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Prevalence and Antimicrobial Resistance of *Escherichia coli* Strains Isolated from Stray Cats in South Spain

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Simple Summary: The fight against zoonoses and antimicrobial resistance (AMR) is a global priority according to the FAO, WOH and WOAH, which promote a One Health approach to address these problems comprehensively. Animals, including stray cats, act as reservoirs of pathogens and resistance genes, representing a risk to public health, especially in urban and peri-urban areas due to their mobility and, in some cases, lack of sanitary control. The objective of this study was to investigate the prevalence of *Escherichia coli* and its AMR from stray cats in Benalmadena city (Malaga province, south Spain). The prevalence rate was 40.23%. *E. coli* strains showed a high percentage of sensitivity except for azithromycin (100% of resistance), followed by sulfonamides (25%) and ampicillin (20.6%). Overall, 13.23% of the isolates exhibited resistance to 3 or more antimicrobial groups (MDR strains). Research work on zoonotic pathogens in feline colonies must contribute to the prevention of potential health risks derived from the handling and management of these animal populations, as well as to the knowledge of the associated AMR.

Abstract: Stray cats act as reservoirs of pathogens and resistance genes, representing a risk to public health, especially in urban and peri-urban areas due to their mobility and, in some cases, the lack of sanitary control. One of the most relevant zoonotic agents is *Escherichia coli*, a pathogen of the gastrointestinal tract of humans and animals that causes infection/disease and can be transmitted from one species to another. We investigated a total of 169 rectal swabs from feline colonies in the municipality of Benalmadena (province of Málaga, south Spain). The microbiological identification process included bacterial isolation on selective media (XLD), standard biochemical tests and the MALDI-TOF identification system. A total of 68 *E. coli* strains were thus confirmed (40.23%). Subsequently, an antimicrobial resistance analysis was carried out using automatized methods (Sensititre); each isolate was evaluated by MIC against 15 antimicrobial agents. *E. coli* strains showed a high percentage of sensitivity. The most relevant finding was the resistance of the strains to azithromycin (100%), as well as a moderate resistance to sulfonamides (25%) and ampicillin (20.6%). The percentage of multidrug resistance (MDR) was found in 13.23% of the strains studied. In relation to the rest of the antimicrobials evaluated, the strains presented high percentages of sensitivity. It can be concluded that our research represents the first confirmation of commensal *Escherichia coli* in stray cats in Spain indicating a moderate-high prevalence as well as a low MDR levels. The control of the health status of these animals under a One Health approach is necessary due to their direct impact on public health.

Keywords: stray cats; *Escherichia coli*; MIC; azithromycin; AMR; MDR; one health

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1. Introduction

Zoonoses from companion animals, especially cats, can arise in the most unexpected way and have serious consequences [1]. Despite all this, it should be remembered that the probability of human beings acquiring an infectious pathology from a domestic cat well controlled by a veterinarian is low, although this risk increases significantly through contact with stray cats [1].

Pet animals are assumed to be potential reservoirs in transferring antimicrobial resistance (AMR) to humans due to the extensively applied broad-spectrum antimicrobial agents and their close contact with humans [2].

Free-living cats, that live outdoors in public or private urban areas, usually live in colonies, using resources of the human activity to feed or predating a broad biodiversity of animals such as birds, rodents, and reptiles [3,4]. Populations of free-living cats could represent an important threat to public health, being an important factor in the transmission of zoonotic diseases due to the close contact with humans and other pets [5,6]. In fact, previous studies, carried out in different countries, have reported cats as a source of zoonotic infectious and parasitic diseases [3,7–9].

In European countries, the number of studies focusing on cats is still very limited, which could result from lower interest in this species compared to dogs, but also logistical challenges such as difficulty in handling and sampling these animals or finding fecal samples [10,11]. Different publications have reviewed the presence of zoonotic pathogens in feline colonies in Europe. Thus, Rosario et al [12] in Spain, made the first description of *Salmonella enterica* subsp. *enterica* in feline colonies in Las Palmas de Gran Canarias, and study its impact on public health. In addition, several works in Italy with a strong One Health approach have investigated specifically the presence of *Escherichia coli* in fecal samples obtained from feline colonies, as well as its AMR phenotype. In fact, Ratti et al [13] and Gargano et al [14], respectively, confirmed the circulation of multidrug-resistant *E. coli* strains in feral cats as well as *E. coli* strains producers of ESBL (Extended Spectrum β-Lactamases).

In Europe, veterinary antimicrobial consumption was monitored by the 'European Surveillance of Veterinary Antimicrobial Consumption (ESVAC)' project since 2009 until 2023 [15]. Currently, in response of European Regulations a modified version of this program has been in force, named 'European Sales and Use of Antimicrobials for Veterinary Medicine (ESUAvet)'. This program developed a harmonized approach for collecting and reporting data on the sale and use of antibiotics in animals. However, the monitoring of AMR among animals is not systematic across Europe countries being focused on food-producing animals, and no for companion animals such as dogs and cats [16,17]. In Spain, the 'Antimicrobial Resistance National Program' [18] has been in force since 2014, and in the period 2025-2027, the PRAN intends to include AMR in companion animals (dogs and cats) in the 'reduce antibiotics program'. On the other hand, despite the excessive use of antimicrobials in dogs and cats and their frequent contact with humans, the role of these species as potential reservoirs of AMR remains poorly studied and understood [19,20].

In this sense, few studies have focused on the role of stray cats as disseminators of resistance to broad-spectrum antibiotics used in veterinary and human medicine [21]. The emergence and spread of AMR are a global problem that requires a One Health approach [22].

Despite several studies in Spain have reported the role of companion animals as reservoirs of AMR [2,23,24], limited information is available regarding the role and circulation of *E. coli* and AMR-linked in free-living cats in Spain.

However, stray cats that are in contact with humans and share the urban environment with them, may act as reservoirs of AMR for humans and their pets. In this context, the aim of this study

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was to investigate the implication of these animals as disseminators of AMR. Thus, we phenotypically assessed the resistance of commensal *E. coli* isolated from stray cat rectal swabs.

2. Material and Methods

2.1. Sample Collection

This collaborative research work was carried out between the Epidemiology, Preventive Medicine, and Health Policy Unit of the Animal Health Department (Veterinary Faculty, University of Córdoba), the Health and Environment area of the Benalmadena City Council (Málaga province) and the Fenix veterinary clinic located in Benalmadena (Málaga province), a 'Cat Friendly Silver Level Centre' Categorized by International Society of Feline Medicine (ISFM).

A total of 169 rectal swabs were collected from cats housed in animal colonies or belonging to shelters located in Benalmadena (Malaga province). These samples were collected at the Fenix clinic at the time of the castration of the animals and from the campaigns carried out on the Malaga province by official institutions for control purposes (Trap-Neuter-Return campaigns) (Figure 1).

Figure 1. Rectal swab sample collection.

All samples preserved in AMIES transport medium were refrigerated at ≤4 ºC and transported to the laboratory of Animal Health Department (University of Cordoba) for microbiological analyses within 24 h of collection.

2.2. Escherichia Coli Isolates Identification

Rectal swabs were pre-enriched in buffered peptone water (BPW; Scharlau, Barcelona, Spain), in 1:10 vol/vol proportion, and incubated at 37 ± 1 ºC for 24 ± 2 h. All the pre-enriched samples were inoculated onto XLD (Xylose Lysine Deoxycholate) agar medium and incubated for 24 hours at 37ºC to identify lactose positive colonies. We must always obtain pure colonies, so if necessary, and if we observe more than one type of colony, those that interest us are taken and replanted in another selective culture plate to separate them. Isolates suspected as *E. coli* (yellow in XLD medium) were transplanted into brain-heart infusion agar incubated at 37 ºC for 24 h and confirmed as *E. coli* by biochemical tests including: Gram stain, catalase test, oxidase test and indole production test, before confirming bacteria species by Matrix-Assisted Laser Desorption–Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF) (Bruker Maldi Blotyper).

2.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was carried out with antibiotics of importance in public health. Thus, each isolate was evaluated by Minimun Inhibition Concentration (MIC) against 15 antimicrobial agents: aminopenicillins (AMP ampicillin), 3rd-generation cephalosporins (CTX cefotaxime, CAZ ceftazidime), carbapenems (MEM meropenem), quinolones (NA nalidixic acid, CIP ciprofloxacin), macrolides (AZM azithromycin), aminoglycosides (GEN gentamicin, AMK amikazim), polymyxins (CL colistin), sulphonamides (SF sulfamethoxazole), diaminopyrimidines (TMP trimethoprim), amphenicoles (CHL chloramphenicol), tetracyclines (TE tetracycline), and glycylcyclines (TGC tigecycline).

AMR was evaluated using MIC assay in *Salmonella/E. coli* EUVSEC Plate (Thermo Scientific Sensititre Plate Guide, Madrid, Spain), which included the antibiotics set out in Decision (EU) 2020/1729 on the monitoring of AMR in zoonotic and commensal bacteria. Sensititre plate results were interpreted according classification criteria followed by the Sensititre™ SWIN™ Software System based on CLSI.

When the strain under study showed acquired resistance to at least one agent from three or more antimicrobial classes, it was defined as multidrug-resistant (MDR) [25].

According to the European Medicines Agency (EMA) about 'Categorisation of antibiotics for use in animals for prudent and responsible use', the antimicrobial used in this research were classified in four different Categories (Table 1): Category D ("*Prudence*"), first line of defence; *Category C* ("*Caution*"), when Category D antibiotics fail; *Category B* ("*Restrict*"), which matches the Highest Priority Critical Important Antimicrobials (HPCIAs) in the Medically Important Antimicrobial (MIA) list, elaborated by the World Health Organization (WHO), (3rd and 4th gen. cephalosporins, quinolones, polymyxins and phosphonic acid derivatives), used when all therapeutic alternatives (D and C) have been exhausted; and finally, *Category A* ("*Avoid*"), matching the antimicrobials not authorised (NA) for veterinary use in the MIA list and commonly known as last-resort antibiotics [26]. In the EU, these antimicrobials are also limited to human medicine and not authorised to treat food-producing animals. However, they can be dispensed in exceptional situations in companion animal clinics, following the prescription order [27].

Table 1. Classification of the 68 commensal *Escherichia coli* strains based on their response to the 15 antimicrobials (AMA) studied.

AMA Group	AMA	EMA*	N.º Strains and Percentage (%)	
			S	R
Aminoglycosides	GEN	C	68/68 (100)	0/68 (0)
	AMK	C	67/68 (98.5)	1/68 (1.5)
Cephalosporins	CTX	B	68/68 (100)	0/68 (0)
	CAZ	B	68/68 (100)	0/68 (0)
Quinolones	CIP	B	64/68 (94.1)	4/68 (5.9)
	NA	B	63/68 (92.6)	5/68 (7.4)
Tetracyclines	TE	D	63/68 (92.6)	5/68 (7.4)
Sulphonamides	SF	D	51/68 (75)	17/68 (25)
Aminopenicillins	AMP	D	54/68 (79.4)	14/68 (20.6)
Carbapenems	MEM	A	68/68 (100)	0/68 (0)
Macrolides	AZM	C	0/68 (0)	68/68 (100)
Polymyxins	CL	B	68/68 (100)	0/68 (0)
Amphenicols	CHL	C	67/68 (98.5)	1/68 (1.5)
Glycylcyclines	TGC	A	67/68 (98.5)	1/68 (1.5)
Diaminopyrimidines	TMP	D	63/68 (92.6)	5/68 (7.4)

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AMA leyend: GEN, gentamicin; AMK, amikacin; CTX, cefotaxime; CAZ, ceftazidime; CIP, ciprofloxacin; NA, Nalidixic acid; TE, tetracycline; SF, sulfamethoxazole; AMP, ampicillin; MEM, meropenem; AZM, azithromycin; CL, colistin; CHL, chloramphenicol; TGC, tigecycline; TMP, trimethoprim. S, susceptible; R, resistant. Classification criteria followed by the Sensititre™ SWIN™ Software System based on CLSI. *EMA (European Medicines Agency). This column indicates the EMA categorisation of antibiotics used in animals to promote their responsible use in order to protect animal and public health.

2.4. Statistical Analysis

The Multiple Antimicrobial Resistance index (MAR index) value was determined for all isolates, according to Matos et al. [28], as follows: *MAR index = N. º antimicrobials to which E. coli isolates were resistant / N. º antimicrobials tested.*

The MAR Index is a measure of a bacterium's resistance to multiple antibiotics. It is calculated as the ratio of the number of antibiotics to which the bacterium is resistant to the total number of antibiotics to which it is exposed. A low MAR index (below 0.2) suggests that the bacteria may have originated in an environment with less exposure to antibiotics, while that a high MAR index (0.2 or higher) indicates that the bacteria may have evolved in an environment where a variety of antibiotics are used [29].

On the other hand, the relationship with resistance to different antibiotics was evaluated using Spearman's rank correlation coefficient with 'Corr Procedure' while considering two levels of resistance—susceptible and resistant—with intermediate isolates being considered resistant for this análisis [29]. As suggested by Heinze and Dunkler [30], manual backward elimination with a p value criterion of 0.157 and without preceding univariable prefiltering was performed to reach the final models which differences were considered significant when $p < 0.05$.

3. Results

3.1. Escherichia Coli Isolates Identification

From 169 rectal swabs collected (colonies and shelters), a total of 68 commensal *E. coli* strains (40.23%) were identified by means of standard biochemical tests and confirmed by means the MALDI-TOF identification system. These strains were stored at -20ºC into brain-heart infusion broth with glycerol (15%) until MIC analysis.

3.2. Antimicrobial Susceptibility Testing

E. coli strains showed a high percentage of sensitivity highlighting the results against gentamicin, cefotaxime, ceftazidime, meropenem and colistin (100%) (Table 1). Of all the antimicrobial groups studied, the macrolides group is the one with the highest AMR being the most relevant finding the resistance detected to azithromycin (100%) (EMA, Category C, *Caution*). Moreover, a moderate resistance rates against sulfonamides (25%) and ampicillin (20.6%) was detected (EMA, Category D, *Prudence*) (Table 1). Furthermore, two of them (meropenem and tigecycline) belonged to the NA category according to MIA list, as well as to category A according to the EMA categorisation, with the lowest percentage of AMR being found for this type of antibiotics (0.0 and 1.5%, respectively) (Table 1).

Overall, ten different resistant phenotypes, grouped by antibiotic, were found in stray cats *E. coli* strains (Table 2). The most prevalent were AZM (63.24%) followed by AMP-AZM and AZM-SF (11.76%, respectively) (Figure 2). Nine strains (13.23%) were resistant to more than three antimicrobial groups and thus considered as MDR strains. These nine strains showed resistance patterns to up to eight antimicrobial groups (Table 2).

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Table 2. Number of commensal *Escherichia coli* strains isolated from stray cats and their resistant phenotypes.

N of Isolates	AMR Phenotypes	N Antimicrobials
43	AZM	1
8	AMP-AZM	2
8	AZM-SF	2
1	AZM-SF-TGC	3
2	AMP-AZM-SF	3
1	AZM-SF-TE-TMP	4
1	AMP-AZM-NA-SF	4
1	AMK-AZM-CIP-SF-TE-TMP	6
1	AMP-AZM-CIP-NA-SF-TE-TMP	7
2	AMP-AZM-CHL-CIP-NA-SF-TE-TMP	8
Total 68		

N, number; AMK, amikacin; CIP, ciprofloxacin; NA, Nalidixic acid; TE, tetracycline; SF, sulfamethoxazole; AMP, ampicillin; AZM, azithromycin; CHL, chloramphenicol; TGC, tigecycline; TMP, trimethoprim.

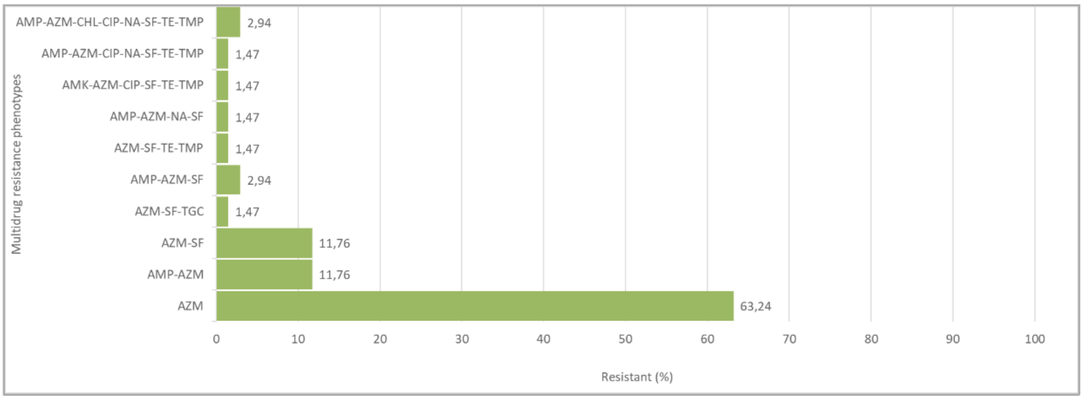

Figure 2. Resistant and multiresistant phenotypes in stray cat's commensal *Escherichia coli* isolates (n=68). AMK, amikacin; CIP, ciprofloxacin; NA, Nalidixic acid; TE, tetracycline; SF, sulfamethoxazole; AMP, ampicillin; AZM, azithromycin; CHL, chloramphenicol; TGC, tigecycline; TMP, trimethoprim.

3.3. Statistical Análisis

In our study, the result of MAR index was 0.6. A MAR index greater than or equal to 0.2 suggests that the bacterium originates from a high-risk source of contamination, where a wide variety of antibiotics are used.

Finally, the correlation between the *E. coli* isolates resistance to different antimicrobials is showed in Figure 3. A very strong correlation was detected between «tetracycline and trimethoprim» (*p* value = 1.0), and a strong correlation between «ciprofloxacin and trimethoprim» and «ciprofloxacin and tetracycline» (*p* values = 0.89, respectively).

	TMP	TGC	CHL	AMP	SF	TE	NA	CIP
AMK	**0,43**	-0,01	-0,02	-0,06	0,22	**0,43**	**-0,03**	**0,49**
CIP	**0,89**	-0,03	**0,70**	**0,34**	**0,45**	**0,89**	**0,73**	
NA	**0,65**	-0,03	**0,70**	**0,49**	**0,45**	**0,65**		
TE	**1,00**	-0,03	**0,62**	0,27	**0,51**			
SF	**0,51**	0,22	**0,31**	0,23				
AMP	0,27	-0,06	**0,34**					
CHL	**0,62**	-0,02						
TGC	-0,03							

Very strong correlation (0.80≤ρ≤1.00)
Strong correlation (0.60≤ρ≤0.79)
Moderate correlation (0.40≤ρ≤0.59)
Weak correlation (0.20≤ρ≤0.39)
Very weak correlation (0.00< ρ≤0.19)
No correlation (ρ≤0)

Figure 3. Spearman correlation between antimicrobial resistances to several antimicrobials. Heat map showing the Spearman *p* value. White bold numbers indicate statiscally significant correlation between coefficients

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($p<0.05$). Leyend: AMK, amikacin; CIP, ciprofloxacin; NA, Nalidixic acid; TE, tetracycline; SF, sulfamethoxazole; AMP, ampicillin; CHL, chloramphenicol; TGC, tigecycline; TMP, trimethoprim.

4. Discussion

Society's changing attitudes toward animals have led many to oppose control and culling measures, viewing cat ownership as an integral part of urban wildlife in so-called Feline Colonies [31]. This phenomenon is not exclusive to Spain; it is a pervasive reality in all European countries. However, the problems associated with feline colonies are diverse [1]: (i) risks to public health; (ii) social conflict; (iii) traffic accidents and run overs; (iv) animal welfare; (v) incidents on local biodiversity. Therefore, sanitary and veterinarian-supervised management is essential for animal welfare and public health. Thus, colony locations, hygiene, and sanitary controls must be met for approval.

The present study underscores the importance of monitoring AMR and MDR in stray cats, as these animals have the potential to disseminate these resistances throughout the environment, thereby facilitating their transmission to humans and other animals. To address this issue, *E. coli* has been used as a sentinel bacterium in different official programs focused on food-producing animals [32], as it constitutes part of the normal intestinal microbiota of humans, and warm-blooded animals and reptiles [33]. In this study, nine strains (13.23%) were considered as MDR strains. Similar results were found in a study conducted in stray cats in indonesia [34]. In Europe, few studies have been focused on presence of *E. coli* and its AMR in stray cats. However, the results obtained in this study are consistent with those reported in other studies conducted in Italy. In Palermo (Sicily, Italy) a phenotypic and genetic characterization study was performed on 75 *E. coli* strains isolated from rectal swabs or fecal samples obtained from stray cats was carried out [14]. A total of 43% of the strains exhibited resistance to at least one of the eight antimicrobials tested. Regarding their MDR profile, six strains (19%) of the 32 resistant isolates exhibited MDR. On the other hand, a study conducted by Ratti et al. [13], examined the prevalence of ESBL/AmpC-Producing *E. coli* in pet and stray cats. The study identified 6/97 ESBL/AmpC-Producing *E. coli*, with two strains belonging to stray cats and four to pet cats. It should be noted that there are more studies focused on pet cats than on stray cats regarding the epidemiological situation of AMR in feline populations, so comparing data on a global level is complicated. However, studies on pet cats serves as basis for understanding their contribution to the epidemiology of AMR.

In a study conducted in East Spain, 72.6% and 34.7% of the *E. coli* strains, isolated from pet cats, showed AMR and MDR, respectively [24]. But not only in this study, contrary to expected, higher MDR patterns have been observed in pet cats, as seen in South Korea (20.9%) [35], Zimbabwe (25%) [36], Panama (29%) [37], Hangzhou, China (30%), [38], Bangladesh (46.34%) [39], Thailand (62.1%) [40], and Poland (66.8%) [41]. These results demonstrate that the global burden of AMR is evident, as it affects both housed and free-roaming animals equally. Therefore, continuos monitoring of companion animals *E. coli* antimicrobial susceptibility is imperative. Furthermore, the integration and application of recommendations for appropriate use of antimicrobials in small animal practice should be essential to minimize the emergence of multidrug resistance among *E. coli* in companion animals.

Concerning the antibiotics to which the strains examined demonstrated the greatest resistance, the percentage observed against azithromycin (100%) is particularly alarming. This antibiotic, which belongs to category C (EMA), is widely used in the treatment of infections in humans and companion animals as an alternative for infectious processes of the upper respiratory tract, genitourinary, oral, and cutaneous systems [42]. Most drugs used to treat respiratory tract disease in cats need daily administration. Azithromycin has a long tissue half-life in cats, and a low dosage is adequate to maintain plasma levels which has led to frequent use of the drug in feline practice [42]. This could be useful in shelter situations where multiple cats need to be treated [42]. The study's high resistance rate contrasts with previous findings in domestic cats, which showed lower resistance rates (i.e. 12.8% in Zimbabwe; less than 5% in Bangladesh; 7.7% in Thailand;) [36,39,43].

Resistance to azithromycin has been previosly described in the Enterobacteriaceae population. In France, emerging resistance to azithromycin in non-typhoidal *Salmonella* as multidrug-resistant *S. enterica* serovar Blockley isolated from poultry has been described [44]; in U.K., Nair et al. [45] showed the resistance to azithromycin, entirely due to acquisition of known macrolide resistance genes (*mph*A, *mph*B, *mph*E or *mef*B) in multiple *Salmonella* serovars.

In other study conducted among the United States that collected 110,423 samples (106,205 from dogs and 4,218 from cats) to study the resistance of *Staphylococci* isolates to antibiotics revealed that 65.5% of *S. aureus*, 78.2% of *S. pseudintermedius* and 14.7% of *S. schleiferi* were resistant to azithromycin [46]. Despite the absence of distinción between dog and cat strins in the study, and in contract to *E. coli*, which is classified as GRAM negative, these bacterial species that are part of the commensal microbiota of the mucous membranes of companion animals. Consequently, it is posible that these bacteria could be transmitting resistance horizontally to other bacteria, such as *E. coli*.

The high AMR to azithromycin observed in feline colonies can be attributed to a multifaceted combination of microbiological, environmental, and veterinary management factors: (i) repeated or prolonged use of azithromycin in cats; (ii) administration without microbiological diagnosis (empirical use); (iii) the administration of sub-therapeutic doses or incomplete treatments; (iv) horizontal transmission between cats in close contact, which facilitates the dissemination of resistant bacteria between individuals by direct or indirect contact (through surfaces, water, food, etc.); (v) selective pressure by other antibiotics; (vi) presence of acquired resistance genes; (vii) environmental contamination and human contact. In this sense, if the colony is near urban or clinical areas, there may be exposure to multidrug-resistant bacteria from humans or other animals. Some resistant bacteria may be zoonotic and can be transferred in both directions. Furthermore, the continued presence of antimicrobials (such as macrolides) within soil and aquatic ecosystems has been documented in previous studies [47,48]. For instance, azithromycin has been shown to accumulate in non-target species (e.g., caddisfly larvae) living in contaminated waters. This antimicrobial is recognized as an emerging contaminant of concern because of its persistence in the environment, minimal biological degradation, slow microbial breakdown, and/or incomplete removal during wastewater treatment [49].

These circumstances described herein may provide a rationale for the unusually high levels of resistance observed in the present stud, which was conducted on feline colonies maintained under free-ranging conditions. It is hypothesized that the access of these animals to a variety of water sources containing antimicrobial residues could be a contributing factor to the observed resistance (Figure 4). On the other hand, one could hypothesize about the risk of acquiring AMR through the consumption of rodents and birds, as well as through close contact with volunteer caregivers of these feline colonies. The transmission of resistant bacteria between feline colonies and other animals, including humans, is a significant challenge. Implementing measures such as regular veterinary check-ups, sterilization programs, and ensuring adequate hygienic conditions can help reduce the spread of these bacteria. It is also essential to promote responsible practices among caregivers and volunteers who interact with these colonies.

Regarding the following antibiotics following azithromycin, the highest AMR observed were against sulfamethoxazole (25%) and ampicillin (20.6%). Both antibiotics belonged to category D (EMA) and are widely used in human medicine but not authorized in companion animals in Spain [50], thus, *a priori* no AMR should be found. However, other studies have reported to have the highest AMR to ampicillin in dogs or cats [24,35]. In fact, sulfamethoxazole is always administered in combination with trimethoprim, enhancing its effect and, therefore, showing lower AMR rates than those found in this study, as seen in some AMR programs in European countries [22]. In addition, is remarkable to address that all the strains studied were sensible to cefotaxime, ceftazidime and colistin, HPCIA in human medicine, and to meropenem, a last-resort antibiotic, contrary to the results observed in pet cats in other studies [24,51,52]. The observed outcomes may be attributable, once more, to the shared environment in which domestic cats and humans coexist, given the proximity and frequent interaction between these two species, which exceeds that generally observed between stray cats and humans.

Figure 4. Shelter for feral cats.

5. Conclusions

It can be concluded that the present research constitutes the first confirmation of *E. coli* in stray cat colonies in Spain. Stray cats, cohabit and interact with humans and their environment, highlighting the importance of monitoring AMR and MDR trends, as they could pose a direct threat to public health due to the spread of AMR genes. These findings underscore the importance of implementing effective AMR control measures within veterinary practices and feral cat colonies management, given the presence of bacteria resistant to commonly used antimicrobials in humans and animals. Despite the need for further studies, the occurrence of resistant *E. coli* provides support for the assumption that stray cats may be a sources of AMR. Consequently, the incorporation of these animals into AMR surveillance programs is imperative. Additionally, molecular based methods are required to investigate the types of circulating AMR genes and the role played by *E. coli* strains in disseminating these genes from pet animals to humans and the environment. For that, other studies about posible presence of resistance genes responsable for the atypical high azithromycin resistance by means real time PCR are necessary.

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