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STUDY OF BACTERIAL CARRIAGE IN DARKLING BEETLES (*ALPHITOBIOUS DIAPERINUS*) COLLECTED FROM INDUSTRIAL BROILER FLOCKS IN NORTH-EAST OF TUNISIA

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Abstract

Alphitobius diaperinus is regarded as a highly resilient pest in poultry production systems that use litter-based rearing. It is also known as a potential reservoir of several avian pathogens. The present study aimed to determine darkling beetle carriage of bacterial pathogens in Tunisian poultry houses. A total of 25 industrial broiler flocks naturally infested by darkling beetles and located in the North-East of Tunisia were sampled. A pooled sample of adult beetles (5 g) was collected from five distinct locations within each broiler house. Insects were placed in sterile containers and transported to the laboratory under cooled conditions. All samples were examined for entomological identification. Bacteriological analysis was performed to detect external and internal bacterial carriage, according to the standard protocols. Antimicrobial susceptibility for all *Escherichia coli* isolates was tested by the disk diffusion method on Mueller–Hinton agar using commercial disks. Antimicrobial susceptibility testing was not performed for the remaining bacterial isolates. Entomological identification showed that all sampled beetles belong to *Alphitobius diaperinus* species. A total of 108 isolates were detected from the surface (57 isolates) and the

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interior (51 isolates) of adult insects. Gram-negative (63.89%) (*Escherichia coli*, *Enterobacter* spp., *Klebsiella pneumoniae*, *Proteus vulgaris*, *Citrobacter* spp., *Pseudomonas* spp., *Pseudomonas aeruginosa*) and coagulase-negative staphylococci (36.11%) (Gram-positive) bacteria were isolated, with the global predominance of coagulase-negative staphylococci, followed by *E. coli* (23.15%), *Pseudomonas* spp. (13.89%) and *Pseudomonas aeruginosa* (10.19%). No *Salmonella* spp. was detected. Antibiotic resistance profile showed that all *Escherichia coli* isolates were multi-resistant. High resistance was observed to doxycycline (25/25; 100%), amoxicillin (24/25; 96%), cephalothin (23/25; 92%), streptomycin (22/25; 88%), tetracycline (22/25; 88%), enrofloxacin (22/25; 88%). A high level of resistance was observed to trimethoprim-sulfamethoxazole (13 out of 25 isolates; 52%) and gentamicin (9/25; 36%). However, a low level of resistance was observed to florfenicol (7/25; 28%) and colistin (3/25; 12%). Our results confirm the potential vector role of beetles in the spread of bacteria pathogens and antibiotic resistance.

Key words: *Alphitobius diaperinus*, broiler chickens, *Escherichia coli*, resistance to antibiotics, Tunisia

ISPITIVANJE PRENOŠENJA BAKTERIJA KOD BRAŠNENIH CRVA (*ALPHITOBIOUS DIAPERINUS*) IZ JATA BROJLERA GAJENIH U INDUSTRIJSKIM USLOVIMA SA SEVEROISTOKA TUNISA

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Kratak sadržaj

Alphitobius diaperinus se smatra veoma otpornim štetocinama u žvinarskoj proizvodnji na prostirci. Takođe je poznat kao potencijalni

rezervoar brojnih ptićjih patogena. Cilj ovog rada je da se utvrdi prisustvo bakterijskih patogena kod brašnenih crva u živinarskim objektima u Tunisu. Ukupno 25 industrijskih jata brojlera, prirodno zaraženih brašnenim crvima sa severoistoka Tunisa uzorkovano je za potrebe istraživanja. U svakom objektu za uzgajanje brojlera prikupljeni su objedinjeni uzorci odraslih jedinki brašnenih crva (po 5 g) sa pet različitih mesta. Insekti su stavljani u sterilne bočice i transportovani do laboratorije u uslovima hladnog laca. Svi uzorci su ispitani u cilju entomološke identifikacije. Bakteriološka analiza je sprovedena kako bi se otkrilo eventualno prisustvo spoljašnjih i unutrašnjih bakterija, u skladu sa standardnim protokolima. Antimikrobna osetljivost svih izolata *Escherichia coli* ispitana je metodom difuzije diska na Mueller–Hinton agaru, korišćenjem komercijalnih diskova. Antimikrobna osetljivost nije ispitivana za ostale bakterije. Entomološka identifikacija je pokazala da svi uzorkovani insekti pripadaju vrsti *Alphitobius diaperinus*. Ukupno je izolovano 108 sojeva - 57 sa površine i 51 iz unutrašnjosti odraslih jedinki. Gram-negativne (63,89%) (*Escherichia coli*, *Enterobacter* spp., *Klebsiella pneumoniae*, *Proteus vulgaris*, *Citrobacter* spp., *Pseudomonas* spp., *Pseudomonas aeruginosa*) koagulaza-negativne stafilokoke (36,11%) (Gram-pozitivne), sa ukupnom dominacijom koagulaza-negativnih stafilokoka, zatim *Escherichia coli* (23,15%), *Pseudomonas* spp. (13,89%) i *Pseudomonas aeruginosa* (10,19%). Nije utvrđeno prisustvo *Salmonella* spp. Profil rezistencije na antibiotike pokazao je da su svi izolati *Escherichia coli* višestruko rezistentni. Visoka rezistencija je zabeležena na doksiciklin (25/25; 100%), amoksicilin (24/25; 96%), cefalotin (23/25; 92%), streptomycin (22/25; 88%), tetraciklin (22/25; 88%) i enrofloxacin (22/25; 88%). Jaka rezistencija je zabeležena i na trimetoprim-sulfametoksazol (13/25; 52%) i gentamicin (9/25; 36%). Međutim, niska rezistencija je detektovana za florfenikol (7/25; 28%) i kolistin (3/25; 12%). Rezultati ove studije potvrđuju potencijalnu ulogu brašnastih crva kao vektora u širenju bakterijskih patogena i rezistencije na antibiotike.

Ključne reči: *Alphitobius diaperinus*, brojleri, *Escherichia coli*, rezistentnost na antibiotike, Tunis

INTRODUCTION

The confinement systems used in industrial poultry farming have created an ideal environment for the proliferation of the mealworm (*Alphito-*

bius diaperinus), turning it into a global issue. In intensive chicken farming, these insects cause sanitary and economic problems, affecting the health and performance of the birds (Skewes and Monroe, 1991). *Alphitobius diaperinus* (Panzer) is a beetle from the Phylum Arthropoda, Insecta Class, Coleoptera Order and Tenebrionidae Family (Krinsky, 2002). Lesser mealworms are omnivorous scavengers that feed on manure, spilled chicken feed, cracked eggs, chicken carcasses, house fly maggots, and detritus (Rueda and Axtell, 1997). They are considered major contributors to the transmission of bacteria among the fauna within poultry house environments and have been confirmed as vectors of various disease agents, including pathogenic bacteria (*Escherichia coli*, *Salmonella* spp., *Clostridium* spp., *Staphylococcus* spp. etc) (McAllister et al., 1995, 1996; Goodwin and Waltman, 1996; Watson et al., 2000; Terra et al., 2023). Indeed, both larval and adult forms have been shown to harbor viable pathogens (*Salmonella* spp., *E. coli*, *Staphylococcus* spp.) on their exoskeleton and in their digestive tract (Donoso et al., 2020; Tamburro et al., 2022).

The darkling beetles can carry pathogenic strains of *E. coli* long enough to infect chicks, as adult insects excrete the bacteria in their feces for up to six days, and larvae for up to ten days. Additionally, *E. coli* was detected both on external surface and internal content (internal organs, intestinal content) in adult and larval stages persisting for as up to 12 days (McAllister et al., 1996). The ability of the living or dead insect to carry *Salmonella* Typhimurium and *E. coli* inside is also evident (Skov et al., 2004; Roche et al., 2009; Smith et al., 2021). Additionally, darkling beetles can act as reservoirs, sustaining *Salmonella* spp. within poultry farms both during and between production cycles (Roche et al., 2009; Barua et al., 2023). Moreover, it has been found that the darkling beetles (larvae and adults) can naturally acquire pathogenic bacteria such as *Staphylococcus* spp. (Tamburro et al., 2022). Similarly, it has been confirmed that *Alphitobius diaperinus* can harbor *Pseudomonas* spp. in the digestive tract of both larvae and adult insects (Cuccini et al., 2020).

Notably, the larvae of these insects have received approval for human consumption, further emphasizing their significance. On 4 July 2022, EFSA confirmed the safety of frozen and freeze-dried *Alphitobius diaperinus* larvae (EFSA, 2022).

The objective of this study is to investigate, for the first time in Tunisia, the external and internal bacterial carriage of adult mealworms collected from industrial broiler houses in the northeast of the country, with the aim of highlighting the role of this pest as a potential vector of poultry pathogens in these farms.

MATERIAL AND METHODS

Study site

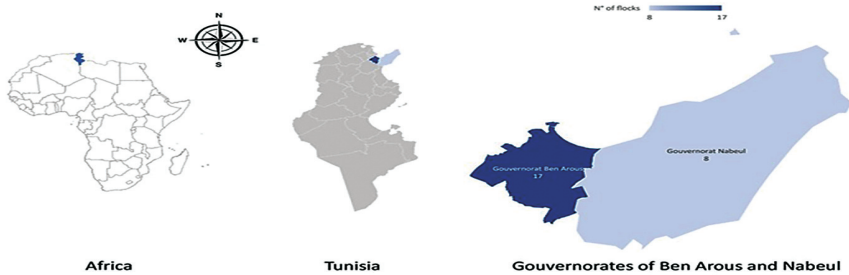


Figure 1. Location of sampled broiler flock

Our study was conducted on 25 poultry flocks located in the region of Nabeul (8 flocks) and Ben Arous (17 flocks) in the North-East of Tunisia (Figure 1), over the period of 6 months, between September 2018 and February 2019.

Nabeul (Coordinates: 36°27'15"N 10°44'5"E) is a coastal town located in northeastern Tunisia, on the south coast of the Cape Bon. It is known for its moderate climate. In January, the temperature reaches 8.4 °C on the minimum average, with the maximum average attaining 15.8 °C. Maximum temperature is 30,6 °C during August. In September, the minimum temperature is 20.9 °C while the maximum is 28.7 °C. Ben Arous (Coordinates: 36°44'50"N 10°20'0"E) is a city in north-eastern Tunisia, part the agglomeration of Tunis, also called "Grand Tunis". It is located south of Tunis city center. The climate is characterized by minimum temperatures recorded in January (7.2 °C) and maximum temperatures reaching up to 32.7 °C in August. In September, the minimum temperature is 19 °C while the maximum is 29.7 °C. These regions were classified as high concentrated poultry production zones (GIPAC, 2010 <http://www.gipac.tn/en/>).

Sample collection

Adult insects were collected from 25 broiler flocks that were naturally infested. In each poultry house, 5 g of adult darkling beetles were collected from

the litter (wood shavings) in five different locations (1 g from each point) then pooled and conditioned in sterile wide mouth bottles and frozen. All the samples were collected using sterile equipment (gloves and anatomical forceps).

Entomological identification

All collected insects were identified entomologically based on morphological criteria described by Schawaller and Grimm (2014) and Nguyen et al. (2019).

Bacteriological analysis

All samples were transported to the laboratory under refrigerated conditions (coolerbox) (+4 °C). Bacteriological analysis was performed immediately. A sample of 1 g of darkling beetles per flock was prepared for bacterial detection. Both Gram-positive and Gram-negative bacteria were isolated from the external surface and internal parts of the insects (organs, digestive tract content) of the darkling beetles. The sampling from surface and interior of adult insects for bacteriological analysis was performed according to experimental design described by Crippen and Sheffield (2006). Five media were used for isolation of bacteria: Mac Conkey agar (Biokar-diagnostics; Beauvais, France) for *E. coli*, mannitol salt agar (Biokar-diagnostics; Beauvais, France) for *Staphylococcus* spp., Cetrimide selective agar (Biokar-diagnostics; Beauvais, France) for *Pseudomonas* spp., and Rappaport-Vassiliadis broth ((Biokar-diagnostics; Beauvais, France) and Xylose Lysine Deoxycholate agar (XLD) (Biokar-diagnostics; Beauvais, France) for *Salmonella* spp. and the classical biochemical gallery for identification (oxidase test, Kligler-Hajna agar, Mannitol-Motility agar, Simmons Citrate agar and urea indole medium) (Biokar-diagnostics; Beauvais, France). In case of identification difficulties, API 20E et API20 NE galleries (BioMérieux, Craponne, France) were used. The antimicrobial susceptibility test was performed using the disk method on Mueller-Hinton agar (Bio-Rad; Marnes-la Coquette, France).

Detection of bacteria from the surface of adult darkling beetles

For external carriage, 1 g of adult insects were sampled, placed in 10 mL of sterile buffered peptone water (BPW) and vortexed for 30 seconds to obtain the wash solution. The darkling beetles were removed with sterile anatomical forceps and placed in sterile bottle. Washing solution was incubated for 24 h

at 37 °C for later bacterial isolation. For the isolation of *Salmonella* spp., 1 mL of washing solution was added to 9 mL of Rappaport-Vassiliadis enrichment broth incubated for 18-24 h at 37 °C. This enrichment broth was then inoculated on XLD agar. MacConkey agar was inoculated directly from the washing solution (0.1 mL) for the isolation of *E. coli*. For the *Staphylococcus* spp. isolation, mannitol salt agar was similarly inoculated (0.1 mL), while cetrimide agar was used for the detection of *Pseudomonas* spp. All these inoculated media were incubated for 18-24 h at 37 °C.

Detection of bacteria from the interior of adult darkling beetles

The same 1 g of adult insects used in the previous analysis was separately washed three times in 15 mL of sterile distilled water, then superficially disinfected by serial treatment with 95% ethanol (Manufacture) and hydrogen peroxide as described by Crippen and Sheffield (2006), after that they had been rinsed three times in 15 mL of sterile distilled water to remove any traces of disinfectants. Each sample was macerated and homogenized with a hand blender in 10 mL of sterile BPW. Dilutions from these main macerating solutions were generated as described previously. Bacterial isolation was then performed using previously described procedures for the external surface of the insects.

Bacterial identification

Bacterial colonies were identified using traditional Gram staining and biochemical techniques. To distinguish between coagulase-positive and coagulase-negative staphylococci, free coagulase detection was performed by mixing three to four staphylococcal colonies with rabbit plasma in a test tube (Bio-Rad; Marnes-la Coquette, France) (0.5 mL) and incubated for 4 h at 37 °C. These isolates are coagulase-positive staphylococci if the liquid solidifies and in case of non-coagulation, the tube is kept at room temperature overnight to avoid negative result.

Antibiotic resistance

Antimicrobial susceptibility was tested by the disk diffusion method on Mueller–Hinton agar using commercial disks for *E. coli* isolates. The plates were incubated for 24 h at 37°C, and inhibition zones were measured and interpreted according to the Clinical and Laboratory Standards Institute guide-

lines (CLSI, 2015). The following antimicrobials were used: amoxicillin 25 µg (AMX), cephalothin 30 µg (KF), gentamicin 10 µg (GEN), streptomycin 10 µg (STR), neomycin 30 µg (NEO), tetracycline 30 µg (TET), doxycycline 30 µg (DOX), florfenicol 30 µg (FFC), colistin 50 µg (CT), trimethoprim-sulfamethoxazole 1.25/23.75 µg (TMP-SMZ) and enrofloxacin 5 µg (ENF).

RESULTS

Entomological identification

Entomological identification was performed on all sampled adult darkling beetles. Morphological key characters of the head, thorax, abdomen and legs were described according to Schawaller and Grimm (2014) and Nguyen et al. (2019) (roughly body 5.5 to 6 mm long and widely oval in shape, black with reddish brown elytra, body surface is dotted with puncture-like impressions, antennae paler at the tips and covered in tiny, yellowish hairs, elytra with shallow longitudinal grooves). The results showed that all examined adult insects collected from broiler houses were compatible with *Alphitobius diaperinus* species.

Bacterial isolation

The present study showed that all sampled darkling beetles (from 25 broiler flocks) carried at least one type of bacteria on the body surface. In addition, 24 samples were positive for at least one type of bacteria detected in interior compartments of adult insects (Table 1). The results of identified pathogens showed the presence of a total of 108 isolates (Gram-positive: 39 isolates; Gram-negative: 69 isolates) detected from the surface and the interior of examined insects. A total of 57 isolates (52.78%) and 51 isolates (47.22%) were detected from the external parts and interior compartments of adult darkling beetles, respectively. The number of isolates per sample varied from one to three bacteria.

Table 1. Prevalence, type and number of bacteria isolated from the surface and the interior of darkling beetles collected from industrial broiler houses (n=25)

Site	Posi- tive	Nega- tive	Gram +	Gram -	1 bac- terium	2 bac- teria	3 bac- teria
Surface	25	0	22	25	3	12	10
Interior	24	1	17	24	4	13	7

The identification of isolated bacteria revealed the presence of eight different species on the external surfaces of the whole samples, while seven distinct bacterial species were identified from the internal parts (organs and digestive tract contents) of the adult insects. Enterobacteriaceae were the most frequently isolated bacteria, accounting for 43 isolates (39.81%) and including *E. coli*, *Klebsiella pneumoniae*, *Citrobacter* spp., *Enterobacter* spp., and *Proteus vulgaris*. This was followed by *Staphylococcus* spp. with 39 isolates (36.11%) and Pseudomonadaceae with 26 isolates (24.08%) (Table 2). Out of Enterobacteriaceae isolates, *E. coli* was the most frequently detected (23.15%), followed by *Enterobacter* spp. (8.33%), *Klebsiella pneumoniae* (3.7%), *Proteus vulgaris* (2.78%) and *Citrobacter* spp. (1.85%). All *Klebsiella pneumoniae* isolates were detected only from surface of analyzed adult insects. Among Gram-negative isolates, *Pseudomonas* spp. was identified in 15 examined samples (13.89%), while *Pseudomonas aeruginosa* was detected in 11 samples (10.19%). *Staphylococcus* spp. was the only Gram-positive isolate in this study (36.11%) and all isolates were coagulase negative.

Type	Family	Species	Surface	Interior	Total	
Gram - (63.89%)	Enterobac- teriaceae (39.81%)	<i>E. coli</i>	10 (9.26%)	15 (13.89%)	25 (23.15%)	
		<i>Klebsiella pneumoniae</i>	4 (3.7%)	0	4 (3.7%)	
		<i>Citrobacter</i> spp.	1 (0.93%)	1 (0.93%)	2 (1.85%)	
		<i>Enterobacter</i> spp.	4 (3.7%)	5 (4.63%)	9 (8.33%)	
		<i>Proteus vulgaris</i>	1 (0.93%)	2 (1.85%)	3 (2.78%)	
	Pseudomona- daceae (24.08%)	<i>Pseudomonas</i> spp.	7 (6.48%)	8 (7.41%)	15 (13.89%)	
		<i>Pseudomonas aeruginosa</i>	8 (7.41%)	3 (2.78%)	11 (10.19%)	
	Gram + (36.11%)	Staphylococ- caceae (36.11%)	Coagulase-nega- tive staphylococci	22 (20.37%)	17 (15.74%)	39 (36.11%)
	Total			57 (52.78%)	51 (47.22%)	108 (100%)

Table 2. Bacteria species isolated from the surface and the interior of darkling beetles collected from industrial broiler houses (n=108)

Antibiotic resistance

The susceptibility of the whole *E. coli* isolates from the surface (n=10) and interior (n=15) was tested for eleven antibiotics (Figure 2). The results indicate that all *E. coli* isolates detected from the surface of the adult insects were susceptible to colistin (10/10; 100%). However, all isolates proved to be resistant to doxycycline (25/25; 100%). Strong resistance to amoxicillin (24/25; 96%), cephalothin (23/25; 92%), streptomycin (22/25; 88%), tetracycline (22/25; 88%), enrofloxacin (22/25; 88%), trimethoprim-sulfamethoxazole (13/25; 52%) and gentamicin (9/25; 36%) was also observed. The *E. coli* isolates showed low percentages of resistance to florfenicol (7/25; 28%) and colistin (3/25; 12%). Moreover, 100% of the isolates proved to be multiresistant, showing resistance to at least three different classes of antibiotics.

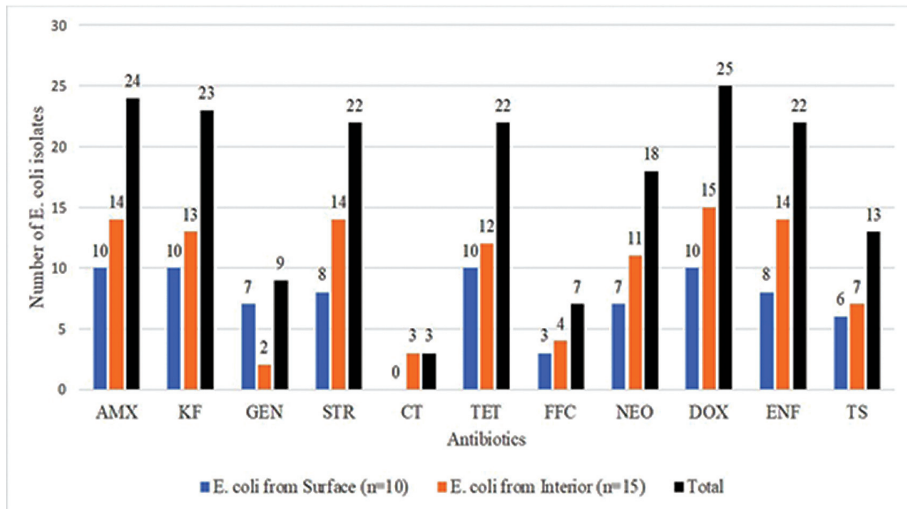


Figure 2. Antimicrobial resistance profiles of the *E. coli* isolates detected from the surface and the interior of the darkling beetles collected from broiler flocks (n=25)

DISCUSSION

Alphitobius diaperinus is a resilient pest commonly found in poultry houses. The field evidence suggests that this arthropod can survive cleaning and disinfection procedures, which represents a risk for its transmission between consecutive flocks (Crippen et al., 2018). Darkling beetles are regarded as either reservoir hosts or mechanical vectors for a variety of poultry pathogens (bacteria, viruses, fungi).

Routine biosecurity measures were implemented in poultry houses in Tunisia. Following insecticide treatment with deltamethrin and cypermethrin, manure and organic matter were removed. Sanitation procedures and disinfection protocols were carried out, and a downtime period of 7 to 14 days was observed. Despite these procedures, darkling beetles have not been eradicated and persist in consecutive flocks. Our findings are in agreement with other studies (Donoso et al., 2020).

All darkling beetles were collected from naturally infested broiler flocks. *Alphitobius diaperinus* was the only species identified in all sampled broiler flocks, which is consistent with findings from other studies conducted in Algeria (Agabou and Alloui, 2010) and Netherlands (Hazeleger et al., 2008). Several beetle species (*Carcinops pumilio*, *Alphitobius diaperinus*, *Gnathoncus*

nanus, etc) have been reported to infest poultry houses, as mentioned in various reports (Pfeiffer and Axtell, 1980). Differences in findings can be explained by the studied geographical zone, the size of samples, the type of production, the type of used litter, the season, etc.

Both adult and larval stages of *Alphitobius diaperinus* serve as particularly effective reservoirs in broiler houses, as they inhabit the litter and manure where they feed on chicken feed, feces, and even carcasses - while also being consumed by the chickens themselves. Consequently, insects can be contaminated and/or spread pathogens. Darkling beetles have been identified as significant vectors for several pathogenic bacteria affecting poultry, including *E. coli*, *Campylobacter*, and *Salmonella* Enteritidis (Donoso et al., 2020; Barua et al., 2023). They can also be reservoirs for some viral diseases like leukosis, Marek's disease, infectious bursal disease, avian influenza, Newcastle disease etc. and also protozoa agents of coccidiosis such as *Eimeria* spp. (Goodwin and Waltman, 1996).

The aim of the surface disinfection after analyzing the external parts of the darkling beetles was to eliminate any surface flora, in order to differentiate bacteria carried externally or internally. The insect integument and presumably the fecal material adhering to the exoskeleton provide refuge to bacterial organisms (Crippen and Sheffield, 2006).

In the present study, 108 isolates were obtained from the surface and the interior of adult darkling beetles. The isolates were identified based on the macroscopic (colony) and microscopic characteristics and biochemical reactions. Microscopic examination of the isolates after Gram staining showed that Gram-negative (69 isolates; 63.89%) were predominant compared to Gram-positive (39 isolates; 36.11%).

Enterobacteriaceae (43 isolates; 39.81%) were the most prevalent isolates in our study, which is in agreement with similar reports (Segabinazi et al., 2005). Among these isolates, *E. coli* was detected in 25 samples (23.15%). In fact, *E. coli* is a ubiquitous and one of the most frequent pathogens in poultry. Several studies, including one by Tayh et al. (2024) conducted in Tunisia, have demonstrated that broiler chickens can act as reservoirs for *E. coli* strains pathogenic to humans, specifically Shiga toxin-producing *E. coli* (STEC), which are capable of causing severe and sometimes fatal hemolytic-uremic syndrome (HUS). Contrary to other reports (Hazeleger et al., 2008; Donoso et al., 2020), we did not isolate *Salmonella* spp. in our study. Lower frequency of other bacteria isolates in sampled beetles may be explained by a competitive exclusion phenomenon, that characterizes the transmission and colonization in poultry and inside the internal organs, in particularly, the gut of *Alphitobius diaperinus*

(Wynants et al., 2018) or by antimicrobial administration. Additionally, litter water activity and pH are considered key factors that promote the growth and multiplication of various important bacteria in poultry farming (Gehring et al., 2020).

Secondly, coagulase-negative staphylococci (36.11%) and *Pseudomonas* spp. (24.08%) were identified on both the surface and interior of adult insects. Beetles carrying bacteria on their exoskeleton or in their guts (Cuccini et al., 2020) can transmit those pathogens, especially to young chicks (McAllister et al., 1996). Furthermore, contaminated darkling beetles can preserve pathogens in their gut for more than five weeks (Crippen et al., 2022).

Pseudomonas aeruginosa (10.19%) is considered as an emerging pathogen in poultry. It is a leading cause of respiratory infection, sinusitis, endocarditis and septicemia (Badr et al., 2020) and it is frequently multiresistant to antibiotics (Atere and Atere, 2024).

Coagulase-negative staphylococci (36.11%) was the only Gram-positive bacterium isolated in the present study, consistent with the findings of Goodwin and Waltman (1996). This pathogen is known to cause osteoarticular infections in poultry (Andreasen, 2020).

In general, antibiotic susceptibility of *E. coli* isolated from darkling beetles showed high to very high resistance to most tested antibiotics (28% to 100%) (doxycycline, amoxicillin, cephalothin, streptomycin, tetracycline, enrofloxacin, trimethoprim-sulfamethoxazole, gentamicin, florfenicol). Several risk factors may contribute to development of antibiotic resistance. The inappropriate use of antimicrobials, whether for treatment or prevention of bacterial infections, is considered the most significant risk factor. Darkling beetles can ingest antibiotics by feeding on contaminated feces and manure (Rawat et al., 2023). Colistin resistance has been detected in three *E. coli* isolates (12%). Commonly used in poultry production in several countries, colistin (polymyxin E) is frequently used in Tunisian poultry sector for the treatment of infection caused by Enterobacteriaceae. Colistin resistance of *E. coli* detected in healthy and diseased chickens is on the rise in Tunisia (Grami et al., 2016; Maamar et al., 2018; Hassen et al., 2020; Di Francesco et al., 2023). Our results confirm the potential vector role of beetles in the spread of colistin resistance in poultry flocks in Tunisia.

Finally, it should be noted that all *E. coli* isolates were multidrug-resistant. Indeed, each bacterium isolate accumulated resistance to several antibiotics of different families, which can lead to therapeutic impasses in poultry and public health. In this way, darkling beetles can carry multidrug-resistant zoonotic bacteria, such as *Salmonella enterica* strains, complicating efforts to control infections in poultry environment and humans (Donoso et al., 2020).

It should be noted that the present study has certain limitations. All samples were collected exclusively from adult insects, although darkling beetle larvae are also recognized as potential vectors of poultry pathogens. In addition, *Campylobacter* spp., a major poultry public health pathogen, was not targeted in our study due to lack of specific media. A serotyping of *E. coli* isolates was not performed because of lack of antisera for the main pathogen serotypes.

CONCLUSION

In conclusion, *Alphitobius diaperinus* was the only darkling beetle species identified in selected industrial broiler houses. A total of 108 bacterial isolates were detected from the surface (57 isolates) and the interior (51 isolates) of adult insects. Gram-negative (Enterobacteriaceae and Pseudomonadaceae) and *Staphylococcus* spp. were isolated, with the predominance of coagulase-negative staphylococci, *E. coli* and *Pseudomonas aeruginosa*. The antibiotic resistance profile of *E. coli* isolates confirms the growing concern of this issue within the Tunisian poultry sector and further supports the potential role of darkling beetles as vectors in the dissemination of bacterial pathogens and antibiotic resistance. Consequently, more drastic measures must be taken in order to rationalize the use of antibiotics in chickens and limit the proliferation of mealworms in farms.

Abbreviations

AMX: amoxicillin; BPW: buffered peptone water; CLSI: Clinical and Laboratory Standards Institute guidelines; CT: colistin; DOX: doxycycline; ENF: enrofloxacin; FFC: florfenicol; GEN: gentamicin; KF: cephalothin; NEO: neomycin; STR: streptomycin; TET: tetracycline; TMP-SMZ: trimethoprim-sulfamethoxazole; XLD: Xylose Lysine Deoxycholate agar.

Author's Contribution

KK: Conceptualization, methodology, results interpretation, writing original draft; MG: sampling, bacteriological analysis, writing; CC: sampling; LM: writing-review, bacteriological analysis supervision and validation. All authors have read and approved the final version of the manuscript for publication.

Competing interest

The authors declare that they have no conflicts of interest.

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