). Also, *Pseudomonas aeruginosa* showed moderate resistance to azithromycin (66%) and high sensitivity to tobramycin, gentamicin, levofloxacin, colistin and ciprofloxacin (100%). While *Pseudomonas fluorescens* was completely resistance to cefotrixon and cefotaxime. But it was sensitive to ciprofloxacin, colistin, levofloxacin, gentamicin, sulfa/trimethoprim and doxycycline 100%.

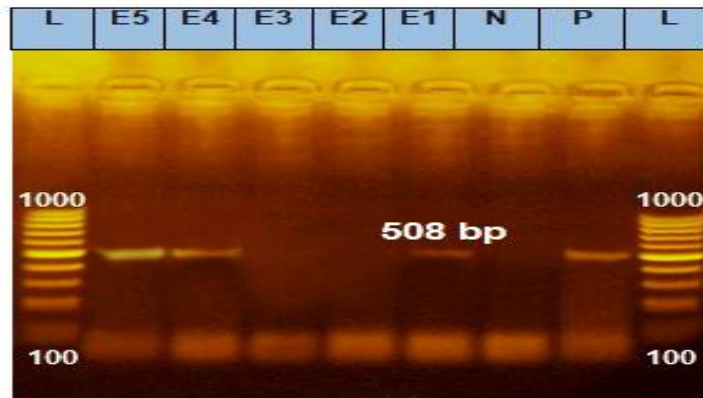
There was a broad resistance (100%) to all tested antibiotics concerning the isolated *Achromobacter xylosoxidans*. In contrast, *Moraxella atlantae* was sensitive to all of them. *Sphingomonas*

*paucimobilis* and *Sphingobacterium spiritivorum* were resistant only to one antibiotic (Aztreoname) and were sensitive to the others. *Ewungella americana* was sensitive to all studied antibiotics, while *Mannheimia hemolytica* showed moderate sensitivity to them. The other recovered bacteria including *Pseudomonas putida*, *Vibrio metschnikovi* and *Shigella* reveal various reactions from resistant, moderate and sensitive.

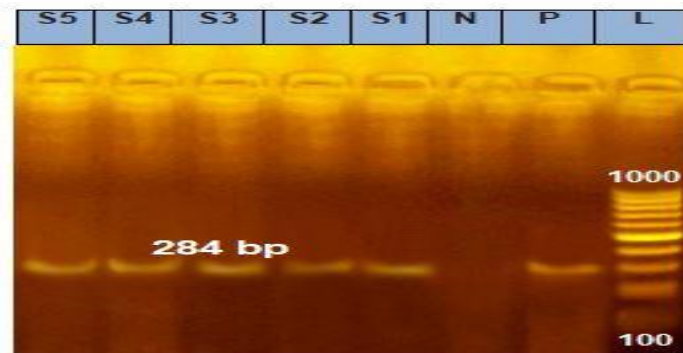
To the point of virulence genes screening, the results reported that, *fimH* gene was noticed in 3 *E. coli* isolates (60%) **photo (1)**. While *invA* gene was detected in all *Salmonella* isolates (100%) **photo (2)**. In the same way, the molecular examination of antibiotic resistant genes indicated that all tested isolates of *E. coli* and *salmonella* were positive for *bla<sub>CTX-M</sub>* and *tetA* **photo (3 & 4)**.

**Table (2):** Total isolated bacteria from Dorcas gazelles and their prevalence in winter and summer seasons.

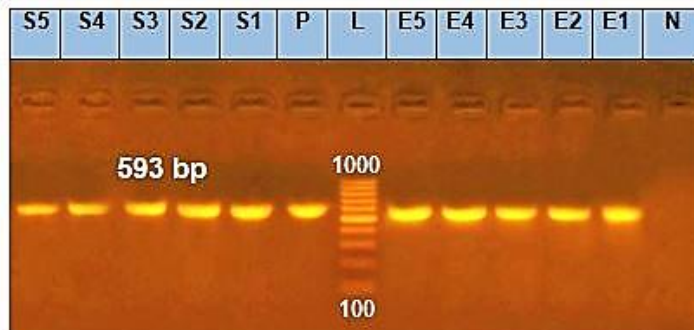
Total isolated bacteria	Winter		Summer		Total No.	%
	No.	%	No.	%		
<i>E. Coli</i>	13	39	12	37.5	25	38.5
<i>Klebsiella</i>	-	-	14	43.8	14	22
<i>Pseudomonas aeruginosa</i>	6	18.1	-	-	6	9
<i>Salmonella</i>	-	-	5	15.6	5	7.7
<i>Pseudomonas fluorescens</i>	4	12	-	-	4	6.2
<i>Achromobacter xylosoxidans</i>	2	6.1	-	-	2	3.1
<i>Shigella</i>	-	-	1	3.1	1	1.5
<i>Mannheimia hemolytica</i>	1	3.1	-	-	1	1.5
<i>Vibrio metschnikovi</i>	1	3.1	-	-	1	1.5
<i>Pseudomonas putida</i>	1	3.1	-	-	1	1.5
<i>Moraxella atlanta</i>	1	3.1	-	-	1	1.5
<i>Sphingomonas paucimobilis</i>	1	3.1	-	-	1	1.5
<i>Borkholderia pseudomallei</i>	1	3.1	-	-	1	1.5
<i>Ewungella americana</i>	1	3.1	-	-	1	1.5
<i>Sphingobacterium spiritivorum</i>	1	3.1	-	-	1	1.5
<b>Total</b>	33	50.8	32	49.2	65	100



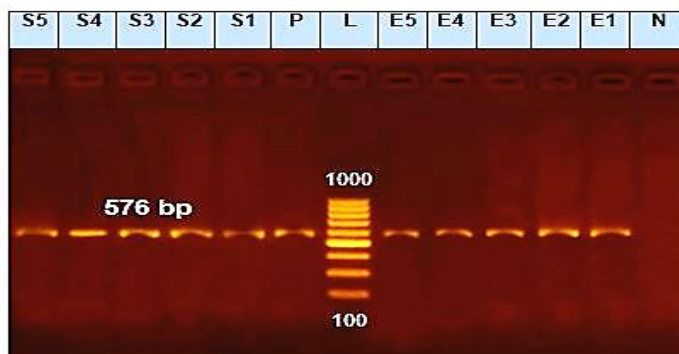
**Photo (1)** Agarose gel electrophoresis of *fimH* gene of recovered *E. coli* (**L**) lane: 100 bp DNA ladder (**P**) lane: positive control (**N**) lane: negative control, (**1,4,5**) were positive for *fimH* gene at 508bp while (**2,3**) were negative.



**Photo (2)** Agarose gel electrophoresis of *invA* gene of isolated *Salmonella* (**L**) lane: 100 bp DNA ladder (**P**) lane: positive control (**N**) lane: negative control. All 5 isolates of *Salmonella* were positive of *invA* gene at 284bp.



**Photo (3)** Agarose gel electrophoresis of *bla<sub>CTX-M</sub>* gene of *E. coli* and *Salmonella* (**L**) lane: 100 bp DNA ladder (**P**) lane: positive control (**N**) lane: negative control, all samples of *Salmonella* and *E. coli* were positive for *bla<sub>CTX-M</sub>* gene at 593bp.



**Photo (4)** Agarose gel electrophoresis of *tetA* gene of *E. coli* and *Salmonella* (L) lane: 100 bp DNA ladder (P) lane: positive control (N) lane: negative control, all samples of *E. coli* and *Salmonella* were positive for *tetA* gene at 576bp.

### Discussion

Animals excrete a variety of bacterial pathogen genera, some of which are important zoonotic pathogens (*Brittingham et al., 1988*), including *Salmonella* spp., *Klebsiella* spp. and *Escherichia coli*. In the current study *E. coli* was the highly prevalent and wide spread bacteria among Dorcas gazelles in Giza Zoo with rate of (38.5%), that disagree with *Mahmoud (2015)* who recorded a high prevalence rate of *E. coli* (63%) in Axis deer.

The importance of *E. coli* infection and its spread in animal habitats is largely based on the organism's ability to persist in soil, water, manure, and feed where other animals nearby the infected species can pick up the microbe (*Hancock et al., 1998*). *Soares et al. (2021)* detected the cause of death in captive sand gazelles which was due to gastro-intestinal infections as *E. coli* (25.9%), although this

percent is less than our prevalence in Dorcas gazelle. The death may occur due to predisposing factors such as low animal immunity, any stress, and bad management.

The second most prevalent bacteria in Dorcas gazelles in Giza Zoo was *Klebsiella* with a rate of (22%) which is lower than that recorded by *Mahmoud (2015)* who isolated *Klebsiella* from Dorcas gazelle with rate of (42%), These results could be attributed to variety of sample size.

In the present study *Salmonella* was isolated from Dorcas gazelle with percentage of (7.7%) which was higher than *Cummings et al. (2021)* who recorded the prevalence of fecal *Salmonella* shedding (1.4%) in 74 wildlife species. The present *Salmonella* percentage was nearly similar to *Soares et al. (2015)* who documented that (10.2%) of the deaths in the Arabian gazelles was due to gastrointestinal lesions caused by *Salmonella* spp. and

concluded that *Salmonella* disease is generally associated with hot periods during the summer. In addition to, **Koochakzadeh et al. (2015)** who investigated the prevalence of *Salmonella* spp. in wild captive herbivores was about (9.7%). This similarity may be due to the same captive condition.

To the best of our knowledge, this study was reported the first isolation of the following bacteria from Dorcas gazelles in Egypt; *Pseudomonas fluorescens* and *Achromobacter xylosoxidans* (6.2% and 3.1%) respectively. *Shigella* prevalence was about (1.5%), although, **Samu et al. (2021)** failed to isolate *Shigella* from feces of different terrestrial mammals within a zoological collection. In addition to, *Mannheimia hemolytica*, *Vibrio metschnikovi*, *Pseudomonas putida*, *Moraxella atlanta*, *Sphingomonas paucimobilis*, *Borkholderia pseudomallei*, *Ewingella americana* and *Sphingobacterium spiritivorum* with prevalence rate (1% for each). As migratory birds travel through or nest in a variety of habitats, **Páll et al. (2021)** hypothesized that they would carry more *Vibrio* strains than sedentary species, with a higher risk of transmission to their contacts or the environment. He also found *V. metschnikovii* with prevalence rate (16%) in wild birds.

In wild animals, low levels of antibiotic resistance are expected as they are seldom subjected to antibiotics, but increased interaction

of those animals with other animals and human may harbored multidrug resistance bacteria (**Dias et al., 2015**). So, in this study, all the recovered bacteria showed various degrees of antibiotic resistance and sensitivity.

The yielded *E. coli* isolates were resistant to tetracycline, erythromycin, and Cephadrine which is different than **Dias et al. (2015)** who recorded the resistance of *E. coli* to some antibiotic as follows, ampicillin (10%), tetracycline (9%). This may be in respect of several treatments by these antibiotics in Giza Zoo.

At the same time, All the recovered *Salmonellae* were resistant to tetracycline which is nearly similar with **Koochakzadeh et al. (2015)** who concluded that (80%) of *Salmonella* which isolated from wild captive herbivores were resistance to tetracycline.

Antimicrobial sensitivity of *klebsiella* revealed that all isolates show resistance to erythromycin and tetracycline. While doxycycline, azithromycin and gentamycin have a wide inhibition zone.

Because *Pseudomonas aeruginosa* has not been isolated previously from Dorcas gazelle especially from Giza Zoo, that explains this bacterium was sensitive to many antibiotics as gentamycin, levofloxacin, ciprofloxacin and colistin. **Ruiz-Roldán et al. (2020)** screened the antimicrobial susceptibility of *Pseudomonas spp*



which showed high resistance to aztreonam (50%), meropenem (12%) and doripenem (11.3%). Whereas in this work, *Pseudomonas* isolates showed high resistance to cefotrixon, azithromycin and cefotaxime. While they were sensitive to colistin, levofloxacin and gentamicin.

Moreover, *Achromobacter xylosoxidans*, *Moraxella atlantae* and *Sphingomonas paucimobilis* were sensitive to all tested antibiotics. This result assures their first time of isolation from *Gazella dorcas* and their disability to resist any antibiotic yet.

Excessive usage of  $\beta$ -lactams in treatment of wild animals lead to the global spread of broad-spectrum beta-lactamase (BSBL) -producing bacteria, they also found on mobile genetic elements, such as plasmids, transposons and integrons, which often also carry additional resistance genes (*Smet et al., 2010*). Among the isolates phenotypically resistant to tetracycline, 100% of the recovered *E. coli* and *Salmonella* were positive for the *tetA* gene, which was nearly similar to *Tawyabur et al. (2020)* who detected high occurrence of multidrug-resistant (MDR) *E. coli* and *Salmonella* in turkey.

*Ohene Larbi et al. (2021)* detected the presence of extended-spectrum beta-lactamase in 28 isolates of *E. coli* that showed phenotypic resistance to aminopenicillins and cephalosporins, only 2 isolates were positive for the *bla<sub>CTX-M</sub>* ESBL

gene (One isolate from poultry and another from cattle), but in this study the prevalence of *bla<sub>CTX-M</sub>* was (100%) in *E. coli* and *Salmonella*. *Clemente et al. (2015)* also recorded the prevalence of *bla<sub>CTX-M</sub>* in *E. coli* and *Salmonella* isolated from different animal species and food products as (32% and 44.4%) respectively.

The severity of disease caused by genus *Salmonella* depends on virulence genes found in it such as *invA* gene that is important for full virulence of *Salmonella* as it allows *Salmonella* to invade, penetrate and cause infection in host epithelial cells (*Mubita et al., 2020*). All *Salmonella* isolated from Dorcas gazelle during the present study harbored *invA* gene. The presence of the gene in the isolated *Salmonella* suggests that the organisms are virulent and have the potential to invade host epithelia cell. Also, *Salah-Eldein et al. (2022)* detected *invA* gene in three isolates of *Salmonella* which isolated from captive wild felids.

### Conclusion:

This study highlighted the status of the vulnerable apparently healthy Dorcas gazelle inhabiting Giza Zoo as a carrier for many GIT bacteria. Some of the isolated bacteria have a zoonotic nature such as *Salmonella* and *E. coli*, which may infect Dorcas gazelle, other animals, and pose a risk to veterinarians and zookeepers. Other bacteria were isolated for the first time in Egypt.

The recovered bacteria were virulent and multi-drug resistant according to the PCR analysis, which represent a risk factor for the failure of the treatment of this species. Additional research is advised to identify the origin of the zoonotic *E. coli* and *Salmonella* infections in the Dorcas gazelles.

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## الصفات الوراثية لبعض البكتيريا المعوية المعزولة من تجمع غزال دوركاس بحديقة حيوان الجيزة

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### الملخص العربي

يعتبر غزال دوركاس من الحيوانات الأكلة للعشب وله دور مهم في التوازن البيئي. وتعتبر أكثر عرضة للإصابة بالبكتيريا مثل البكتيريا المعوية وميكروب السالمونيلا والكلبيسيلا. وقد أجريت هذه الدراسة على 17 حيوان من الغزال المصري في حديقة الحيوان بالجيزة، حيث كانت الحيوانات لا يظهر عليها أي علامة من علامات المرض أو الاسهالات. تم جمع 70 عينة براز على مدار مواسم مختلفة وإخضاع العينات لطرق العزل القياسية للعزل البكتيري والتعرف الكيميائي والتحليل الجزيئي واختبار حساسية المضادات الحيوية المختلفة للأنواع المعزولة. وأوضحت الدراسة أن ميكروب الإيشريشيا القولونية هو السائد من عائلة انتيروباكترياسي يليه ميكروب الكلبيسيلا ثم السالمونيلا. أظهرت البكتيريا المعزولة مقاومة عالية متعددة بدرجات متفاوتة للإريثروميسين والتتراسيكلين والسيفتازيديم والكلينداميسين والسيفاكلور. وتم الكشف عن جينات المسؤولة عن الضراوة *fimH* و *invA* في عزلات الايكولاي والسالمونيلا على التوالي علاوة على ذلك، أكد الفحص الجزيئي للجينات المقاومة للمضادات الحيوية وجود *tetA* و *blaCTX-M* في جميع عزلات الايكولاي والسالمونيلا. هذه هي الدراسة الأولى التي نتج عنها عزل هذه البكتيريا من براز غزال دوركاس في مصر. وهناك حاجة إلى مزيد من الدراسات لتقييم العبء البكتيري الآخر الذي يصيب هذه الأنواع المعرضة لخطر الانقراض.