Cross-sectional study to identify risk factors associated with the occurrence of antimicrobial resistance genes in honey bees *Apis mellifera*) in Umbria, Central Italy

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Highlights

- The use of antimicrobials has exerted selective pressure in recent years to determine the onset and spread of antimicrobial resistance genes.
- The use of antimicrobials in humans and animals over time may be associated with the presence of antimicrobial residues in the environment.
- Honey bees (*Apis mellifera*) are considered an excellent biological indicator of the state of pollution of the territory in which they live thanks to their peculiar characteristics as they can act as collectors and disseminators of antimicrobial resistance genes.
- Honey bees form complex, social networks, the health of which is closely linked to the associated microbial communities living in their gut and can be used as an indicator of the prevalence and distribution of antimicrobial resistance genes in the environment.
- In the study area (region of Umbria, Central Italy), *aph*, *blaZ*, *tetM*, *sul1*, and *sul2* genes are frequent in honey bees' microbiome, and their prevalence is associated with presence of farms nearby.

Abstract

The use antimicrobials for therapeutic and metaphylactic purpose in humans and agriculture exerts selective pressure on animal and environmental microbiota resulting in the survival and spread of antimicrobial resistance genes among bacteria and subsequent development of resistance in bacteria. Previous studies have shown that honey bees' microbiota (*Apis mellifera*) can accumulate antimicrobial resistance genes in their microbiome and act as collectors and disseminators of resistance genes. The aim of this study was to investigate to what extent honey bees act as reservoir of select antimicrobial resistance genes. This study was conducted on 35 groups of bees. Bees were collected from 35 sites in Umbria, Italy. PCR was used to screen pooled ground bees' specimens for genes that code for resistance against antimicrobials that are commonly used in humans and in veterinary medicine including aminoglycosides (*aph*), beta-lactams (*blaZ*), tetracycline (*tetM*) and sulphonamides (*sul1* and *sul2*). Twenty-four samples out of 35 (68.57%) were positive for at least one antimicrobial resistance gene. Two samples were positive for the *aph*, 5.71%; eight for *blaZ*, 22.86%; three for tetM, 8.57%; ten for sul1, 28.57% and eighteen for sul2, 51.43%. Positivity to more than one antimicrobial resistance gene was observed in nine samples, 25.71%. The multivariate analysis identified "presence of farms nearby" as the factor most closely related to PCR positivity. Honey bees (*Apis mellifera*) from Umbria, Italy, carry antimicrobial resistance genes and can be used as indicators of the presence of resistance genes in the environment.

Introduction

Human and animal health and the production of food of animal origin are strongly dependent on the effective use of antimicrobials. Increasing antimicrobial resistance in human and animal pathogens poses a serious threat to human health and food production, as the traditionally used antimicrobials are becoming ineffective in the face of rapidly evolving populations of bacteria (Baker 2015). The growing presence of antimicrobial-resistant bacteria is linked to the use of antimicrobial compounds in clinical medicine and agriculture (Taylor et al. 2019). Antimicrobials exert selective pressure on bacterial populations which leads to survival and spread of resistant bacteria in animals and the environment. Furthermore, antimicrobial use in agriculture may pollute the environment with antimicrobial residues that are detrimental to the fauna (Bogdanov et al. 2008). In the veterinary field, the development of antimicrobial resistance has been attributed to the vast use of antimicrobials for metaphylactic purposes or as growth promoters (Cambiotti et al. 2014; Cenci-Goga et al. 2004; Iulietto et al. 2016). In fact, the occurrence of resistant bacteria as a consequence of antimicrobial use, abuse and misuse is probably the best documented case of a contemporary evolution in progress (Hiltunen et al. 2017). Currently antimicrobial resistance is a global public health threat to humans and animal health (WHO *Antimicrobial Resistance Global Report on Surveillance*: *2014* summary 2014). However, little is known about evolutionary consequences of the use of antimicrobials on the environment. Numerous studies have shown that bacteria populations in the wild can serve as environmental reservoirs and indicators of antimicrobial resistance (Bengtsson-Palme et al. 2018). Regarding insects, to date, only a few studies have investigated the occurrence of transferable AR genes in their microbiome (Milanović et al. 2016; Osimani et al. 2017; Roncolini et al. 2019). These studies revealed the massive presence of genes conferring resistance to tetracyclines and other antibiotics. However, the mechanisms on how antimicrobial resistance genes are disseminated and maintained in natural environments and how these genes are transferred between clinical settings and farming environments and from one agricultural environment to another remain unclear (Woolhouse et al. 2015). Furthermore, investigations on the role of farmed insects

such as honey bees as reservoirs and indicators of antimicrobial resistance genes remain scanty.

Honey bees (*Apis mellifera*) form complex, social networks, the health of which is closely linked to microbial communities living in their gut (Babendreier et al. 2007; Cox-Foster et al. 2007). Honey bee microbiota is highly stable and specialized and appears to have low variations in different seasons and geographical regions (Gilliam 1997; Kwong et al. 2017). Gram-negative bacteria are the most common bacterial group in honey bees, followed by Gram-positive, and only 1% of fungi and yeasts (Borsuk et al. 2013). Bacterial species colonizing honey bees are recognized as carriers of antimicrobial resistance genes and good biological indicators of the complex evolutionary processes occurring among environmental bacteria and their hosts that are very difficult to measure by other means. Honey bees, especially foraging bees, can easily intercept and accumulate environmental contaminants as a result of contact with air, water, soil and vegetation (Ceauśi et al. 2009; Giglio et al. 2017; Goretti et al. 2019).

The aim of this cross-sectional study was to identify potential risk factors associated with the occurrence of antimicrobial resistance genes in honey bees in a selection of apiaries. PCR was used to detect the presence of genes coding different antimicrobial compounds belonging to antimicrobial classes that are commonly used in humans and animal agriculture for therapeutic and metaphylactic purposes.

Materials and methods

Samples were taken from apiaries of *Apis mellifera ligustica* (Spinola, 1806) from 35 different sites in Umbria, Italy (Table 1 and Fig. 1). The sample size was calculated using the formula $n = Z^2 * p * (1-p)/C^2$, where Z is the Z-value (e.g. 1.96 for a 95% confidence level), p is the expected prevalence, expressed as a decimal, and C is the absolute precision, expressed as a decimal (Mariano et al. 2009). With approximately 200 registered permanent apiaries in Umbria and with an expected prevalence for PCR-positive samples of 20% (0.2), a precision of 12% and a confidence level of 0.95, a sample size of 35 apiaries was then required. The 35 apiaries were then randomly selected using premise-identification numbers apiary database at the onset of the study (specifically all numbers for given area were printed, cut out and drawn from a “hat”). Sampling was carried out in summer (16 samples) and in autumn (19 samples) in 2014 (22 samples) and 2015 (13 samples), in detection stations where no antibiotics were used neither for prophylaxis nor for therapy. Sampling sites were selected on the basis of the different altitudes (23 apiaries situated in hill areas and 12 in lowlands) and the different degrees of pollution, due to the presence or absence of production sites or agricultural land (6 in industrial areas and 29 in agricultural area) or farms nearby (19 with farms within 1 km radius and 16 without farms in a radius of 5 km) (Table 2). All samples were collected once the consent had been acquired of the beekeepers, who had joined the study anonymously. Honey bee sampling was carried out according to the safety rules and in respect of the colonies, avoiding to open the hives and trying not to disturb the insect activity. The sampling operations focused on specimens of foraging bees, which are the bees responsible for collecting nectar and pollen and, therefore, more easily exposed to environmental contamination. For each apiary, about 100 forager bees were taken from a single central hive as a representative sample of the sampling site. Before being submitted to the analytical procedures, each honey bee specimen was cleaned from the pollen that could be present on the pollen baskets of the hind legs, and eventual parasites, i.e. *Varroa destructor* (Anderson and Trueman 2000), were removed. In the laboratory, they were transferred to Petri dishes

sealed with Parafilm tape (Bemis, Inc., Neenah, WI, USA), marked with an alphanumeric identification code and placed in a freezer at the temperature of − 80 °C. Before moving on to the DNA extraction phase, the samples were ground separately in liquid nitrogen, in order to obtain a homogeneous pulverized pool.

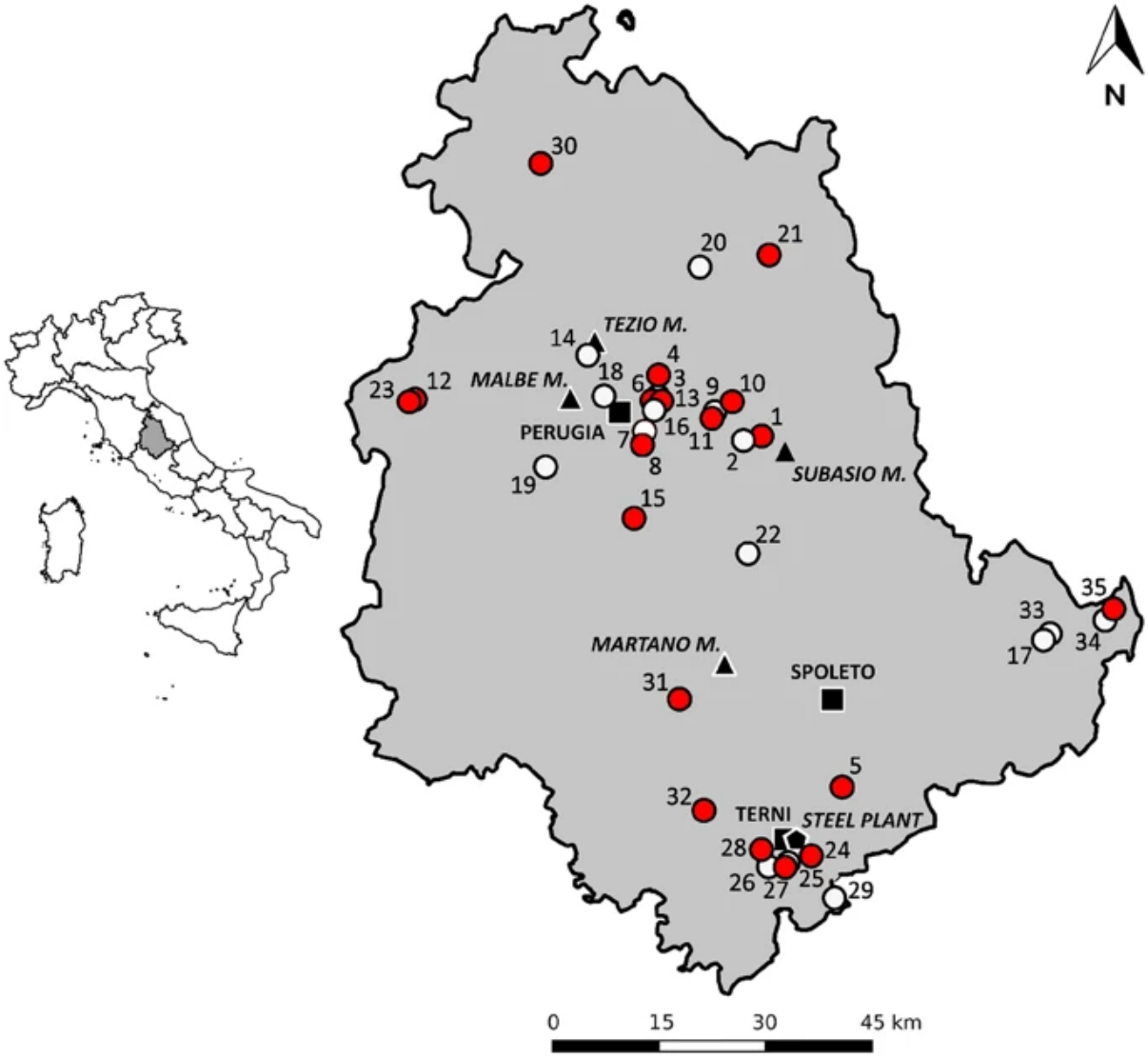

Fig 1. Location of the sampling sites (apiaries) – Umbria Region, Italy. Squares, triangles and pentagons indicate cities, mountains and steel plant, respectively. Red dots are the apiaries located nearby farms

Table 1 Sampling sites

Samples	Place	Coordinates UTM	Sampling date
1	Assisi	33 T 307018 4,771,395	Oct-14
2	Assisi	33 T 304419 4,770,961	Oct-14
3	Montescosso	33 T 292866 4,777,995	Sep-14
4	Villa Pitignano	33 T 293180 4,780,975	Sep-14
5	Terni-Spoleto	33 T 314766 4,721,485	Aug-14
6	Bivio Valvitiano	33 T 291991 4,777,471	Sep-14
7	Balanzano	33 T 290663 4,773,249	Sep-14
8	San Martino in campo	33 T 290205 4,771,367	Sep-14
9	Santa Tecla Assisi	33 T 300703 4,775,280	Oct-14
10	Mora Palazzo di Assisi	33 T 303236 4,776,508	Oct-14
11	Petrignano Assisi	33 T 300224 4,774,381	Oct-14
12	Castiglione del Lago	33 T 258847 4,780,028	Sep-14
13	Ponte Valleceppi	33 T 293354 4,777,306	Oct-14
14	Monte Tezio	33 T 283458 4,784,459	Aug-14
15	Fosso Provancio Deruta	33 T 288311 4,761,180	Sep-14
16	Ponte Valleceppi	33 T 292194 4,776,109	Oct-14
17	Norcia	33 T 344402 4,740,040	Jul-15
18	San Marco (PG)	33 T 285323 4,778,573	Aug-14
19	Poggio delle Corti Magione	33 T 276441 4,769,242	Sep-14
20	Gubbio	33 T 300053 4,795,646	Oct-14
21	Padule Gubbio	33 T 309868 4,796,661	Oct-14
22	Bevagna Alta	33 T 303921 4,755,101	Oct-14
23	Castiglione del Lago	33 T 257991 4,779,748	Sep-14
24	Larviano Terni	33 T 309757 4,712,168	Jul-15
25	Stroncone San Rocco Terni	33 T 306467 4,711,441	Jul-15
26	Collescipoli Terni	33 T 303673 4,711,053	Jul-15
27	Stroncone Santa Lucia Terni	33 T 306007 4,710,830	Jul-15
28	Terni	33 T 302853 4,713,549	Jul-15
29	Prati Stroncone Terni	33 T 312591 4,706,046	Jul-15
30	Città di Castello	33 T 278821 4,811,779	Jul-15
31	Collevalenza Todi	33 T 292858 4,735,409	Jul-15
32	Collepizzuto Terni	33 T 295188 4,719,595	Jul-15
33	Fontevena Norcia	33 T 345394 4,740,859	Jul-15
34	Piana Castelluccio di Norcia	33 T 353201 4,742,203	Jul-15
35	Piana Castelluccio di Norcia	33 T 354527 4,743,705	Jul-15

Table 2 Sampling sites split by risk factors and presence of five antimicrobial resistance genes (*aph*, *blaZ*, *tetM*, *sul1* and *sul2*) in honey bees' samples analysed by nested PCR

	Total count		PCR + ve		aph + ve		blaZ +ve		tetM +ve		sul1 + ve		sul2 + ve	
Environment	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Industrial	6	17,1	3	12,5	0	0,0	2	25,0	0	0,0	2	20,0	1	5,6
Agricultural	29	82,9	21	87,5	2	100,0	6	75,0	3	100,0	8	80,0	17	94,4
Total	35	100,0	24	100,0	2	100,0	8	100,0	3	100,0	10	100,0	18	100,0
Elevation														
Hill	23	65,7	15	62,5	1	50,0	6	75,0	3	100,0	5	50,0	11	61,1
Lowland	12	34,3	9	37,5	1	50,0	2	25,0	0	0,0	5	50,0	7	38,9
Total	35	100,0	24	100,0	2	100,0	8	100,0	3	100,0	10	100,0	18	100,0
Farms nearby														
No	16	45,7	7	29,2	0	0,0	2	25,0	0	0,0	3	30,0	5	27,8
Yes	19	54,3	17	70,8	2	100,0	6	75,0	3	100,0	7	70,0	13	72,2
Total	35	100,0	24	100,0	2	100,0	8	100,0	3	100,0	10	100,0	18	100,0
Season														
Autumn	19	54,3	13	54,2	2	100,0	5	62,5	1	33,3	7	70,0	9	50,0
Summer	16	45,7	11	45,8	0	0,0	3	37,5	2	66,7	3	30,0	9	50,0
Total	35	100,0	24	100,0	2	100,0	8	100,0	3	100,0	10	100,0	18	100,0
Year														
2014	22	62,9	14	58,3	2	100,0	5	62,5	2	66,7	8	80,0	10	55,6
2015	13	37,1	10	41,7	0	0,0	3	37,5	1	33,3	2	20,0	8	44,4
total	35	100,0	24	100,0	2	100,0	8	100,0	3	100,0	10	100,0	18	100,0

The HipurA™ Insect DNA Purification Kit from the HiMedia company (Mumbai, India) was used to extract the DNA. The quantification of the extracted genetic material was performed using the NanoDrop ™ Lite spectrophotometer (Thermo Fisher Scientific, USA) with 1 µl of sample. The DNA amplification was conducted on a volume of 25 µL using 12.5 µL of RED Taq (10 mM Tris HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.001% of gelatin, 0.2 mM each of deoxyribonucleoside triphosphate), 0.5 µL (1 µM) of each primer, 5 µL of extracted DNA and 6.5 µL of H2O. The PCR reaction was carried out in a thermocycler Gene Amp, PCR System, 9700 Gold (Applied Biosystems, Foster City, CA).

The primers and the amplification conditions used are listed in Table 3 (Cenci-Goga et al. 2004; Enne et al. 2001; Kao et al. 2000; Kozak et al. 2009; Martineau et al. 2000; Sköld 2000). The PCR reaction was carried out in a Thermocycler Gene Amp, PCR System, 9700 Gold (Applied Biosystems, Foster City, USA). The amplifications were analysed by electrophoretic run on 1.5% agarose gel containing ethidium bromide (0.5 µg/ml); 10 µl of each PCR sample was loaded with 2 µl of 6× loading buffer (Fermentas-VWR-Italy) and 5 µl of marker PCR as reference DNA (Fermentas-VWR-Italy); the run was carried out at a voltage of 100 V for about 1 h in TBE 10× (Trizma base, boric acid, EDTA 0.5 M pH 8). At the end of the run, the bands were viewed with the UV transilluminator (Fotodine 3–3102 Celbio, Milan, Italy).

In order to improve the sensitivity and the yield of the amplification, a second reaction was carried out using the Nested PCR technique. Starting from the products of the first amplification, a second pair of internal primers was used. The amplifications were analysed by electrophoretic run on 1.5% agarose gel containing ethidium bromide (0.5 µg/mL). 10 µL of each PCR sample was loaded with 2 µL of 6× loading buffer (Fermentas, Thermo Fisher Scientific, Waltham, MA) and 5 µL of marker PCR as reference DNA (Fermentas). The run was carried out at a voltage of 100 V for approximately1 h in TBE 10× (Trizma base, boric acid, EDTA, Euroclone, Siziano, Italy; 0.5 M pH 8). At the end of the run, the bands were viewed with the UV transilluminator (Fotodine 3–3102 Celbio, Milan, Italy).

To identify risk factors associated with PCR positivity, first a univariate analysis of the variables of interest was conducted with binary logistic regression followed by multiple logistic regression performed with StatView 5 for Mac OS (SAS Inst. Inc., Cary, NC, USA).

Results

The results of the analysis are reported in Tables 2 and 4. After the first amplification, none of the samples were positive for *aph*, *blaZ*, and *tetM* genes, one sample (2.86%) was positive for *sul1* gene, and five (14.29%) were positive for *sul2* gene. In contrast, a higher number of positive samples were obtained after nested PCR: two samples out of thirty-five (5.71%) were positive for the *aph* gene, eight out of thirty-five (22.86%) were positive for the *blaZ* gene, three out of thirty-five (8.57%) were positive for the *tetM* gene, ten out of thirty-five (28.57%) were positive for the *sul1* gene, and eighteen out of thirty-five (51.43%) were positive for the *sul2* gene. Twenty-four samples out of thirty-five (68.57%) were positive for the presence of at least one of the antimicrobial resistance genes researched. Nine samples out of thirty-five (25.71%) were positive for the presence of more than one of the antimicrobial resistance genes researched. One (2.86%) of the samples was positive for the presence of all the genes researched.

Table 3 PCR primers and amplification conditions

Target gene	Description	Nucleotide sequence (5′-3′)	Ampl (bp)	Amplification
aph	*aac(6′)-aph(2″)* gene, which encodes for a bi-functional, aminoglycoside modifying enzyme (Kao et al. 2000)	GAGCAATAAGGGCATACCAAAAATC CCGTGCATTTGTCTTAAAAAACTGG	505 bp	94 °C × 5′; (94 °C × 30″, 55 °C × 30″, 72 × 30″) × 35 cycles, 72 °C × 7’
blaZ	*blaZ* gene, which encodes for the predominantly β-lactamase in *S. aureus* (Martineau et al. 2000)	ACTTCAACACCTGCTGCTTTC TGACCACTTTTATCAGCAACC	173 bp	94 °C × 4′; (94 °C × 30″, 58 °C × 30″, 72 × 30″) × 30 cycles, 72 °C × 7’
tetM	*tetM* gene, which encodes for a tetracycline resistance protein (Cenci-Goga et al. 2004)	ACCCGTATACTATTTCATGCACT CCTTCCATAACCGCATTTTG	1115 bp	95 °C × 3′; (95 °C × 1′, 48 °C × 1′, 72 × 1′) × 35 cycles, 72 °C × 10’
sul1	*sul1* gene normally found in class 1 integrons, which encodes for a form of dihydropteroate synthase responsible for sulphonamide resistance in gram-negative bacilli (Kozak et al. 2009)	CGGCGTGGGCTACCTGAACG GCCGATCGCGTGAAGTTCCG	433 bp	94 °C × 3′; (94 °C × 15″, 69 °C × 30″, 72 × 1′) × 30 cycles, 72 °C × 7’
sul2	*sul2* gene, usually located on small non-conjugative plasmids (Sköld 2000) or large transmissible multi-resistance plasmids (Enne et al. 2001), which encodes for a form of dihydropteroate synthase, responsible for sulphonamide resistance in gram-negative bacilli	GCGCTCAAGGCAGATGGCATT GCCTTTGATACCGGCACCCGT	285 bp	94 °C × 3′; (94 °C × 15″, 69 °C × 30″, 72 × 1′) × 30 cycles, 72 °C × 7’

Table 4 Presence of five antibiotic resistance genes (*aph*, *blaZ*, *tetM*, *sul1*, and *sul2*) in honey bees' samples analysed by classic and nested PCR

Samples	*aph*		*blaZ*		*tetM*		*sul1*		*sul2*	
	Classic PCR	Nested PCR	Classic PCR	Nested PCR	Classic PCR	Nested PCR	Classic PCR	Nested PCR	Classic PCR	Nested PCR
1	–	–	–	+	–	–	–	–	–	+
2	–	–	–	–	–	–	–	+	–	+
3	–	–	–	–	–	–	–	–	–	–
4	–	+	–	+	–	–	–	+	+	+
5	–	–	–	–	–	+	+	+	+	+
6	–	–	–	+	–	–	–	+	+	+
7	–	–	–	+	–	–	–	+	+	+
8	–	–	–	–	–	–	–	+	–	–
9	–	–	–	–	–	–	–	–	–	–
10	–	–	–	+	–	+	–	+	–	–
11	–	–	–	–	–	–	–	–	–	–
12	–	–	–	–	–	–	–	–	–	+
13	–	+	–	–	–	–	–	–	–	–
14	–	–	–	–	–	–	–	–	–	–
15	–	–	–	–	–	–	–	–	–	+
16	–	–	–	–	–	–	–	–	–	–
17	–	–	–	–	–	–	–	–	–	–
18	–	–	–	–	–	–	–	–	–	–
19	–	–	–	–	–	–	–	–	–	–
20	–	–	–	–	–	–	–	–	–	+
21	–	–	–	–	–	–	–	+	–	–
22	–	–	–	–	–	–	–	–	–	–
23	–	–	–	–	–	–	–	–	–	+
24	–	–	–	–	–	–	–	–	–	+
25	–	–	–	–	–	–	–	–	–	+
26	–	–	–	–	–	–	–	–	–	+
27	–	–	–	–	–	–	–	–	–	+
28	–	–	–	–	–	–	–	–	–	+
29	–	–	–	–	–	–	–	+	–	–
30	–	–	–	+	–	–	–	–	–	+
31	–	–	–	–	–	–	–	–	–	+
32	–	–	–	+	–	+	–	+	+	+
33	–	–	–	+	–	–	–	–	–	–
34	–	–	–	–	–	–	–	–	–	–
35	–	–	–	–	–	–	–	–	–	–

The multivariate analysis identified the "presence of a farm nearby" as the factor most closely related to PCR positivity: OR 12.764, CI = 1.524–106.894 (Tables 5 and 6). Moreover, the similarity for odd ratio from multiple logistic regression with the simple logistic regression indicates that there is little confounding effect of the environment (agricultural or industrial), of the elevation and of the sampling season or year on the relationship between the presence of farm nearby and PCR positivity.

Table 5 Factors associated with PCR positivity: results of logistic regression for each variable

Elevation	OR (95% C.I.)	*P*
Hill	1*	0.5557
Lowland	1.600 (0.335–7640)	
Environment		
Industrial	1*	0.2921
Agricultural	2.625 (0.436–15.813)	
Farms nearby		
No	1*	0.0080**
Yes	10.921 (1.867–63.979)	
Sampling season		
Autumn	1*	0.9833
Summer	1.0151 (0.242–4.256)	
Sampling year		
2014	1*	0.4168
2015	1.905 (0.402–9.024)	

OR odd ratio, *P P* value

* Reference level, ** $P < 0.05$

Table 6 Factors associated with PCR positivity: results of multiple logistic regression

Elevation	OR (95% C.I.)	*P*
Hill	1*	0.8690
Lowland	1.203 (0.134–10.774)	
Environment		
Industrial	1*	0.7003
Agricultural	1.587 (0.151–16.668)	
Farms nearby		
No	1*	0.0188**
Yes	12.764 (1.524–106.894)	
Sampling season		
Autumn	1*	0.7009
Summer	0.560 (0.029–10.799)	
Sampling year		
2014	1*	0.1119
2015	21.125 (0.491–908.899)	

OR odd ratio, *P P* value

* Reference level, ** $P < 0.05$

Discussion

Our results confirm that the extreme sensitivity of the nested PCR allowed the number of positive samples to be increased for most of the target antimicrobial resistance genes, in contrast to the classical PCR, in agreement with the results of previous studies (Milanović et al. 2016; Osimani et al. 2017). The fact that after the first amplification there were positive samples only for *sul1* and *sul2* genes suggests a high contamination of those samples with bacteria carrying antimicrobial resistance genes against sulphonamides.

Most published data on antimicrobial resistance focus on bacteria isolated from humans or food-producing animals. Instead, there is little data available in literature on antimicrobial resistance genes in bacteria from the environment and environmental indicators. Honey bees can easily come into contact with bacteria carriers of antimicrobial resistance genes present in water, soil or materials from farms during their foraging travels. There is evidence in literature that environmental and/or feeding conditions might influence the high occurrence of antimicrobial resistance genes in insects. As reported by Vaz-Moreira et al. (2014), water could constitute an important route for the spread of antimicrobial resistance genes in the environment. Kang et al. (2016) found that different fertilizers significantly impacted the spread of tetracycline resistance genes and tetracycline-resistant bacteria in soil rhizospheres. The number of PCR positivity was associated with the presence of farms nearby. This association may be due to unmeasured management or environmental factors related to antimicrobial use in farmed animals.

A joint report by the European Centre for Disease Prevention and Control (ECDC), the European Food Safety Authority (EFSA) and the European Medicines Agency (EMA) on the integrated analysis of the consumption of antimicrobial agents and the occurrence of antimicrobial resistance in bacteria highlighted a positive association between the use of tetracyclines in animals and the emergence of resistances to the same class of antimicrobials in bacterial isolates from these animals (Anonymous 2015).

The high prevalence of antimicrobial resistance genes in our samples (66.67% of the samples were positive for at least one gene, 25.71% were positive for the presence of more than one of the antimicrobial resistance genes researched, and one sample, 2.86%, was positive for the presence of all the genes researched, is similar to data obtained in studies on edible insects recently reported by Milanović et al. (2016) and Osimani et al. (2017), and with data reported by the cited joint report by the European Centre for Disease Prevention and Control (ECDC), the European Food Safety Authority (EFSA) and the European Medicines Agency (EMA) (Anonymous 2015) on antimicrobial resistance in bacteria isolated from environmental indicators.

Conclusions

Wild bacteria populations may serve as environmental reservoirs for antimicrobial resistance genes, but the mechanisms by which these genes are distributed and retained in natural environments and how the exchange between medical or agricultural environments and nature are still not very clear. Honey bees are excellent biological markers for determining the condition of the world in which they live, due to their particular behaviour. In particular, they can easily come into contact with antimicrobial resistance gene carried by bacteria and can serve as a reservoir and/or spread those genes. Our study shows that *Apis mellifera* can be used as a measure of the prevalence and distribution of genes of antimicrobial resistance in

the environment and that these genes are widespread in the environment in the territory studied (region of Umbria, Central Italy).

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Conflict of interest

The authors declare that they have no conflict of interest.

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