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Citizen Science to Raise Antimicrobial Resistance Awareness in the Community: The MicroMundo Project in Spain and Portugal

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ABSTRACT

Antimicrobial resistance (AMR) poses a global threat to human, animal and environmental health. Among the multidisciplinary tasks aimed at collectively tackling the AMR crisis, surveillance, research and education stand as major priorities. Based on a crowdsourcing research strategy, the MicroMundo project, a partner of the Tiny Earth initiative in Spain and Portugal, has been developed and consolidated with success in the academic environment. The objectives are focused on promoting research and, especially, on bringing knowledge of One Health and microbiology concepts, as well as AMR awareness to the community. Following a service-learning approach, MicroMundo integrates university and secondary/high school students in a citizen science-based research project to collectively isolate microorganisms with the potential to produce new antibiotics from soil environments. Over the last 7 years, 32 MicroMundo hubs operating across 31 different Portuguese and Spanish universities have recruited thousands of teenagers in this quest. Here we review the outcome of this unprecedented effort from a scientific and an educational perspective.

1 | Introduction

The overuse or misuse of antimicrobial compounds, essentially antibiotics, in human health, animal health or food production may lead to unnecessary overexposure to these molecules in diverse microbial habitats. If sustained, this situation leads to the rapid evolution of microorganisms, notably bacteria but also viruses, fungi and parasites, towards antimicrobial resistance

For affiliations refer to page 13.

(AMR). The forecast of the O'Neill report in 2016 (O'Neill 2016), predicting a mortality burden of 10 million deaths worldwide by the year 2050 as a cause of infections by drug-resistant bacteria if the problem is neglected, raised a warning echoed by the World Health Organization (WHO), which had launched a Global Action Plan on Antimicrobial Resistance in 2015 (World Health Organization 2015). Although National and International agencies have started programs to fight AMR, recent estimates based on 2019 data raised its global burden to more than 1.27 million deaths (Murray et al. 2022). The COVID-19 pandemic may have even worsened this situation (Bocabella et al. 2024). Furthermore, in a hyperconnected world, AMR is considered a 'silent pandemic', as bacteria harbouring multiple genetic determinants for antibiotic resistance can travel as part of the microbiota of carriers via international transportation, food trade or animal migration, thus spreading all over the world.

To face the threat of AMR, a multilateral strategy is required, involving (i) surveillance of AMR worldwide, which can be challenging in low-income countries that lack a strong healthcare system; (ii) prevention of a potential further raising of multidrug-resistant bacterial isolates, especially of the 24 bacteria within the WHO bacterial priority pathogen list (World Health Organization 2024), by promoting a rational use of these drugs and (iii) research on new antibiotics or alternative antimicrobial strategies, such as phagotherapy, to ensure an efficient treatment of infectious diseases worldwide (MacNair et al. 2024).

Most bacteria of the highest AMR risk cycle between the environment and our bodies, either by the food chain, contact with animals, or by other means. Thus, to stop the rise of AMR, the use of antimicrobial drugs must be regulated in human health, as well as in animal welfare, food production and the environment. This multilateral approach is referred to as the One Health perspective and is common to the prevention of other key global health problems such as emerging viral pandemics (Pitt and Gunn 2024). The efforts made over the last decade by national and international organisations in this direction have led to improvements in the management of antibiotics in the clinics and in food-animal production in high-income countries, but a global commitment to this cause is still a major goal. Moreover, the pipeline for the discovery of new antimicrobial drugs is scarce. The cost of launching a novel antibiotic to the market is estimated at 1.4 billion dollars, whereas the return of such investment in the market is low compared to other drugs. This has led pharmaceutical companies to abandon their antibiotic development pipelines. Paradoxically, the recent outstanding progress achieved in exploring the chemical space and defining novel microbial targets by applying to the field the latest advances in functional genomics and metagenomics, or structural biology boosted by deep learning approaches, does not seem to translate into the registration of new antimicrobial drugs (reviewed by Brüssow 2024). In spite of growing awareness and investment by public agencies, the academic actors responsible for this knowledge lack the necessary financial and management resources to push candidates down the pipeline. According to a recent WHO report (World Health Organization 2023), among 32 antibiotics under development in pre-clinical or clinical trials, only 12 can be considered innovative, and only four of these 12 are presumably active against pathogens categorised as top risk.

In this scenario, two tasks come up as essential to ensure that we will be able to face infectious diseases in the future. First, to promote research on either new antibiotics or new alternative antimicrobial strategies (Hibbert et al. 2024), and second, to preserve the efficiency of the existing antibiotic arsenal by using them rationally (Laborda et al. 2024). Fulfilling such aims requires increasing the scientific education of citizens towards the causes and consequences of AMR, so that society meets the required scientific culture status to face the challenge. A pioneering strategy to achieve these goals in North American universities was devised by Jo Handelsman and her team, under the eventual denomination of Tiny Earth (Hurley et al. 2021). This antibiotic discovery project applies concepts of hands-on collaborative learning and citizen science (CS) within the University campus to motivate students towards a Science, Technology, Engineering and Mathematics (STEM) curriculum (Fernández-Fernández et al. 2023; Kolokithas et al. 2023). The experimental basis of the Tiny Earth initiative aims to engage students in the exploration of antibiotic bioactivities in natural ecosystems, in which competitive interactions among microbes are still expected to be a key source of novel antibiotic compounds (Van Goethem et al. 2024). Thus, by involving students in a community of researchers in the way that CS applies crowdsourcing to achieve research goals, it has been tagged by its promoters as a 'studentsourcing' discovery platform.

Inspired by Tiny Earth and associated with it in Spain and Portugal, the MicroMundo project has developed a strategy that targets pre-university students by adding the standpoints of service-learning to those of CS (Valderrama et al. 2018). Service-learning is considered an innovative pedagogic strategy of extraordinary value to train students on One Health multidisciplinary concepts (Cai et al. 2024). It implies that students must achieve knowledge and skills by implementing a real hands-on project with a direct impact in their community. Thus, MicroMundo couples the value of Tiny Earth CS protocols for antibiotic discovery with service-learning by involving teams of university students in training younger secondary high school students in microbiology techniques. By this means, a hierarchical learning community is established, in which secondary or high school students receive from university degree or master students the know-how to isolate potentially antibiotic-producing microorganisms from soil samples and transfer onto them the mission to spread knowledge on AMR and One Health concepts to their friends and families, and eventually to the community. In this way, it contributes to the discovery of antimicrobial bioactivities and brings scientific culture on AMR and One Health to the youngest members of society and their communities. Here, we present the results of 7 years of MicroMundo activity in the Iberian Peninsula.

2 | Tackling AMR From the Academics

When starting the project in 2016, we realised that it was necessary to work beyond the gates of our university campuses in order to inspire STEM vocations in younger students, as well as to expand the outreach of a crowdsourcing project aimed at isolating microorganisms with the potential to produce antibiotics from environmental samples. Thus, we focused on adapting the principles and experimental protocols of Tiny

Earth (formerly the Small World Initiative) to the reality of Spanish secondary and high schools. This involved simplifying the experimental procedures in general terms but, beyond the general guidelines, it posed an open challenge for each MicroMundo hub to innovate. During the academic year, each team of volunteer university students was assigned to a particular off-campus school to freely implement the antibiotic discovery program in four or five 1-or 2-h sessions. These includedlectures on AMR, One Health and basic microbiology, genetics and pharmacology, together with laboratory practices to isolate microorganisms and assay their ability to produce antibiotic activities, as well as post-activity evaluation surveys. It was agreed that this routine should be accompanied by appropriate creative strategies to disseminate awareness on AMR in the community around each particular school. A graphical view of the overall MicroMundo activities through one academic year for each team is shown in Figure 1. On this basis, as different universities in different regions joined the initiative, this scheme was adapted and diversified. Annual meetings sponsored by the Spanish Society for Microbiology (SEM) served as discussion hubs to share different views and experiences on how to enhance the efficiency of this essential structure for a CS program.

3 | The Spread and Outreach of MicroMundo

The pilot project was successfully tested at the Complutense University of Madrid (UCM) on 21 secondary/high schools in the 2016–2017 academic year, in which the service-learning strategy was coupled to the crowdsourcing CS tactic (Valderrama et al. 2018). Afterwards, the Spanish Strategic Plan for Antimicrobial Resistance (PRAN) and the SEM sponsored a workshop to start hubs in other universities and create a network, which was renamed MicroMundo in 2018 when the first Portuguese hub started at Porto University (Antunes et al. 2021). The MicroMundo network comprised 16 hubs in its first year and has kept growing to 32 hubs, covering most regions in Spain and Portugal (Figure 2). Although some hubs have discontinued the program, especially during the peak years of the COVID-19 pandemic, others have been very constant, active and innovative in implementing it or developing complementary approaches (Maicas and Fouz 2024). Besides the pioneering team at UCM, the hubs in Toledo (Castilla-La Mancha University), Valencia (two hubs at the Universities of Valencia and Cardenal Herrera-CEU), Barcelona (especially at the Autònoma University), Sevilla, León, Salamanca and Porto have been active through the years, working on average in more than five educational centres in their surroundings. From the 2017–2018 academic year, the first in which the network was in operation, the program has been implemented in 114.57 (±37.04) schools per year on average throughout the Iberian territory (Figure 2A). The contribution of each MicroMundo hub to the whole network activity is graphically represented in Figure 2B. Plotting these data on a map reveals the broad geographical distribution of the project (Figure 2C).

The evolution of the MicroMundo project and its outreach in the community is displayed in Figure 3 on a year-by-year time scale. Since the academic year 2017–2018, an average of 486.71 university students per year (reaching a maximum of 656 in 2023–2024) configured teams of four (3.94 total average) university student members. Additionally, an average of 2613.43 secondary or high school students was enrolled yearly across various geographic locations in Spain and Portugal. As an average per year, a total of 1378.71 soil samples were processed, with 20,791.71 microbial isolates tested as an average per year. As a whole, 9651 soil samples were analysed and 145,542 microorganisms tested in the 7 years within the MicroMundo network. The outcome of this collective endeavour is double. On the one side, we have gathered a valuable collection of local isolates with potential interest for antibiotic discovery, which is kept at the laboratories of partnering Universities; on the other side, we have widely contributed to the dissemination of scientific culture on AMR awareness, One Health, and basic microbiology, pharmacology, genetics, and epidemiology concepts. Although measuring objectively the impact of the project in this latter aspect is difficult, the MicroMundo network won an Antibiotic Guardian Prize in 2019, and the pioneering Madrid team (UCM) was recognised with the PRAN Prize for Communication of AMR to Society in 2021. Additionally, the MicroMundo group from the University of Porto was also awarded the Pedagogical Innovation Prize by its University in 2019.

4 | Scientific Results of the MicroMundo Project

In CS, the obvious goal of a project is the involvement of members of the community in the collection of data of scientific value. In our case, the deliverables are microbial strains of diverse origins that yielded positive results in the antibiosis assays performed by students. The basic program involves testing at least one Gram-positive and one Gram-negative 'ESKAPE dummy' bacterium, typically *Bacillus subtilis* or *Staphylococcus epidermidis* as Gram-positive, and *Escherichia coli* or *Acinetobacter baylyi* as Gram-negative testers, although other GRAS (Generally Recognised as Safe) microorganisms are used, even the yeast *Saccharomyces cerevisiae* as a tester for antifungal activities. Generally, it is more frequent to find activity against Gram-positive than against Gram-negative bacteria. The hub at Autonomous University of Barcelona found that among 124 strains confirmed positive upon re-testing, 50% were active against both Gram-negative and Gram-positive testers, and 49.2% inhibited exclusively Gram-positive bacteria, whereas just one isolate was active solely against Gram-negative. In the University of Valencia hub, through their first 2 years of activity, the percentages of active isolates against *Bacillus cereus* or *E. coli* were, respectively, 6.4% and 1.2% out of 7002 isolates tested (Maicas et al. 2020). Although results depend on the tester strains chosen, these findings reflect the general trend that antibiosis against Gram-negative bacteria is a rarer event, whereas inhibitors of the Gram-positive tester are more frequently found. The UCM hub in Madrid observed that using carotenoid-producing *Kocuria* spp. as a Gram-positive tester at schools enhances even more the rate of success compared to *Bacillus* or *Staphylococcus* strains, giving rise to a more encouraging experience for younger students.

The basic CS and service-learning program ends up with the freezing of pure culture samples of positive isolates for their conservation, together with the filing of the sampling data,

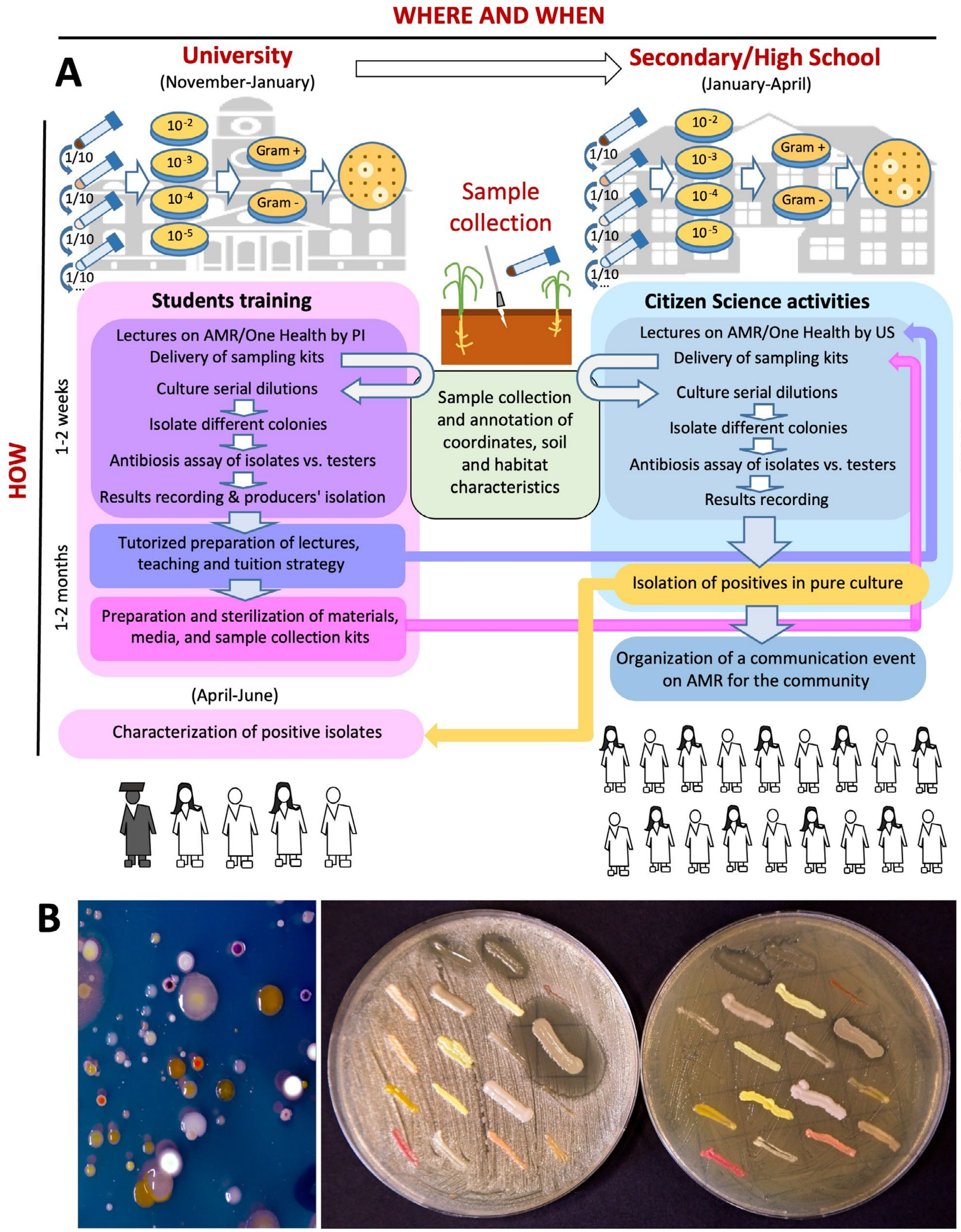


FIGURE 1 | Legend on next page.

to be available for the scientific community. However, each MicroMundo hub, and even individual MicroMundo teams, may take on further tasks once the off-campus activities are accomplished, especially the identification of the isolates by microbiological, molecular (16S rRNA sequencing) or spectroscopic techniques (MALDI-TOF MS), or the elucidation of their activity spectra by challenging them against a battery of testers, including real ESKAPE strains in BSL-2 facilities at the universities, GRAS mycobacteria, such as *Mycobacterium smegmatis*, or opportunistic pathogenic fungi, like *Candida* spp.

FIGURE 1 | Basic flowchart of MicroMundo routines for a team in an academic year. (A) Teams of university students led by a school member consist of 3–6 members. Once students are recruited for the project, they enrol in lab training sessions where they perform at the University labs the experimental procedures that they will later teach. The routine involves soil sampling, preparation of serial dilutions in aseptic conditions, and spreading them onto culture medium in Petri dishes, followed by the recovery of diverse colonies and antibiosis assays on a lawn of at least one Gram-positive and one Gram-negative tester bacterium. The observation of halos denotes growth inhibition by positive antibiotic-producing isolates. Once trained, they arrange and plan the activities at the assigned secondary or high school for the following semester, preparing lectures and lab materials. Their objective once at the secondary/high school is to communicate to younger students (usually a classroom of 20–30 students) the scientific basis for AMR and its prevention in the frame of a One Health perspective, as well as to lead them on the same routine of microbiology lab experiments for the isolation of potential antibiotic producers from the environment. Sample collection kits are delivered to school students with anticipation to personally choose the sampling site. After completion of the project along 3–4 weeks, post-activity surveys are carried out. Positive isolates are frozen and kept at university labs, where the team may further characterise them. Typically, a MicroMundo team tests 10–15 soils, isolating and testing for antibacterial bioactivity around 100–200 microorganisms. An ultimate dissemination goal of the joint team is to celebrate a final activity aimed at communicating AMR to the community (other students in the school, families, local campaigns in the district or on the internet, social networks, etc.). PI, Principal Investigator; US, University Student. (B) Typical aspect of isolation plates (left) and antibiosis assays (right). In the left image, a 10^{-3} dilution on a trypticase soy 10% agar plate shows microbial diversity of a given soil after 1 week of incubation. In the right image, typical antibiosis plates show positive isolates on a tester lawn of a Gram-positive (*Staphylococcus epidermidis*, left) and a Gram-negative (*E. coli*, right).

Considering positiveness in antibiosis for all the isolates investigated by the whole network over 7 years, a total of 6244 potential antibiotic-producing strains have been unearthed by the network. This involves a positiveness rate of 4.17%; approximately one out of 25 microorganisms isolated from the soil displayed some degree of activity against at least one of the tester bacteria in the primary screen. When investigated, the most frequent isolates positive for antibiosis were actinobacteria (*Streptomyces* spp. and related genera), Gram-positive bacteria phylogenetically affiliated to the genus *Bacillus* and *Bacillus*-like (e.g., *Bacillus* spp., *Paenibacillus* spp., *Brevibacillus* spp., *Lysinobacillus* spp. or *Peribacillus* spp.) (Fernández-Fernández et al. 2023; Pino-Hurtado et al. 2024) and, less frequently, Gram-negative bacteria, commonly of the genera *Pseudomonas* and *Serratia*, as well as Gram-positive cocci (Antunes et al. 2021; Pino-Hurtado et al. 2024). Although to date there is neither a common repository for all the antibiosis-positive isolates in the MicroMundo network nor a specific coordinated project to systematically characterise the putative molecules produced, individual initiatives have been started to exploit this wealth of microbial strains. Within the UCM hub, Lafuente et al. (2024) have recently described a novel bacteriocin produced by a *Bacillus altitudinis* strain isolated by pre-university students, which has received the name of altitudin A. Similarly, within the MicroMundo project at the University of Porto, a promising soil isolate of the *Pseudomonas* genus with significant antimicrobial activity against various pathogenic strains has been identified (unpublished data). Moreover, the team at Jaume I University is testing the isolates against plant pathogens, such as *Xanthomonas vesicatoria*, *X. arboricola*, *Clavibacter michiganensis, Pseudomonas syringae* or *Rhizobium radiobacter*, in search of bioactivities potentially useful to control crop diseases.

5 | Improving the Protocols for Crowdsourcing Antibiotic Discovery

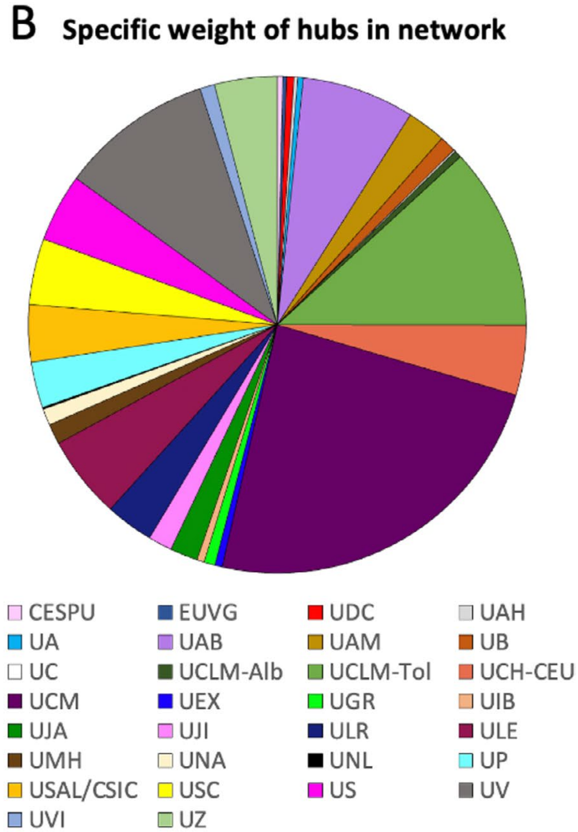
Several MicroMundo hubs have provided improvements and adaptations of the basic bacterial isolation and testing protocols for a better performance and success rate. Notably, the hub at Castilla-La Mancha University in Albacete, Spain, modified the protocol to preferentially isolate actinobacteria by using selective media for this group of prokaryotes instead of general media, typically Nutrient Agar (Davis et al. 2017) or Trypticase-Soy Agar (Valderrama et al. 2018). Furthermore, they implemented an agar disk-based technique for the antibiosis assays on testers. This change allowed the isolation of 12 strains of *Streptomyces* spp. and one *Amycolatopsis* spp. isolate from 38 soil samples (de Groot et al. 2019). The same group introduced innovations in the screening technique by setting up alternatives to the classic antibiosis assay. Rather than patching the colonies onto a freshly prepared lawn of the tester on agar, these innovative techniques take advantage of pre-diffused antibiotic compounds in the original isolation plates and involve either spraying the tester 'ESKAPE dummies' on the pre-grown isolation plates or a so-called 'reverse antibiosis assay'. The modification involved turning the agar of the isolation plate upside down into an empty plate and spreading the tester in the former agar bottom, now facing up, to detect the activity of previously diffused antibiotic substances (Alvarado et al. 2020). Other teams, like the ITQB NOVA at Oeiras, Portugal, have expanded the project by introducing a few additional steps to the protocol to identify bacteria resistant to antibiotics relevant to human and animal health (like ciprofloxacin, amoxicillin and tetracycline) in soil samples collected mainly around the Lisbon area. Likewise, the University of Valencia team also complemented the basic MicroMundo work frame with the isolation of amoxicillin-resistant bacteria in soils surrounding pig farms (unpublished data). These alternative experiments illustrate to participating students the ubiquity of multidrug-resistant bacteria in the environment.

6 | Scientific Education at Various Levels and Dissemination of AMR Awareness: The Value of CS and Service-Learning

According to the European Citizen Science Association (ECSA), CS programs are evaluated not only for their scientific output and data quality but also for their participant experience and wider societal or policy impact (Gold 2022). Although master's and doctorate students have also enrolled in MicroMundo teams over the years, around 95% of the students engaged in this widespread CS and service-learning project were undergraduate students of diverse levels. Students

A Number of secondary/high schools involved

University	2016-17	2017-18	2018-19	2019-20	2020-21	2021-22	2022-23	2023-24
	Number of Secondary/High Schools involved							
CESPU							1	2
CEU-CH		5	7	7	4	5	5	5
EUVG							1	1
UAB		15	14	2	8	10	7	5
UAH								2
UA				1				
UAM			1	7		5	3	5
UB		3	2		4			
UC		1						
UCLM-Alb			4	2				
UCLM-Tol		3	5	13	12	14	23	29
UCM	21	25	29	20	24	34	34	42
UDC		1					1	2
UEX								4
UGR						1	2	3
UIB						4		
UJA		5	4	2		1	1	2
UJI					2	2	5	4
ULE			3	8	5	11	9	9
ULR				1	11	4	5	5
UMH			3	3		1	2	2
UMU								
UNA		9						
UNL								1
UP			7	6		3	5	4
UPV								
US		2	4	4		5	11	11
USAL/CSIC		3	5	5		5	4	9
USC		3	3	3	3	6	8	10
UV		10	13			13	27	20
UVI			3	2	1	1	1	
UZ			6			12	8	8
Total	21	85	113	86	74	137	163	185



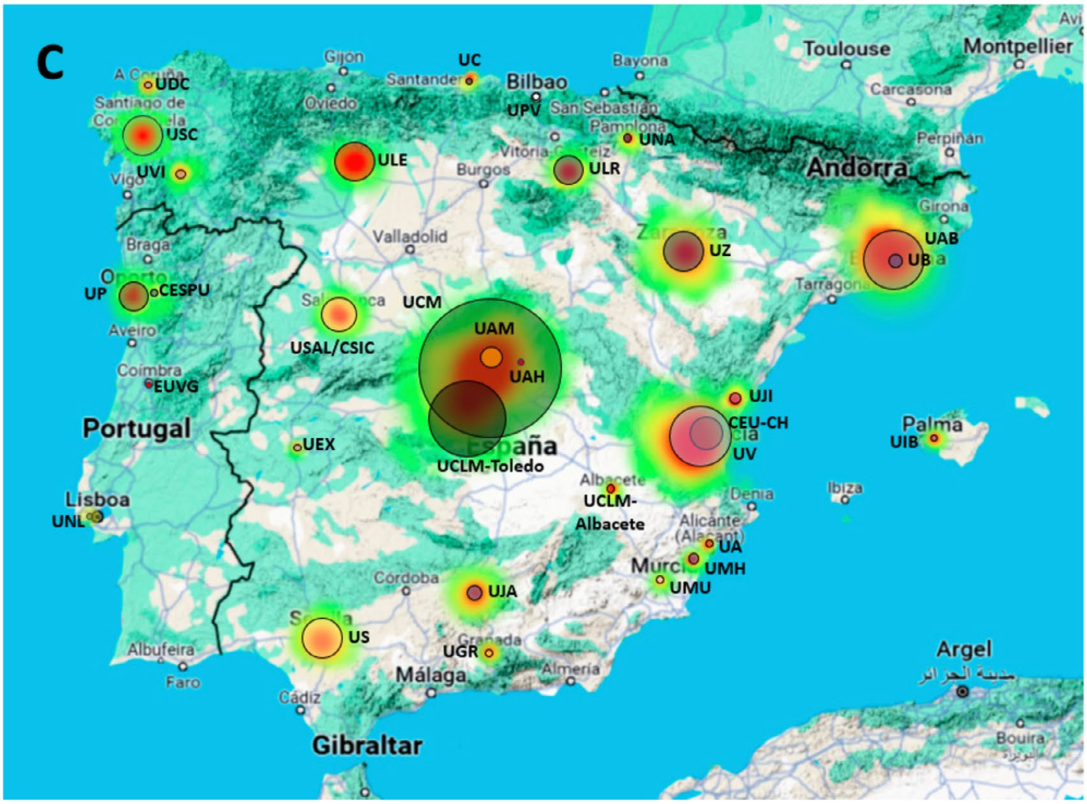


FIGURE 2 | Legend on next page.

FIGURE 2 | Development of MicroMundo hubs in Spain and Portugal. (A) Heat chart displaying the number of secondary or high schools reached per hub per academic year. Brown shades are proportional to the local outreach of the project. Grey colour denotes years in which other activities besides the off-campus CS project have been carried out. Blank spaces are years in which MicroMundo was not implemented in that hub. The sum of all schools reached in Spain and Portugal is displayed at the bottom line on a green scale. Note that the COVID-19 pandemic slowed the expansion of the project from 2019 to 2021. Some hubs involved several teams (classrooms) in the same school, so the figures relate to the number of teams rather than to the number of schools involved. (B) Chart displaying the specific contribution of each hub to the whole activity of the network along its 7 years of existence. Data were obtained by calculating the average of secondary/high schools attended by each hub per year and calculating the percentage over the network average (114.57 schools per year). (C) Map chart representing graphically the data in A and B to show geographical coverage of the project. Each spot corresponds to the hub noted, and the diameter of the spot and heat halos are proportional to the specific weight of each hub as in B. CESPU, University Institute of Health Sciences-CESPU (Gandra); EUVG, Escola Universitaria Vasco da Gama (Coimbra); UA, University of Alicante; UAB, Autonomous University of Barcelona; UAH, University of Alcalá de Henares (Madrid); UAM, Autonomous University of Madrid; UB, University of Barcelona; UC, University of Cantabria; UCH-CEU, CEU-Cardenal Herrera University (Valencia); UCLM-Alb, University of Castilla-La Mancha (Albacete); UCLM-Tol, University of Castilla-La Mancha (Toledo); UCM, Complutense University of Madrid; UDC, University of A Coruña; UEX, University of Extremadura; UGR, University of Granada; UIB, University of the Balearic Islands; UJA, University of Jaén; UJI, Jaume I University (Castellón); ULE, University of León; ULR, University of La Rioja; UMH, Miguel Hernández University (Elche); UMU, University of Murcia; UNA, University of Navarra; UNL, NOVA University Lisbon, Oeiras Municipality; UP, University of Porto; UPV, University of the Basque Country; US, University of Sevilla; USAL/CSIC, University of Salamanca/Spanish National Research Council; USC, University of Santiago de Compostela; UV, University of Valencia; UVI, University of Vigo; UZ, University of Zaragoza.

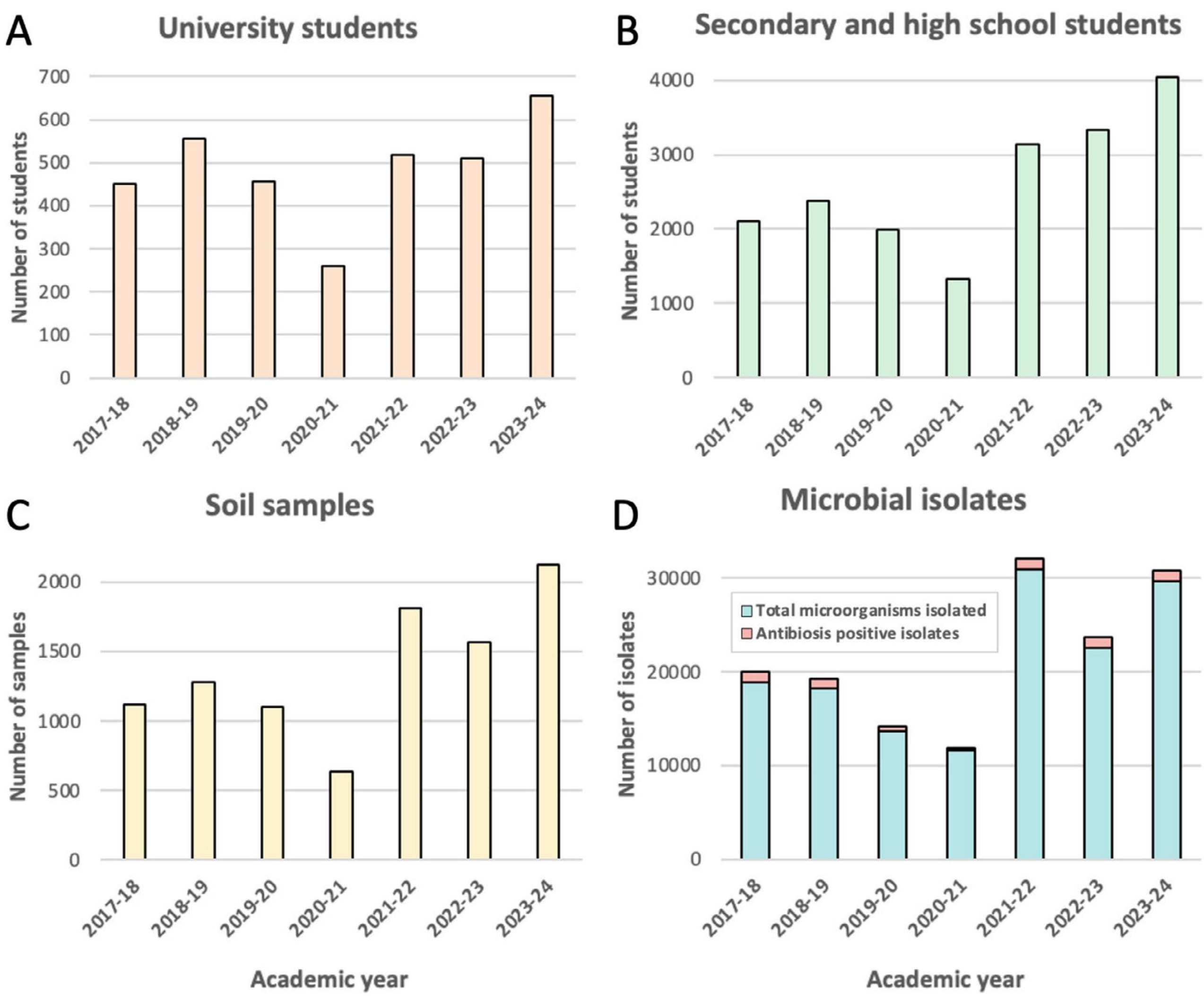


FIGURE 3 | Overall activity and results in Spain and Portugal of the CS MicroMundo activities in seven years (2017–2024). (A) Global number of university students (degree and master students) enrolled in the whole MicroMundo hubs per academic course. (B) Global number of pre-university students (secondary or high school students) enrolled in all the MicroMundo hubs per academic course. (C) Number of soil samples collected, representing the sum of all operative MicroMundo hubs per year. (D) Total number of microbial isolates tested for antibiosis per year in the MicroMundo network. The salmon colour represents the proportion of positive isolates.

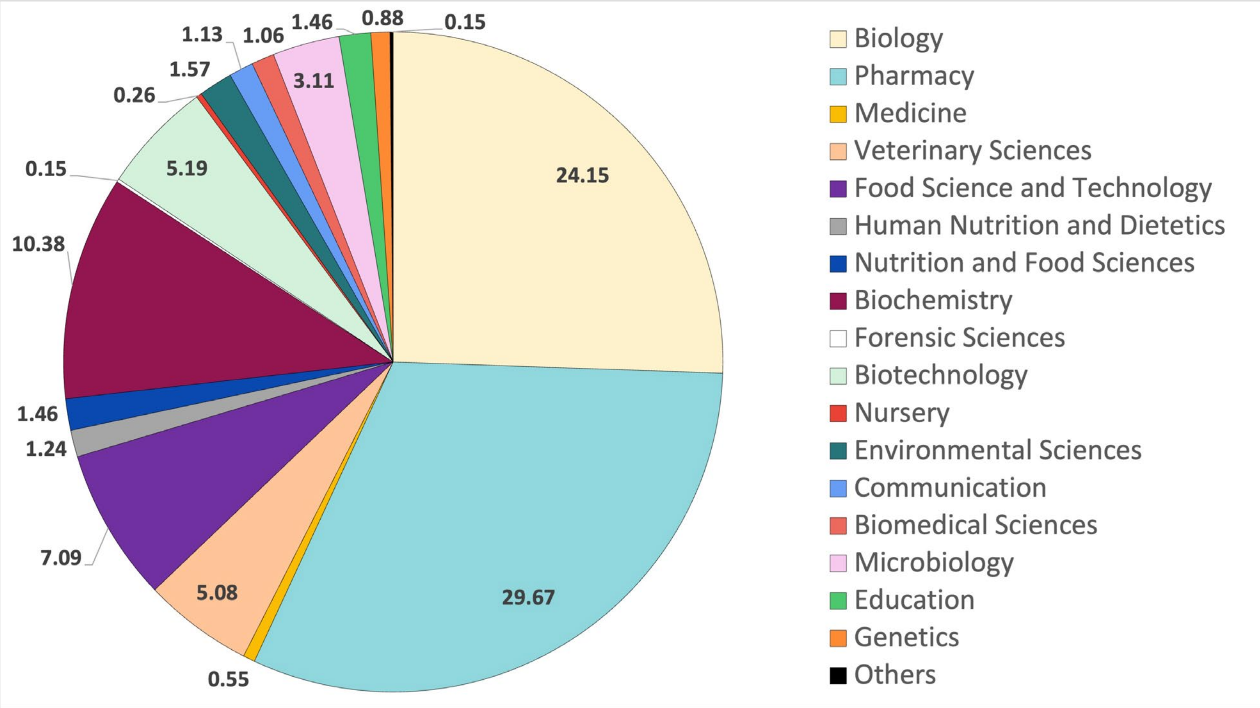


FIGURE 4 | Distribution of undergraduate students at Spanish and Portuguese universities according to their corresponding degrees. The graph represents the percentage of the total ($n = 2737$ students reported). The category 'Others' includes students of Odontology, Design Engineering and Physiotherapy degrees.

usually volunteered or were enlisted by School members when attending a microbiology subject, which is a core one in most degrees in the biomedical field. As shown in Figure 4, the most significant degrees through Spanish and Portuguese universities that contributed to MicroMundo were Pharmacy and Biology. These two fields account for more than half of the students, followed by Biochemistry, Food Science and Technology, Biotechnology and Veterinary Sciences. These data are obviously biased by the affiliation of School members leading the project in each hub. It is remarkable, for example, the low number of Medicine students enrolled in the project. This is probably due to the fact that academic clinical microbiologists are involved in medical societies but rarely members of the basic research-oriented SEM, whose teaching and dissemination division vertebrates the MicroMundo network. The specific academic offer of each university was also a cause of heterogeneity. For example, a degree in Microbiology only exists in Spain at the Autonomous University of Barcelona. Thus, it greatly contributed to the project locally but got diluted in the context of the network. In some universities, teams were comprised of students and teachers from diverse degrees (e.g., Pharmacy, Veterinary, Biology) to reflect the multidisciplinary One Health perspective.

The value of the MicroMundo service-learning strategy to gain specific competences and dexterities in microbiology, as well as general skills such as leadership, teamwork abilities and confidence in the communication of science was evaluated by the individual hubs. In the team at UCM, for example, over 90% of the participating undergraduate students claimed that their ability to communicate science had benefitted from interaction with lower education levels, and 98% considered that their interest in microbiology and their scientific vocation had been promoted (Valderrama et al. 2018). Results published by other hubs followed the same trend (Maicas et al. 2020; Antunes et al. 2021; Tarín- et al. 2022a, 2022b). These data were extracted from surveys of university students, a necessary source of data for the School teams to evaluate the impact of the service-learning strategy on their students. The completion of the survey is usually required for students to obtain credits. These surveys helped both to provide feedback to improve the project in successive academic years and to assess the outcome of the project to funding agencies, as many hubs are commonly supported by local grants in campus for innovation in higher education teaching, service-learning or science outreach.

However, to assess the value of CS, other surveys must be targeted to the individuals of the community collaborating in the research, in our case the secondary/high school students, who are the essential participants of the project. Regarding the influence of the project on future academic decisions of the participants, that is, their commitment to choosing an STEM path, the MicroMundo project should have a higher impact on secondary schools than on high schools in our environment because upon entry into high school, they must already have chosen between an experimental or a social sciences path. The indicators for assessing CS initiatives should be process-related as well as results-related (Wehn et al. 2021). Post-activity surveys were generally used to monitor the impact of the project on participants, often applying online tools like Google Survey and eAnalyzer. These also included gamified online quiz tools such as Socrative or Kahoot!. Figure 5 displays the questions most frequently included in such surveys and the

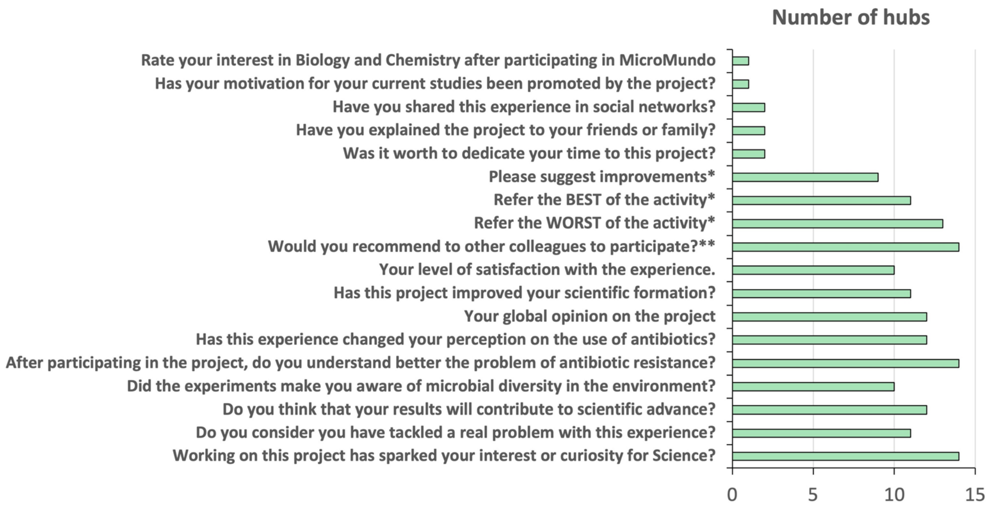


FIGURE 5 | Typical questions in MicroMundo post-activity surveys for off-campus participants and frequency of use by hubs. All questions were to be answered by rating on a scale, typically from 1 to 5, except those marked with one asterisk (*), that required a short-written answer, or two asterisks (**), that were answered as 'yes or no'. Scale indicates the number of hubs using this question in their surveys.

use made of them by 14 MicroMundo hubs enquired. To evaluate the impact of the project on knowledge gain, some hubs used pre- and post-activity surveys at participating schools as a means of objectively assessing the increase in scientific knowledge and AMR awareness (Bueso-Bordils et al. 2020; Tarín- et al. 2022a, 2022b). As an example, Figure 6 shows a representative outcome of such surveys, performed with the Google Survey platform by the UCM team through the 2023–2024 academic year. Comparing the results of over 600 pre- to almost 400 post-activity surveys, more students gave the right answers in post- than in pre-surveys in 17 out of 19 questions of general scientific culture about AMR and One Health. The global average of right answers to this multiple-option test of 19 questions was 49.76 (±17.83) % before and 60.20 (±18.62) % after participating in the CS project. This implies a 10.44% improvement in knowledge, even though the result was biased by the fact that teachers had been preparing the students, or students had commonly been pre-selected based on their interest in the field. Hence, they cannot be considered a standard naïve representative cohort of teenagers regarding basic knowledge on AMR.

Assessing the outreach of the project in the community is a challenging task. Nevertheless, some hubs, like that at León University, devised surveys for the families, thus involving the students in communicating scientific culture on infectious diseases to the community. These surveys, however, were designed with a focus on educating the population in AMR awareness rather than evaluating the impact of the project itself.

Finally, besides involving and integrating diverse levels of education, this project also offers an opportunity at higher academic levels. The scientific results of the project or the evaluation of AMR awareness in the community through the project have been the subject of degree and master theses in several hubs. Also, both the school and students have presented multiple communications to national and international congresses, either oriented towards microbiology or public health, or involving service-learning, CS and innovation in education.

7 | Beyond CS: Boosting AMR Awareness in the Community

Table 1 summarises a series of satellite activities that were performed by different MicroMundo hubs with the aim of taking advantage of the off-campus CS experience in schools to disseminate scientific culture on AMR to the community. These activities were boosted by the COVID-19 pandemic, as the lockdown and person-to-person contact limitations imposed by the epidemiological situation mostly brought to a halt the CS activities in most hubs during the 2019–2020 and 2020–2021 academic years (note the lower record of quantitative parameters measured during to the peak pandemic years in Figure 3). The experimental procedures of the project were often substituted during these years by activities that could be coordinated online and tutorised by video conferences. Most of these alternative activities involved strategies to enhance the outreach of MicroMundo goals towards the community, including the production of videos, games, materials for campaigns, both physical and in the virtual space (social networks or web pages). In this regard, one of the most active groups is the CEU-Cardenal Herrera University hub, whose activities are described in Tarín-Pello et al. (2022b). Other activities were performed routinely every year by most hubs as a complement to the experimental work, usually involving the dissemination of the results and goals of the project in the school (towards students of other levels or curricular itineraries), or outside the school to families or the community, by the organisation of scientific fairs and workshops, or participation in scientific dissemination events or in local media (TV, radio, digital media, social networks or local newspapers).

Special initiatives have been taken to expand the CS project beyond the geographical area of influence of the campus or beyond the profile of the standard student participants. The Autonomous

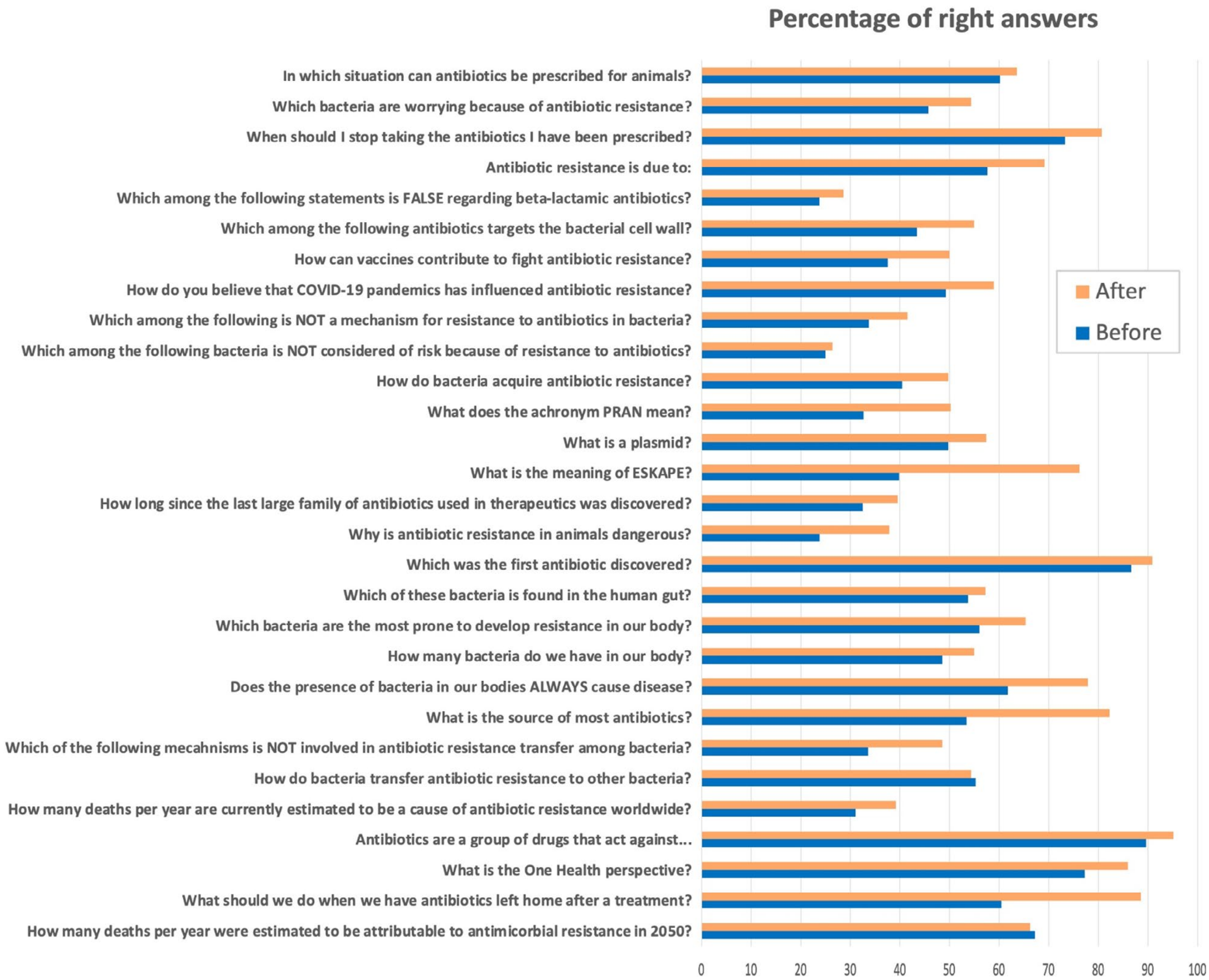


FIGURE 6 | Outcome of typical pre- and post-activity surveys performed by the UCM hub. The same survey was filled online by secondary/high school participants before starting ($n=643$) and after completing ($n=395$) the MicroMundo project. The questions were multiple-choice with four options, only one of which was correct. The graph shows the percentage of right answers referred to the total.

University of Barcelona launched a 'do-it-yourself' program so that teachers from rural schools, located far from metropolitan areas where university teams could not easily reach, were able to implement the project autonomously by providing remote formation and tuition and delivering to these areas the required material by courier transport. The University of Cantabria-CSIC node devised a successful pilot experience with students from the senior campus, retired persons usually over 65 years old who sought formation at university programs. This and other hubs also targeted special groups of students at schools that were included in high learning potential (HLP) groups, whereas the Salamanca hub included people with disabilities in the teams, interweaving the goals of the initiative with inclusion and diversity aims in education. Thus, around the core of an experimental CS program, a plethora of initiatives emerged to enhance the preparedness of society to face a global health problem.

8 | Conclusions and Future Perspectives

Since its beginnings in 2016, MicroMundo has consolidated as the most widespread AMR-focused CS project in the Iberian Peninsula, reaching thousands of pre-university students nationwide in Spain and Portugal and collecting hundreds of bacterial isolates with potential antimicrobial bioactivities.

The rationale followed by MicroMundo for the discovery of antibiotics, originally developed by the Small World Initiative and Tiny Earth initiatives in the USA, is the same one that was successful in the isolation of our current antimicrobial arsenal in the decades following the auspicious discovery of Fleming's penicillin: searching for bioactivities in soil microorganisms. Pharmaceutical companies largely abandoned this strategy from the 1980s on, claiming that such a search was no longer cost-effective. CS is a strategy that brings a necessary diversification and expansion of sampling. However, the main limitation is still our inability to cultivate the vast majority of microorganisms present in the environment, and consequently, our incapability to assess their putative bioactivities. It is of strategic importance to achieve the experimental conditions to culture the yet obscure constituents of microbial consortia in the environment. Efforts in this regard, as well as adaptation of the experimental procedures to the exploration of aquatic microbial niches may advance the scope of MicroMundo in the future.

TABLE 1 | Alternative AMR dissemination activities linked to the MicroMundo programme.

Type of activity	Hub*
Survey in the community on the knowledge about antimicrobial resistance	UCH-CEU UCM, ULE, UNL, US, USC, UZ
Dissemination session for primary school students	UAB, UCH-CEU UCM, UJA, UJI, US, UV, UVI, UZ
Scientific fairs and workshops in schools, or at the university involving pre-university students	IUCS-CESPU, UCH-CEU, UAM, UCM, ULR, UCLM-Tol, UJA, UJI, ULE, UNL, UP, US, UV, UVI, UZ
Outreach activities organised by students towards the community	IUCS-CESPU, UCH-CEU UCM, UCLM-Tol, UP, USAL/CSIC, USC
Participation in preuniversity research conferences	UAH, UCH-CEU, UCM, UNL, USAL/CSIC, UV
Performance of a karaoke with songs to raise awareness about AMR	UCH-CEU
Participation in programmed science dissemination activities (Week of Science, Science Festivals, Women in Science Day, etc.)	UCH-CEU, UCM, UCLM-Tol, UJA, UJI, UNL, UP, US, USC, UV, UVI, UZ
Writing articles for the secondary/high school newsletter or webpage	UCH-CEU UCM, UCLM-Alb, UDC, USAL/CSIC, USC, UV, UZ
Antimicrobial Awareness Week campaigns (November)	UAH, UCH-CEU, UP, USC, UV, UVI
Elaboration of posters, brochures and various materials for dissemination of scientific culture to citizens through family, friends, libraries, senior centres, etc.	UAB, UCH-CEU, UCM, UJI, USC
Elaboration of microhistories using different formats (movies, songs, etc.)	UCH-CEU
Interviews to students, teachers and school in local televisions	IUCS-CESPU, UAB, UCH-CEU, UCLM-Tol, UJI, ULE, UV
Interviews to school or students in the radio	UAB, UAM, UCH-CEU, UCM, UCLM-Alb, US, USAL/CSIC, UV
Interviews and articles in printed or digital newspapers	UCH-CEU, ULE, UCLM-Alb, UNAV, UP, US, USC, USAL/CSIC, UV, UVI, UZ
Producing videos for YouTube, social networks and/or webpages of secondary/high schools	IUCS-CESPU, UAB, UCH-CEU, UCM, UJI, UP, USC, UV
Student dissemination through social networks	UAH, UCH-CEU, UCM, ULE, UNL, US, USAL/CSIC, USC, UV, UZ
Symposia organisation (e.g., 'You can be part of the change: Improving literacy about Antimicrobial Resistance and Science Communication')	IUCS-CESPU
Keeping didactic virtual blogs	UCM, UCH-CEU
Designing card games or board games	UB, UCH-CEU, UCM, UV

(Continues)

TABLE 1 | (Continued)

Type of activity	Hub*
Designing videogames and 'ESKAPE Rooms'	UCH-CEU, UCM, UJI, US
Organising communication contests (infographics, memes and cartoons)	UCH-CEU, UP, UV
Open forum at the University: Student presentations, awards, microtalks, etc.	UAH, UAM, UCH-CEU, UCM, UCLM-Alb, UCLM-Tol, UJA, UJI, ULE, UNL, UP, USAL/CSIC, USC

*See legend to Figure 2 for hub nomenclature.

Although current priorities set the focus on the antibiotic crisis, the aims of the project can be expanded beyond the initial clinical interest of human/animal health towards the environment and food production, testing isolates against plant pathogenic fungi in search of new fungicidal strategies in biocontrol (Pino-Hurtado et al. 2024).

Moreover, the effort set on adapting the Tiny Earth postulates into Spanish and Portuguese languages could be exploited to extend the project to Latin America and Portuguese-speaking African countries if the paths for cooperation are found in the future.

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Jessica Gil-Serna: writing – review and editing, data curation, formal analysis, project administration, funding acquisition, conceptualization, investigation, writing – original draft. **Patricia Antunes:** funding acquisition, writing – review and editing, data curation. **Susana Campoy:** funding acquisition, writing – review and editing, data curation. **Ángeles Cid:** funding acquisition, writing – review and editing, data curation. **Antonio Cobo-Molinos:** data curation, writing – review and editing, funding acquisition. **Paulo Durão:** funding acquisition, writing – review and editing, data curation. **Carmen Fajardo:** funding acquisition, writing – review and editing, data curation. **Belén Fouz:** funding acquisition, writing – review and editing, data curation. **Ana R. Freitas:** funding acquisition, writing – review and editing, data curation. **Filipa Grosso:** funding acquisition, writing – review and editing, data curation. **Piet W. J. de Groot:** methodology, writing – review and editing, funding acquisition, data curation. **Trinidad de Miguel:** funding acquisition, writing – review and editing, data curation. **Bruno González Zorn:** conceptualization, writing – review and editing, data curation. **Belén Hinojosa:** funding acquisition, writing – review and editing, data curation. **Maria João Leão:** funding acquisition, writing – review and editing, data curation. **Montserrat Llagostera:** conceptualization, funding acquisition, writing – review and editing, data curation. **Rosa de Llanos:** funding acquisition, writing – review and editing, data curation. **Ainhoa Lucía:** funding acquisition, writing – review and editing, data curation. **Sergi Maicas:** funding acquisition, methodology, writing – review and editing, data curation. **Irma Marín:** funding acquisition, writing – review and editing, data curation. **Magdalena Martínez-Cañamero:** funding acquisition, writing – review and editing, data curation. **Carla Miranda:** funding acquisition, writing – review and editing, data curation. **José Manuel Molina-Guijarro:** funding acquisition, writing – review and editing, data curation. **Diego A. Moreno:** funding acquisition, methodology, writing – review and editing, data curation. **María de los Llanos Palop:** funding acquisition, writing – review and editing, data curation. **María José Pérez-Álvarez:** funding acquisition, writing – review and editing, data curation. **Pedro M. Pereira:** funding acquisition, writing – review and editing, data curation. **María Teresa Pérez-Gracia:** funding acquisition, writing – review and editing, data curation. **Sandra Quinteira:** funding acquisition, writing – review and editing, data curation. **Carmen Rioboo:** funding acquisition, writing – review and editing, data curation. **Beatriz Robredo:** funding acquisition, writing – review and editing, data curation, methodology. **José María Rodríguez-Calleja:** funding acquisition, writing – review and editing, data curation. **Rafael R. de la Haba:** funding acquisition, writing – review and editing, data curation. **Sandra Sánchez:** funding acquisition, writing – review and editing, data curation. **Manuel Sánchez Angulo:** funding acquisition, writing – review and editing, data curation. **Cristina Sánchez-Porro:** funding acquisition, writing – review and editing, data curation. **Félix J. Sangari:** funding acquisition, writing – review and editing, data curation. **Beatriz Santos:** funding acquisition, writing – review and editing, data curation. **Eduarda Silveira:** funding acquisition, writing – review and editing, data curation. **Begonya Vicedo:** funding acquisition, writing – review and editing, data curation. **Víctor J. Cid:** conceptualization, investigation, funding acquisition, writing – original draft, methodology, validation, visualization, formal analysis, project administration, data curation, supervision, resources.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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