Learnings from the pilot citizen science and AMR project

Part of the Citizen Science for Food Standards Challenges Funding Call

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Executive Summary

Background

Antimicrobial resistance (AMR) is a global societal challenge which can be characterised as a 'One Health' problem as it has implications not only for human health but also that of animals, the environment and, ultimately, the economy. Despite the significance of this threat, there remain substantial knowledge gaps in relation to transmission pathways for AMR within the food system, and home-growing is a particularly understudied space. Citizen Science and Antimicrobial Resistance (CSAMR) was a pilot project designed to collate data on the cultivation and food preparation practices of home-growers which could enrich existing knowledge on how AMR bacteria move through the food system. CSAMR sought equally to prove the efficacy of citizen science methodology to contribute to the evidence base in this research area.

Methodology

CSAMR adopted a collaborative citizen science approach, with participants being involved in multiple stages of the scientific process. Project methods comprised a pre- and post- project questionnaire, a Question & Answer (Q&A) series and swab collection exercise. The project Q&A series was designed to build participants' understanding of AMR in order to empower them to co-design and refine the project's central data collection instrument (swab questionnaire) which they subsequently completed as part of the swab collection exercise.

Participants collected swab samples from the surface of home-grown lettuces and submitted them to the research team for analysis. To examine the effects of participants' food-handling practices, each lettuce was swabbed twice (once before, and once after, preparation for consumption). Although the sampling protocol was stipulated by the team, we gave no instruction on 'preparation for consumption' in order to capture data on the diversity of preparation practice. Lettuces were selected for sampling both because their intrinsic qualities made them particularly interesting for this research (as a 'ready-to-eat' crop, many of the practices measures which might otherwise mitigate the risk of consuming pathogenic microorganisms do not apply) and for logistical reasons (since lettuces are a widely grown crop this broadened the pool of potential participants and their typical harvest period aligned well with the project timeline).

Samples received (n=254) were tested for the presence of selected target bacteria (*Escherichia coli, Salmonella* and *Listeria monocytogenes*) which, if detected, were analysed for susceptibility against a range of antimicrobials.

These methods were designed to answer the following core research questions:

1) How do people grow and prepare crops at home?

2) Are there home growing or food preparation practices which increase the likelihood of finding AMR bacteria?

3) Can citizen science methods provide quality data helping to fill evidence gaps in antimicrobial resistance research?

Key Findings

A total of 127 lettuces were swabbed by 84 participants, generating 254 samples for analysis (one sample each pre- and post- preparation for consumption). Target bacteria were detected on 38 (15.0%) of the 254 samples. The most common of our target bacteria was *E. coli,* which was detected 37 times. *Listeria* and *Salmonella* were both detected much less often (6 and 5 times respectively).

Interestingly, *E. coli* was detected more frequently on samples collected after preparation for consumption (n=22) as compared with samples collected pre-preparation (n=15). Statistical analysis of available data yielded no insight into this pattern, with no statistically significant relationship between any of the processing measures undertaken (washing method or drying method) and the detection of *E. coli*.

Out of the 48 instances where target bacteria were detected, 44 cultures (91.7%) were resistant to one or more of the antimicrobial agents tested, with multi-drug resistance (which we have classified as resistance to 3 or more antimicrobials) demonstrated in 28 (58.3%) instances.

Statistical analysis provided limited insight into factors associated with the presence of bacteria exhibiting AMR, though possible links with wild/companion animal presence, and the effects the time for which water was standing (e.g. in water butts) were highlighted. This substantiates the importance of using a One Health lens when considering AMR.

Factors affecting AMR prevalence suggested by the literature such as manure application were not supported by this study with our small sample size. This warrants further investigation in larger scale studies.

Outcomes of and Reflections on Citizen Science

The FSA and UKRI required grant recipients to partner and co-create projects with citizens. Some of our team have extensive experience of running citizen science projects, whilst for others it was their first time. All found it a very valuable learning experience, with interesting results. Challenges included the short time frame of the project and budget, along with low attendance at some of our Q&A sessions.

Conclusions

The FSA should consider running more projects using a citizen science approach as it proved a useful way of collecting data that would not otherwise be easily available, whilst improving participants' knowledge about AMR. Larger scale projects could investigate different pathogens of interest or employ other techniques such as whole genome sequencing to enable analysis of the microbiome and resistome of samples, thereby improving the evidence base.

Background

Background

Antimicrobial resistance (AMR), the insusceptibility of microorganisms to substances designed to kill them or halt their growth, is increasingly recognised not only as a global health problem, but a transdisciplinary challenge which will require the adoption of 'One Health' approaches and multi-sectoral cooperation at the human-animal-environment interface (see for example O'Neill, 2016, World Bank, 2017, DoHSC, 2019).

AMR is relevant to the agri-food sector because of the potential for AMR to be transmitted to consumers via food. As Hudson et al. (2017, p.136) summarise this can occur via 3 main routes, namely "1) the consumption of contaminated food 2) contact between humans and treated animals or 3) environmental contamination", with horizontal gene transfer (HGT) muddying the distinction between each route by readily permitting the transmission of resistance between microorganisms.

In the context of vegetable production (the focus of this study), the first and third of these routes are the most immediately relevant with several sources of AMR contamination well established in the literature. The amendment of soils with manure as fertiliser, for example, is one potential means by which antimicrobial resistant bacteria (ARB) and/or antimicrobial resistance genes (ARGs) can be introduced to crops (Tien et al., 2017), which may be relevant whether or not treated with antimicrobials (Guron et al., 2019). A similarly well-established route is via irrigation water (Holvoet et al., 2013, Arajúo et al., 2017). There is also a growing body of research on growing conditions which may co-select for resistance including pesticide application (Qiu et al., 2022) and soil type (Guron et al., 2019). Nevertheless, there remain significant evidence gaps, and possible mitigation measures often yield mixed results. For example, while some research suggests composting manures prior to application decreases the likelihood of crop exposure to manure-borne ARB (Tien et al., 2017), others have concluded that this provides incomprehensive, and therefore insufficient, protection against the risk of ARG transfer (Keenum et al., 2021). Similarly, while thorough washing of vegetables prior to consumption is endorsed in FSA guidance on controlling the spread of AMR (FSA, 2018), a recent study found washing with only tap water hardly reduced bacterial load and even sanitiser-water mixes could not reduce pathogen load to zero (Dhardmarha et al., 2019).

Much of the research to date has focused on the commercial context and often in simulated or laboratory conditions, meaning there is little data on how these risks translate to the home-growing context. Meanwhile, little is known how about home-growers handle their produce as compared with these mitigation measures, with consumer food handling behaviours being the least well-studied part of the food system (Redmond and Griffith 2003, Byrd-Bredbenner et al., 2013).

Seeking to generate evidence which could provide preliminary insight into these matters, Citizen Science and Antimicrobial Resistance (CSAMR) worked with home-growers across the UK to investigate the prevalence of AMR on home-grown produce. In so doing, CSAMR also aimed to assess whether a citizen science approach could yield high quality data relevant to research questions about how AMR bacteria moves through the food system. This research is particularly urgent given the increasing popularity of home-growing (see for example Fletcher and Tilly, 2020), including during lockdowns when significantly more people reported growing their own food; a change which, alongside obtaining more food from local producers, they expected to continue after the influence of COVID-19 on other aspects of household daily life abated (Rivington et al., 2021 p. 27 reporting UK survey data for the 'Our relationships with food during the COVID-19 pandemic' study). Equally, as an example of "ultra-local food systems" home-growing is gaining increasing attention in the context of societal and environmental pressures to reduce the environmental impacts of food production (Jarzebowski et al., 2020).

A citizen science approach was chosen because it offered multiple co-benefits including providing the opportunity for participants to learn about food safety and AMR and enabling the rapid collection of data which would ordinarily be time-intensive and difficult for researchers to collate.

Aims and Objectives

The overall aims of CSAMR were twofold:

1. To generate evidence on the cultivation and food preparation practices of home-growers which provides further insight into the ways in which AMR moves through the food system.

2. To assess the efficacy of using a citizen science approach in antimicrobial resistance research.

These aims positioned CSAMR to contribute evidence relevant to FSA Research Priority Areas 1 and 2 (as described in the FSA's 2022 statement on Areas of Research Interest), which are themselves highly relevant to the delivery of the UK National Action Plan on Antimicrobial Resistance 2019-2024 (Department of Health and Social Care, 2019). Research into the risks associated with vegetable crops, and leaf crops in particular, was also highlighted as a high priority recommendation in the Advisory Committee on the Microbiological Safety of Food's report to the FSA in 2018 (ACMSF, 2018).

To achieve these aims CSAMR established the following objectives:

1) Organise a series of project activities to support participants' understanding of AMR, how it moves through the food chain and the possible impacts of their growing and preparation practices

2) Collaboratively design and refine research methods with participants, benefitting from their expertise

3) Engage home-growers across the UK in the process of bacterial sampling and provision of relevant contextual data

4) Analyse samples provided by home-growers for the presence of antimicrobial resistant bacteria and, if present, investigate possible relationships with growing practices and preparation practices

5) Capture any changes in participant understanding and/or behaviours through a pre-post project survey

Definition of citizen science

Citizen science is where scientists and non-scientists work together to answer questions about the world. The funding call text required projects to 'be a collaboration between researchers, a specific group of citizens and, where appropriate, relevant partners from outside academia' and for citizens and partners to be involved in co-creating the projects. The FSA and UKRI provided the following documents as a guide:

- ECSA's **ten principles of citizen science (PDF, 193KB)**
- **ECSA characteristics of citizen science**
- the recent FSA publication **citizen science and food: a review**.

The specific citizen science approach adopted by CSAMR was ‘collaborative’, by which we mean that participant involvement included, but was not limited to, data collection (see Bonney et al., 2009, West and Pateman, 2017). Our collaborative approach manifested itself in two main ways. Firstly, we worked with two community partners (growing organisations, one local and one national) as members of the project team who were involved right from the project’s conception and helped to design the project and decide on our research focus. Moreover, we invited all of our participants to contribute to the design of our central data collection instrument (swab questionnaire) and to attend Q&A sessions through which they were able to share in the interpretation of our findings and shape the direction of further analysis by indicating areas of particular interest.

Methodology

Methodology

CSAMR ran from January – September 2022, with participant recruitment spanning from March – July 2022. To monitor and evaluate the effects of the project on participants’ understanding of AMR and capture any changes in their growing and food preparation practices, a pre-post survey method was adopted. This complemented the project’s central swab collection exercise which involved participants swabbing the surface of home-grown lettuces and completing an accompanying ‘swab questionnaire’ which they co-created. Participant-team interaction took place exclusively online, centred around an online platform (Padlet) and series of Q&A sessions supported by a weekly-fortnightly project newsletter.

Pre-project survey

All prospective participants were asked to complete our pre-project survey designed to assess their pre-existing understanding of AMR (including sources and causes of AMR), as well as their attitudes towards science generally. This portion of the survey was designed with reference to national scale surveys on antimicrobial resistance awareness, including the FSA’s AMR Consumer Awareness Survey (Gillespie and King, 2021) and the Public Health England (PHE) 2017 national household survey (PHE, 2020), to help contextualise our participants’ understanding of AMR by enabling comparisons with that typically exhibited by members of the general public.

Critically, the pre-project survey also collected detailed information about participants' cultivation practices including, for example, where they grow, what soil amendments they apply (as well as when they apply them within a harvest cycle), what is in their compost and what pest control measures they use.

Padlet 'Project Home'

To create a hub for project activities and provide a central repository for project information we created an online project 'home' using Padlet which operates essentially as a virtual noticeboard. Padlet's core functionality was well suited to this role; its adaptable layout and flexible posting structure allowed for the integration of a wide range of multimedia resources including embedded videos and documents, while its commenting and upvoting features enabled collaboration and communication between users. Creating this online platform as a centre point for project interactions was an integral part of our project strategy as it enabled us to facilitate asynchronous and flexible interactions, improving accessibility for time poor participants.

Padlet was chosen in favour of familiar alternatives, such as Facebook for example, on the advice of one of our community partners, who indicated that they felt Padlet offered a more secure platform for project interactions because security settings allow you to restrict access to the board to those with the link only and make it undetectable via public search. Furthermore, Padlet enables users to contribute to boards without creating an account, thus removing a potential obstacle to participation, allowing users to contribute anonymously. This feature was highly valued by the team in recognition of its potential to encourage contributions from less confident participants. An additional benefit of choosing Padlet was that we didn't have to compete with other content or navigate unpredictable algorithms to get our content seen (problems pervasive to social networks such as Facebook).

These benefits were, however, accompanied by trade-offs, the most significant of which was that, since Padlet boards are accessible independently of creating an account, the inbuilt notification mechanism cannot be relied upon to notify participants when new content has been added to the board.

Zoom Q&A series

Between May and September 2022, participants were invited to join us for a series of 15 online Q&A sessions featuring relevant experts including members of the team and external guests. Q&A sessions typically lasted half an hour and were scheduled for 7.30pm following participants' feedback that this time was preferable to them. The sessions did not follow a strict format and were instead led by participant questions, helping to balance power dynamics. Zoom was chosen both because of benefits to the participants, including familiarity and facility for flexible participation (including dial-in by mobile and text-based communication via the meeting chat), and those to the team (integrated subtitling functionality expedited the compilation of transcripts).

To maximise participation and increase the usefulness of the sessions to participants, we shared information about the speaker we'd invited and their area of expertise in advance of each session via our newsletter and by adding posts to our project Padlet. As well as peaking interest, this allowed participants time to reflect on and prepare questions making the sessions more productive. Anyone unable to attend was invited to submit questions via email or on Padlet which would be raised on their behalf by a member of the team. Despite these endeavours, attendance at Q&As was consistently low with sessions garnering 5 attendees on average (attendance ranging from 2-15 participants).

Following each session, we also posted a transcript of the session and a recording to Padlet for the benefit of any participants wishing to catch up. Using data from Zoom and our post-project questionnaire we can see that these options were well used by participants, with Zoom recordings receiving an average of 13 views and ~60% of post-project survey respondents reporting that they read a write-up from a Q&A.

The overall purpose of the Q&A series was to encourage participants to think critically about gardening practices such that they could identify specific areas of research interest and, ultimately, help to shape our swab questionnaire. Initial sessions built foundations for later work and served principally to solidify participants' understanding of key concepts including AMR itself, what is (and is not) known from the existing research in terms of associated risks and possible transmission pathways. Later sessions explored topics such AMR as a 'One Health' issue, pesticide use, pest management approaches and soil types to encourage a holistic consideration of conditions potentially co-selecting for AMR. These included topics suggested by our participants.

The Q&As were also an opportunity for us to familiarise participants with the research process more generally and allowed us to communicate with participants transparently about timescales, barriers to progress such as waiting for ethics approval and data analysis protocols. Moreover, interactions with participants in these Q&As gave us invaluable insight into their thinking, and indeed their concerns, allowing us to continually improve and adapt the materials we shared with them.

Communication

To support the Q&A series, encourage engagement with Padlet, and maintain interest throughout the project lifetime we communicated regularly with participants via the Padlet project home and an email newsletter. The newsletter typically comprised a series of short items on project events with relevant calls-to-action, including invites to take part in polls. The principal function of the newsletter was to encourage participants to engage with the Padlet and to remind them of Q&A topics and dates.

Co-designing the swab questionnaire

A core element of our collaborative approach was the co-design of our swab questionnaire. Participants were invited to get involved in this stage of the project by leaving their suggestions on a Padlet and/or attending a dedicated Q&A session. The purpose of the session was to help refine and finalise the question set, as well as to manage expectations. While we were happy to be led by participants and welcomed their expertise, the session also provided a useful opportunity to reiterate the necessary limitations of a project at this scale.

Swab collection

Participants collected from the surface of their home-grown lettuces over two weekends in early July 2022. Lettuces were selected for sampling both because their intrinsic qualities made them particularly interesting for this research (as a ‘ready-to-eat’ crop, many of the practices measures recommended by the FSA (FSA, 2018) to mitigate the risk of consuming AMR microbes do not apply i.e. lettuces are unlikely to be cooked before eaten and will not be peeled) and for logistical reasons (since lettuces are a widely grown crop this broadened the pool of potential participants, and their typical harvest period aligned well with the project timeline). In some cases, due to adverse events such as significant pest or weather-related damage causing crop failure, participants were

unable to sample lettuces as planned. In these instances, we permitted swab collection from the surface of other leafy vegetables with a preference for other varieties of vegetable which are typically consumed raw.

Sample swabbing

Two swabs (Amies Charcoal), which were labelled ‘Sample 1’ or ‘Sample 2’ were sent out to each participant along with a unique ID number. Participants were instructed to pick and swab one of the outermost leaves of their lettuce (or whichever leafy vegetable they were using), in order to maximise the likelihood of detecting AMR bacteria on the basis of previous research (e.g. Guron et al., 2019) that degree of contact with soil could be a key factor in antimicrobial resistance spread. Participants were instructed to pick a leaf and, holding the stalk end, participants were then instructed to swab (using swab from ‘Sample1’ tube) over the entire surface of the leaf, front and back, before placing back into the tube. The leaf was then ‘processed’ by whatever method is typically carried out by that participant before consumption. The leaf was then swabbed again (using swab from ‘Sample 2’), before placing back into the tube (see our swab instruction video). Swabs were sent to the Royal Veterinary College overnight and stored at 4°C for no longer than 72 hours before processing.

Bacterial culture

Each swab was removed and placed into 10 ml of buffered peptone water (BPW) and then incubated overnight at 37°C. Culture for *Salmonella* spp. was carried out according to ISO 6579-1:2017. Briefly, 100 µl was inoculated into Rappaport-Vassiliadis medium with soya (RVS broth; Oxoid) and incubated at 42°C for 24 h. A 1 ml sample from the BPW was also inoculated into Muller-Kauffmann tetrathionate-novobiocin (1 ml; MKTTb; Oxoid) broth and incubated at 37°C for 24 h. Samples (100 µl) from each enrichment culture were plated onto XLD agar (Sigma) and incubated at 37°C for 24 h. Typical colonies were selected and plated onto BHI agar and incubated at 37°C for 24 h. Samples were then confirmed as *Salmonella* spp. using *Salmonella* Test Kit using Latex Agglutination (Oxoid).

Listeria monocytogenes was cultured according to ISO 11290-1:2017. Briefly 1 ml of pre enriched BPW samples was added to 9 ml pre-warmed Half Fraser Broth (Oxoid). The samples were incubated at 30°C for 24 h. Samples (100 µl) were inoculated into 10 ml of Fraser Broth (Oxoid) and incubated at 37°C for 48 h. The samples were then sub-

cultured onto Palcam Agar (Oxoid) and incubated at 37°C for 24 h. Presumptive colonies were plated onto Horse Blood agar (Oxoid) and incubated at 37°C for a further 24 h. Identity of the *L. monocytogenes* was confirmed by Maldi-TOF analysis.

The enrichment cultures were transported to the University of Reading for the culture of *E. coli*. Samples (100 µl) were transferred to MacConkey agar and incubated at 37°C for 20 h. Presumptive coliforms (pink colonies) were transferred to nutrient agar and incubated at 37°C for 20 h. They were then confirmed as being *E. coli* if they tested negative for cytochrome oxidase enzyme (Remel BactiDrop Oxidase test kit, Thermo Scientific) and positive for the production of indole (RapID Spot Indole reagent, Thermo Scientific).

Disc diffusion testing

Cultured bacteria were tested for antibiotic resistance using the disc diffusion method (EUCAST). Using a sterile loop several fresh colonies from a nutrient agar plate were picked and suspended in a sterile peptone buffered saline (PBS) to a concentration of McFarland 0.5. The suspension (100 µl) was evenly spread onto Mueller Hinton (MH) agar. Antibiotic impregnated discs were placed onto the surface of the agar. The antibiotics used were; apramycin (15 µg), imipenem (10 µg), cefpodoxime (10 µg), tetracycline (30 µg), trimethoprim (5 µg) and ampicillin (10 µg). The plates were incubated inverted at 37°C for 24 h. Diameters were measured to the nearest millimetre and resistance was determined using EUCAST breakpoints.

Analysis and communication workshops

To bring the project Q&A series to a close we scheduled two ‘Analysis and Communication workshops’ using Zoom, during which we planned to work with participants in small groups to analyse project data together, consider how best to communicate our findings and identify groups with which we should share our findings. In advance of these sessions we invited participants to comment on a Padlet board regarding what they were most interested in finding out and add ideas about specific questions they wanted to investigate using the data. Although the sessions themselves were poorly attended (with only 2 attendees at each session) they nevertheless provided a useful opportunity to jointly reflect on what we had learned during the project.

Post-project survey

To capture any changes in participant behaviour and understanding we ran a post-project survey in September – October 2022. This survey also featured questions allowing participants to reflect on their experience and critique project design.

Citizen Scientist profile and recruitment

In total, CSAMR recruited 124 home-growers on a rolling basis from March – July 2022. Criteria for participation were that home-growers had to be over 18, have access to the internet (so that they could complete questionnaires and participate in online Q and A sessions), and have access to 'home-grown' lettuce for sampling. By 'home-grown' we mean not commercially grown and included in our project scope lettuces (and other salad leaves) grown in allotments, community gardens and other shared growing spaces.

A key reason for adopting this broader definition was to widen participation in recognition of the significant disparities which exist regarding access to gardens across ethnic and socio-economic groups (data from Natural England's Monitor of Engagement with Natural Environment survey 2014-19 demonstrates that while "one in eight British households has no garden", Black people in England are four times as likely as White people not to have any outdoor space at home, and "people in semi-skilled and unskilled manual occupations, casual workers and those who are unemployed are almost three times as likely as those in managerial, administrative, professional occupations to be without a garden (20% compared with 7%)" (ONS, 2020a).

To recruit participants, we prepared some short promotional text incorporating explicit links to key motivating factors for taking part in environmental citizen science projects (as discussed in West and Pateman, 2016), with a core focus on opportunities for personal development (learning new skills) since it has been suggested that this is particularly influential for groups typically underrepresented in citizen science (West, Pateman and Dyke, 2021). Recognising that time constraints often prove a barrier to participation and are likely to disproportionately affect those with caring responsibilities and those in lower socio-economic groups (Pateman, Dyke and West, 2021), we also clarified the minimum time commitment and emphasised that participants could be involved as much, or as little, as they liked.

This text was shared by project partners via a multitude of different mechanisms. Initially the invite was shared via various Garden Organic communications channels (social media, email newsletter, magazine and online), posts in relevant Facebook groups and researchers' personal social media accounts (Twitter and Facebook). To counteract the self-selection bias intrinsic to this 'scattergun approach' (West and Pateman, 2016), and in recognition of the value attributed in the literature to enlisting the help of gatekeeper organisations for improving the diversity of participation, (highlighted by Unell and Castle, 2012) we dedicated funding to community partners reaching out to their pre-existing contacts with a view to recruiting traditionally 'hard-to-reach' groups specifically.

When these methods proved slow despite the large audiences of groups targeted, we supplemented them by sharing via internal University mailing lists (Staff digest, RVC mailing) which we had initially been resistant to do and by mailing Garden Societies and community growing groups directly with a specific focus on areas with high proportions of ethnic minorities (based on UK census data). To bolster these efforts, we dispensed with the staggered recruitment process we had initially adopted, whereby prospective participants were encouraged to indicate their interest via email and sent an invite to complete our pre-project survey in response, in favour of hosting the survey online and making it available for completion directly. The drawback of this approach was that it obscured the relationship between promotional channel used and level of sign-ups in response. Equally, we adapted our promotional material several times over in response to potential barriers highlighted by team members after reflection.

Participant demographics

In total one hundred and twenty-four home-growers completed our pre-project questionnaire. Based on the data from this questionnaire we can see that typically our participants were:

- White (including any white background) (93.5%)
- Female (69.34%)
- Aged 45+ (62.1%)
- Educated to undergraduate degree level or higher (88.7%)

Throughout the project the most common participant profile was Female, White, and educated to undergraduate degree level of higher, accounting for 59.7% of pre-project respondents, 61.0% of swabbers, and 66.7% post project respondents).

Ethics

This project was subject to ethics review at the University of York, via applications made by Dr Sarah West (project lead) to the Department of Environment and Geography Ethical Review Committee. Ethics approval was granted for three separate applications submitted in sequence (approved on 03/03/2022, 01/06/2022 and 05/08/2022 respectively), each covering different elements of the project. This stepwise approach facilitated the evolution and refinement of project strategy as the project progressed and allowed for the co-creation of our central data collection instrument (swab questionnaire).

The project's first ethics application covered initial activities (pre-project survey, Zoom Q&A series and creation of the Padlet 'project home') and addressed overarching ethical issues including, in particular, that the project's core focus (antimicrobial resistance) has been shown repeatedly to be poorly understood by the general public (see for example FSA (2021) and PHE (2020)) which meant that our project activities were likely to alert participants to the existence of harmful bacteria of which they may otherwise have been unaware, leading to potential distress. Subsequent applications covered mechanisms associated with swab collection activities (swab registration form, swabbing activity and swab questionnaire), the post-project survey and analysis and communications workshops.

Alignment with citizen science principles

CSAMR aligned with ECSA's 10 principles of what constitutes citizen science in the following ways:

Principle 1. Citizen science projects actively involve citizens in scientific endeavour that generates new knowledge or understanding. Citizens may act as contributors, collaborators, or as project leader and have a meaningful role in the project.

The capacity of CSAMR to adduce data relevant to antimicrobial resistance research was reliant on, and considerably improved by, the active involvement of home-growers both in their capacity as data collectors and, equally, in their role as co-designers of our swab questionnaire.

Principle 2. Citizen science projects have a genuine science outcome

By exploring the prevalence of AMR bacteria on home-grown produce with reference to different growing and food-handling practices, CSAMR sought to enrich the existing literature on transmission pathways for AMR bacteria within food systems. This is novel and important research because the home-growing context has received little research attention thus far, and yet the incidence of home-growing is demonstrably on the rise. Equally, as a pilot project seeking to gauge the feasibility of using citizen science approaches for antimicrobial resistance research, CSAMR aimed to introduce a new audience of researchers, and future researchers, to citizen science methodology.

Principle 3. Both the professional scientists and the citizen scientists benefit from taking part.

By adopting citizen science methodology, we were able to collect a greater volume of data, over a greater geographical scale, than we would otherwise have been able to achieve in such a short project cycle. The involvement of our participants in multiple stages of the research process (see next point) was invaluable as it enabled us to benefit from their considerable expertise. Participant insight into the reality of home-growing had a particularly favourable impact on the development of our swab questionnaire. Indeed, as well as helping to fine-tune proposed questions, participant input led to the removal of some questions proposed by the team and the addition of new questions to capture data on variables we had not initially considered.

Through inviting home-growers to participate in multiple different stages of the project (see next point) CSAMR provided opportunities for participants to develop new skills and hone existing strengths and potentially also to improve their understanding both the subject of our research (AMR), and the research process more generally. Furthermore, our project Q&A series and process of co-design allowed participants to shape the research and explore areas of particular interest to them in more detail.

Principle 4. Citizen scientists may, if they wish, participate in multiple stages of the scientific process.

CSAMR offered participants the opportunity to get involved in multiple stages of the scientific process beyond data collection including helping co-design our core data

collection instrument (swab survey), thereby refining the focus of our research, and assisting with the interpretation and analysis of project data.

Principle 5. Citizen scientists receive feedback from the project.

We communicated with participants regularly throughout the project lifetime and were able to offer them feedback in a variety of ways. We discussed the research process openly with participants, including project logistics and barriers to progress. When we worked with participants collaboratively to design the questionnaire, we shared an annotated copy of the final version with them indicating all the instances in which their input had been influential as a means of acknowledging the value of their contributions. As soon as we had them, we shared initial findings with our participants and discussed with them the limitations of what we were able to say without further analysis. At the end of our project we followed this up with a summary of our results.

Research Findings

Home-growing and food preparation practices

Through our project questionnaires participants provided in depth information about their home-growing practices. Of particular interest are

- **Data on diversity of growing space used** - using data from our pre-project questionnaire, we found that home-growers cultivate produce in a variety of spaces including - outside (62.9%), in a Green-house or Polytunnel (8.1%), inside house (e.g. windowsill or conservatory) (0.8%), or more than one of these spaces (28.2%). Our swab questionnaire data (focusing on the specific growing conditions of samples swabbed) shows similarly that the final growing location of participants' lettuces (when lettuces had reached maturity and swab samples were collected) was typically outdoors (81.6%) - whether in raised beds, pots or trays, or directly in the soil - while 17.6% grew lettuces inside or in covered spaces - including in Polytunnels and Green-houses. However, having amended our questionnaire options to better reflect growing practice we found that for many samples (64 or 51.2%) more than one growing space had been used during the lettuce's lifetime.
- **Soil amendments applied** - see Table 1 below.

Table 1. Soil amendments applied by participants

Soil amendment type	Number of times reported	As % of samples
Homemade garden compost	83	66.4%
Animal manure (e.g. horse manure, chicken manure)	48	38.4%
Commercial compost (e.g. potting or multipurpose)	45	36.0%
Commercial fertiliser made from plant products (e.g. seaweed, comfrey, rapeseed meal)	25	20.0%
Homemade liquid fertiliser (e.g. comfrey or nettle liquid)	25	20.0%
Green manures (plants grown to improve the soil, e.g. clover, phacelia, vetch)	20	16.0%
Chicken manure pellets	18	14.4%
Homemade leafmould	15	12.0%
Other	15	12.0%
Commercial soil improver made from (green waste) compost	11	8.8%
Commercial fertiliser made from animal products (e.g. hoof and horn, bonemeal, dried blood)	9	7.2%
Top Soil	9	7.2%
Commercial fertiliser made from mineral products (eg potash, rock phosphate)	8	6.4%
Lime	7	5.6%

Soil amendment type	Number of times reported	As % of samples
Soil improver made from anaerobic digestate (this is likely to be stated explicitly on the packaging e.g. Plantgrow)	3	2.4%

- **Data on food preparation practices undertaken** - we asked participants to prepare their lettuce for consumption in the way that they normally would and describe this preparation process for us. For 112 samples (89.6%) some form of washing was reported - ranging from washing under a tap, in a bowl, or using a salad spinner - with the remaining 10.4% (13) of samples not undergoing washing of any kind. For samples where no washing was reported a wide variety of practices were described such as brief visual inspection, manual preparation (including brushing off dirt and removing pest-damaged areas) and, in 7 cases, no preparation of any kind. For 77 samples (61.6%) some form of drying was also reported, again encompassing a wide variety of practices such as shaking dry, patting dry on kitchen towel or on a cloth and salad spinning.
- **Data on food preparation practices in alternate scenarios** - Having recorded how they had actually prepared their lettuce sample for consumption, participants were also asked to report how their preparation methods would have differed (if at all) if the produce was 1) going to be cooked or 2) shop-bought. In both scenarios, we found that there was a fairly even split between the number of participants stating that the preparation would have been the same and those stating preparation would have differed (51.2% and 50.4% reporting preparation would have been the same for each scenario respectively).

  For produce destined to be cooked prior to consumption, the most commonly reported points of difference were that no drying method would have been applied and that additional preparation measures such as peeling or scrubbing would have occurred. For shop-bought produce, two key trends emerged with some participants stating that produce would not be washed (in some cases depending on the instruction on the packaging, if any), while others stated either that that they

would have washed shop-bought produce, with 9 (7.2%) specifying that they would have washed shop bought veg more thoroughly. Various reasons were given for those stipulating they would have taken extra care, including concerns about handling of produce and pesticide residues.

Frequency of target bacteria detection

Over two weekends in early July 2022, 84 participants collected 254 swab samples from the surface of their home-grown lettuces (or other leafy vegetables) and submitted them for analysis by the team (samples were collected in pairs, yielding 127 samples pre-, and 127 samples post-, preparation from consumption).

Target bacteria – *Escherichia coli* (E. coli), *Salmonella* subspecies and *Listeria monocytogenes* (*L. monocytogenes* – were detected on 38 samples (15.0%). Out of our target bacteria *E. coli* was the most frequently detected, present on 37 samples, while *L. monocytogenes* was detected on six samples and *Salmonella* on five.

Table 2. Frequency of target bacteria detection (*E. coli*, *Salmonella* and *L. monocytogenes*) on home grown lettuce samples

Detections	***E. coli***	***L. monocytogenes***	***Salmonella***
Total number of times detected*	37	6	5
Of which pre-preparation for consumption (Plate 1)	15	5	4
Plate 1 frequency	15/127 (11.8%)	5/127 (3.9%)	4/127 (3.1%)

Detections	*E. coli*	*L. monocytogenes*	*Salmonella*
Of which post-preparation for consumption (Plate 2)	22	1	191.7
Plate 2 frequency	22/127 (17.3%)	1/127 (0.8%)	1/127 (0.8%)

*Note: total number of times target bacteria were detected differs from the number of samples on which target bacteria were detected because some participant samples had more than one bacterium per plate or bacteria on both plates

E. coli was detected more frequently on samples collected after preparation for consumption (n=22) as compared with samples collected pre-preparation (n=15). Not only was it detected more frequently post-preparation for consumption, in 15 instances *E. coli* was detected post- preparation for consumption when it had not been detected pre-preparation for consumption (see table 3 below). Statistical analysis of available data yielded no insight into this pattern, with no statistically significant relationship between any of the processing measures undertaken (washing method or drying method) and the detection of *E. coli.*

Table 3. *E. coli* detection patterns

***E. coli* detection patterns**	**Number of samples**
E. coli detected on plate 1 only	8
E. coli detected on both plates	7
E. coli detected on plate 2 only	15

Antimicrobial susceptibility

Out of the 48 instances where target bacteria were detected, 44 (91.7%) were resistant to one or more of the antimicrobial agents tested, with 28 of those samples (58.3%) demonstrating resistance to 3 or more antimicrobials, (which we have classified as 'multi-drug resistant'). This pattern varied across target bacteria with 64.9% of *E. coli* samples demonstrating multi-drug resistance, compared with only 40.0% of *Salmonella* samples and 33.3% of *L. monocytogenes* samples. Summary results from susceptibility testing are shown in Table 4 with further detail on the specific antibiotic to which resistance was demonstrated in Table 5. This shows that Cefpodoxime resistance was widespread (72.9% of samples demonstrating cefpodoxime resistance), while tetracycline resistance the least common (14.6% of samples). The most commonly observed resistance profile (accounting for 6 samples) was imipenem, apramycin, ampicillin and cefpodoxime.

Table 4. Resistance of target bacteria to antimicrobials.

Shaded cells indicate samples demonstrating multi-drug resistance

Number of antimicrobials to which resistant	Frequency (number of target bacteria demonstrating this resistance profile)	As proportion of samples where target bacteria detected
0	4	8.3%
1	7	14.6%
2	9	18.8%
3	14	29.2%
4	12	25.0%
5	2	4.2%

Table 5. Number and percentage of samples demonstrating resistance grouped by antibiotic to which resistant

Antibiotic	Number of target bacteria demonstrating resistance	As proportion of samples where target bacteria detected (n=48)
CPD cefpodoxime (10 µg)	35	72.9%
APR apramycin (15 µg)	29	60.4%
AMP ampicillin (10 µg)	25	52.1%
IPM imipenem (10 µg)	16	33.3%
W trimethoprim (5µg)	13	27.1%
TE tetracycline (30 µg)	7	14.6%

Statistical analysis

For samples where antimicrobial resistance was demonstrated the following findings are of particular interest:

- **Application of manures -** Statistical analysis of our data determined that there was no significant association between the use of manure and whether target bacteria detected demonstrated AMR, either when considered as a binary variable (whether or not it had been applied), or looking at source of manure.
- **Water source and time standing -** Water source was indicated by participants as a factor of particular interest. Our analysis found no significant association between water source (Mains water from a tap or tank, Ground water (from a well or borehole), rainwater from a water butt or any combinations thereof) and AMR

bacteria detection, despite there being 80% lower odds of AMR when rainwater was used compared with mains water (p=0.64). When participants reported using rainwater from water butts, we also collected data on the estimated amount of time water typically stood in their water butts. Analysis of this data shows that there was a significant reduction in odds when water stood for weeks or months compared with days *(*p=0.025 and p=0.002 respectively).

- **Animal activity -** We asked home-growers to give details of any animals or birds which they were aware had access to their growing area. Our analysis found that the reported presence of both cats (OR=3.01, p=0.009) and foxes (OR=6.94, p<0.001) were significantly associated with increased odds of AMR. However when adjusting for the presence of foxes, the presence of cats was no longer significant (OR=2.04, p=0.119). The presence of foxes remained significant after adjusting for cats (OR=5.36, p=0.003). This finding should be taken cautiously, as some participants' reports of animal activity were speculative, and the effects of pest prevention measures they described is uncertain.
- **Food preparation methods -** no statistically significant relationships were found between the detection of AMR bacteria and any of the various food preparation methods reported by participants. This encompasses both washing and drying when taken as binary variables, (X^2 = 0.008, df = 1, p =1.000 and X^2 = 0.104, df = 1, p =0.747 respectively), and is equally true for specific practices reported such as manual drying i.e. shaking and salad spinning for example (X^2 = 0.041, df = 1, p =0.840 and X^2 = 0.322, df = 1, p =0.554 respectively).

Changes in participant knowledge or behaviour

Only 33 out of 124 participants completed our post-project survey. Although we envisaged a tiered participation structure (in line with the 80:20 law acknowledged by many citizen science practitioners), and expected a degree of attrition, this is disappointing. With such a small sample size, it is not possible to draw conclusions about changes in knowledge or behaviour for our participants as a whole.

For those completing the post-project survey we can say:

- For the majority of participants, participation in the project did not lead to any changes in confidence regarding their relationship with science (assessed through questions on participants' self-reported understanding of science stories in the

news, confidence discussing science stories with others and the degree to which they felt able to make contributions to science). This lack of change is likely explained by the high percentage of participants educated to Undergraduate degree level or higher (87.9%). Certainly, several participants' volunteered information via our pre-project survey that they had either degrees in science subjects or were currently employed as scientists.

- When asked whether the way they garden has changed as a result of the project, only 21% (7 participants) answered positively. This is not altogether unsurprising because, as a pilot project, we were only able to draw tentative conclusions from the microbiological analysis we conducted rather than offer categorical recommendations.
- A more appropriate indicator of the project's success therefore lies in participants' reflections on how much they learned, with ~70% of post-project respondents reporting that they had learned something from the project.

Outcomes of and Reflections on Citizen Science

Key things learned

This pilot project suggests that citizen science is an appropriate method for gathering data about the prevalence of antimicrobial resistant bacteria in home-grown vegetables, and that through this method, participants can learn about this complex and important topic.

Recruitment and participation levels

We struggled with recruitment, and participation in the project as a whole was lower than anticipated (only attracting ~120 participants compared with a target of 300), although the number of people swabbing produce was more than originally intended. We have reflected on this as a project team and we consider that this could be because as society is still recovering from the COVID-19 crisis, appetite to consider bacterial contamination is reduced. Indeed, anecdotal evidence from our PI's experience of promoting the project at an in-person event was that prospective participants expressed that they 'just weren't interested in finding out what was on their lettuces'. Equally, since it is well evidenced that gardening offers a multiplicity of well-being benefits, including stress relief (even

outside of organised therapy schemes e.g. Genter et al., 2015, Dobson et al., 2020) and these benefits are also self-reported (with 44% of adults surveyed across Great Britain reporting this something that helped them cope whilst staying at home during lockdown in April 2020 (ONS, 2020b), and approximately 1 in 3 people with access to a garden saying they spent time in it for their mental health and well-being according to People and Nature Survey data covering March 2020 – April 2022 (Natural England, 2022), we surmise that home-growers may have been reluctant to introduce stress in this environment by considering levels of harmful bacteria on their produce.

We are also aware that our pre-project questionnaire constituted a significant hurdle for participants to overcome and recognise on reflection that it introduced uncomfortable power dynamics. Our desire to assess pre-existing understanding of AMR and potential changes as a result of participating meant we could offer little explanation of terms we were aware were quite poorly understood. This may have had the effect of putting off those who don't know much about AMR by making them think that the project is not for them (and may in part explain why our participants are not as representative as we'd hoped). Indeed, some participants told us in Q&A sessions that although they feel confident about AMR they would be reluctant to promote the project to their peers since it is a potentially scary topic. We also received critical reflections on the pre-project questionnaire via email; in the words of one participant:

“I found the whole [pre-project questionnaire] quite intense with a lot to read. The opening section should surely explain what AMR is and why the project matters? I’m still not clear actually….I think this could be more inviting and user-friendly.”

We also struggled with low levels of participant interaction throughout the project which may partly be down to our choice of platform, as Padlet is not a place people ‘hang out’ online, like Facebook for example. Previous projects, for example, Parenting Science Gang (Collins et al 2020) which used Facebook had a much larger pool of people contributing to study design and analysis than actually took part in experiments.

Learnings for researchers

The Q&A sessions were a useful way of increasing the whole team’s knowledge about AMR and factors that could influence it. These were enjoyable sessions, attended by

between 2 and 15 participants, and the session recordings were viewed a total of 129 times[1], indicating that those not attending in real-time were interested in their content.

Different groups in the team gained different things from the project. One of our microbiologists noted “I found this project to be a great way to learn about how to engage with citizen scientists in a variety of different ways, such as evening meetings, newsletters, speaking with organisations. I was really impressed with the input and engagement we had from them.

Working with people from a range of different fields of work was interesting and useful to get a wide range of inputs into what we were trying to achieve and why.

Difficulties have been trying to report our results in an accessible way and in a way that would not induce ‘panic’ or worry regarding the presence of bacteria in home grown produce.

It has also been interesting getting across the scientific process and trying to reiterate that it is an ongoing process of asking questions and trying to design experiments to answer them.“

Our PI noted: “I have lots of experience running citizen science projects but I’ve not done anything with home-growers or microbiology before, so I learned a lot about AMR as a result of this project. The value of regular contact with participants (in our case via a newsletter and Q&A sessions) was reiterated to me, and although the Q&A sessions weren’t as well attended as we hoped, people found them really valuable and the experts really enjoyed them so we will incorporate more of them into future projects”.

Our project co-ordinator said “I had not used Padlet before but found it intuitive to set up and our participants found it easy to use. As a result of this project, I’m now using it on a big citizen science project we are running with school teachers.”

Learnings for participants

In their own words, here are some of our participants’ reflections grouped by topic:

Things learned:

[1] For the 11/15 project Q&A sessions for which this data is available a total of 129 views have been recorded.

- I have learnt lots about AMR both through the project and from other sources. I also feel that the subject can seem quite frightening, but the project has made me feel that with more research there are practices that could be implemented that could limit the extent of the problem.

- [I] Had never specifically thought about resistant bacteria contaminating produce.

- I learnt more about AMR, and about the different scientific work going on around AMR. I've also learned about how data is presented and analysed.

Things gained (what if anything do you feel you got out of the project):

- I enjoy participating in citizen science projects and like the idea that the public can contribute to scientific analysis. I found it interesting learning more about AMR and listened to radio podcasts on the subject to widen my understanding.
- Satisfaction of contributing to the experiment
- A greater understanding of how scientific research works, and feeling like I have contributed to important research.

Ethics

As with any citizen science project, ethical issues need careful consideration. We were very aware that for many people AMR was a scary topic, and so spent a lot of time ensuring that our language was clear, that people had opportunity to ask any questions, that we explained what the data showed (and didn't show), and about the low risks that AMR bacteria provided for healthy individuals. Nonetheless some participants who had AMR bacteria on their produce were concerned, and were not reassured by the generic guidance that was provided by the FSA about vegetable preparation, as in most cases they had washed their produce.

Conclusions and Implications

This study confirms the power of using citizen science approaches for microbiological research. Not only is the methodology uniquely suited for giving insight into understudied areas by providing researchers with access to hard-to-reach spaces and data on lived

experiences, but it also offers potential for improving awareness of a topic and enthusing participants about the research process.

Given our small sample size, factors highlighted as potentially linked with the frequency of detection of AMR bacteria warrant further investigation in future studies. Further investigations could equally probe how variables relevant to AMR prevalence suggested by the literature, such as manure use, translate to the home-growing sphere since our data does not support these associations.

Future studies could be designed specifically to test hypotheses surrounding post-processing methods, allowing investigation into topics such as whether, for example, processing of the samples could also be contributing to the frequency with which AMR bacteria are found by removing competing non-target bacterium allowing our target bacteria to grow. Equally, future studies could investigate other pathogens of interest or employ techniques such as whole genome sequencing to enable analysis of the microbiome and resistome of samples gathered.

The possible links found between AMR bacteria and the presence of wild/companion non-human animals highlight the importance of viewing areas such as this through a One Health and systems lens as humans, plants, other animals and the environment are all linked.

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Annex

Statistical appendix

Variables	X^2	df	p
Target bacteria detected (on either plate) * Lettuce type (best match from classification e.g. 'Tight, compact, head of leaves (Crisphead or Iceberg)', 'Loose, soft and ruffled (Butterhead)')	3.910	6	0.678
Target bacteria detected (on either plate) * Grown in Pot, Bed or Container (as binary)	0.707	1	0.400
Target bacteria detected (on either plate) * Use of animal manure (as binary)	2.098	1	0.147
Target bacteria detected (on either plate) * Use of home-made compost (as binary)	0.015	1	0.901
Target bacteria detected (on either plate) * Heat of compost (best match from classification: 'not much hotter than air temperature' – 'too warm to touch')	1.041	3	1.000
AMR on either plate * Use of animal manure (as binary)	1.873	1	0.171
AMR on either plate * Use of home-made compost (as binary)	0.165	1	0.685
AMR on either plate * Heat of compost – (best match from classification: 'not much hotter than air temperature' – 'too warm to touch')	1.255	3	0.967
AMR on Plate 1 * Lettuce type (best match from classification e.g. 'Tight, compact, head of leaves (Crisphead or Iceberg)', 'Loose, soft and ruffled (Butterhead)')	1.826	6	0.958
AMR on Plate 1 * Grown in Pot, Bed or Container (as binary)	1.034	1	0.309

Variables	**X^2**	**df**	**p**
AMR on Plate 1 * Most recent application of home-made compost (‘In the last month’ – ‘over a year ago’)	3.391	5	0.558
AMR on Plate 1 * Final Growing Location (‘Inside (or covered) including polytunnel or greenhouse’, or ‘outside’)	0.812	1	0.354
AMR on Plate 1 * Commercial soil improver made from green waste compost (as binary)	0.066	1	0.679
AMR on Plate 1 * Green Waste in Homemade compost (as binary)	0.359	1	0.549
AMR on Plate 1 * Use of water from a water butt (as binary)	0.695	1	0.405
AMR on Plate 2 * Washing (as binary)	0.008	1	1.000
AMR on Plate 2 * Washing in bowl (as binary)	0.002	1	0.968
AMR on Plate 2 * Drying of any kind (as binary)	0.104	1	0.747
AMR on Plate 2 * Manual drying (as binary)	0.322	1	0.554
AMR on Plate 2 * Use of a salad spinner for drying (as binary)	0.041	1	0.840

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