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HOUSEHOLD ARTHROPODS AND THEIR ASSOCIATED BACTERIAL COMMUNITIES

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Abstract Considering the ability of arthropods, to act as vectors of pathogens, an understanding of bacterial species associated with household arthropods and hence their significance to public health is also limited. This study aimed to investigate household arthropods in the United Kingdom, exploring their populations in homes located within a 15-mile radius of Birmingham UK over a 12-month period, and to investigate their associated microbial communities. Arthropods were collected by trapping, with light-emitting diode (LED) light flytraps and sticky traps, and by volunteers making active captures. Bacterial communities were explored through culture-based methods and 16S rRNA metabarcoding. In 11 months of collection 2,125 arthropods were sampled from 19 households that provided results. Diptera were the most abundant arthropods collected, representing 30.1% of the total, followed by Zygentoma (16.2%), Araneae (13.9%) and Collembola (8%). There were 324 bacterial isolates recovered from the arthropods analysed. Of isolates, 48.5% were Gram-positive cocci, followed by Gram-positive rods (24.1%), Gram-negative rods (13.6%) and Gram-negative cocci (5.6%), the remaining represented by filamentous bacteria, yeasts and Gram-negative coccobacilli. The isolates with a high bacterial load were identified, to the species level, revealing the presence of opportunistic pathogens such as *Staphylococcus* spp, *Acinetobacter* spp and *Serratia* spp. Work is to be developed regarding the epidemiological analyses of culture independent microbiological metabarcoding data, which will assist in informing and prioritizing pest control strategies in housing in relation to public health risks.

Key words Insects, disease, microbiology, housing, pest control

INTRODUCTION

The human population, particularly in urbanized areas, spend 90% of their time indoors and built structures are known to be rich sources of microbial species (Shan et al., 2019). A better understanding of this environment, from an arthropod-microbe interaction and therefore public health pest control point-of-view, is especially appropriate as arthropods are the most common animals present indoors (Bertone et al., 2016). Arthropods and humans have a long history together, but our knowledge related to their presence indoors is biased towards pest species, such as those that damage people, pets or belongings, causing economic loss or health concerns (Bertone et al., 2016). Surprisingly, a recent study showed that the majority of the arthropods found in 50 homes, located in Raleigh (North Carolina, USA), were casual intruders typically associated with the outdoor environment with only a low percentage of the arthropod populations found in the surveyed households being represented by pest species (Bertone et al., 2016). Madden et al., (2016) investigated the household arthropod populations in hundreds of homes, across the United States, by utilising a gene marker sequencing approach to analyse dust samples. The work provided deeper insights into the composition, and diversity of, household arthropod populations while revealing results like the more traditional sampling approach of Bertone et al. (2016). Indeed, aphids (*Aphis* spp), which are plant-associated insects, were found in the 30% of the homes studied by Madden et al., (2016). This finding showed that the most common arthropods detected are not necessarily those described, traditionally, as being associated with the indoor environment. The authors found statistically significant differences between rural and urban homes, with rural homes harbouring 50% more arthropods than urban households, and homes with a basement showed a higher diversity compared to homes without it (Madden et al., 2016). Leong et al., (2017) investigated the factors that affect the diversity of household arthropods. According to their analysis the presence of cats and dogs did not significantly affect the composition of the arthropod community, in the 50 homes surveyed, while on the contrary Madden et al. (2016) showed that the arthropod diversity observed in the 1,462 homes studied was greater in homes with dogs and lesser in homes with cats.

The knowledge related to arthropods indoors in the United Kingdom, in terms of diversity and abundance, is still scarce. The attention is pointed mainly towards pest species that lead to economic loss in commercial situations (Freeman, 1948; Tebb, 1968) or public health risks such as flying insects carrying antibiotic-resistant bacteria in hospitals (Boiocchi et al., 2019). The references in the scientific literature about arthropod populations and their associations with microbes in indoor human environments, such as homes, are also scarce. An aim of this study, therefore, is to increase the knowledge related to the composition of arthropod populations in the UK domestic environment and potential threats they may or may not pose to public health. One objective was to monitor the arthropod populations in several households in Birmingham UK over 12 months. A further objective being to provide a preliminary evaluation of the possible health risks associated with the bacteria harboured by the collected arthropods, via exploring their bacterial communities, using culture-dependent techniques and 16S rRNA gene metabarcoding.

MATERIALS AND METHODS

Recruitment of citizen scientists. Citizen science volunteers, for the 12-month arthropod collection, were recruited via an email sent to Aston University staff and students. Volunteers were trained, at a workshop, in insect sampling and provided with relevant documentation and materials. Crawling insect monitors (Killgerm Chemicals Ltd, Ossett, UK) and ultra-violet light-emitting-diode (UV LED) flying insect monitors (Hohto Shoji Co. Ltd, Tokyo, Japan) were provided, including a guide for identification of the most common household arthropods, forceps, magnifying glass, 35 ml of Ethanol 70%, in sterile pots (Thermo Fisher Scientific, Whaltham, USA) and test tubes, one pooter (Ocean, Southampton, UK), one 'spider-catcher' (Brainstorm, Gisburn, UK), a marker pen and gloves.

Study site, period and households' information. The study was conducted in 20 households within 15 miles of Birmingham city centre (United Kingdom) over a period of 12 months, from November 2018 to October 2019. The households were classified as urban and sub-urban according to their location. Details of the households were collected through a questionnaire completed by the volunteers.

Arthropods collection. *Passive capture.* The passive capture was performed using crawling insect monitors and UV LED flying insect monitors. After the collection, the arthropods were identified to the species level when possible, and to genus or family otherwise, using a stereo micrscope and preliminary entomological references (Chinery, 2012; Roberts, 1996). *Active capture.* Volunteers actively captured the arthropods inside the household using the provided pooter or 'spider-catcher'. The arthropods collected were put in a sterile pot and kept in the domestic freezer. The pooter and 'spider-catcher' were then wiped with Ethanol 70% to prevent cross-contamination among the captured specimens. Volunteers were provided, each month, with a polystyrene box and ice packs for bringing the frozen arthropods to Aston University for identification.

Analysis of the arthropods' culturome

Isolation of bacteria. Following the identification of the arthropods, the active captures were selected for the analysis of culturable bacteria. A variable number of active captures belonging to the same morphospecies, captured in the same household and in the same month, were pooled together in 1ml of sterile water. The pool was vortexed at maximum speed (minimum speed for Pholcidae and Lepismatidae) for 30 seconds and this step was repeated in order to increase the recovery of surface bacterial cells present on the arthropods. These external washings were then serially diluted down to 10^{-4} and 0.1 ml of the Neat, 10^{-2} and 10^{-4} dilutions inoculated onto the surfaces of Nutrient Agar, Mannitol Salt Agar, Violet Red Bile Glucose Agar and Blood Agar N° 2 (Thermo Fisher Scientific Oxoid Ltd, Basingstoke, UK). The pooled specimens were then surface sterilised with Ethanol 70%, rinsed with sterile water, and crushed in 1ml of sterile water using a micro pestle (Eppendorf, Hamburg, Germany). The above process of dilution and inoculation was repeated for the macerates. All the inoculated agar media were incubated at 37°C for 48h in aerobic conditions, blood agar plates were incubated both in aerobic and anaerobic conditions. The remaining external washings and macerates were stored at -20°C prior to total DNA extraction for the microbiome analysis.

Identification of bacteria. Gram staining and the microscopic examination of the colony and cell morphology were performed for all the strains isolated. The most abundant bacterial colonies (CFU/ml/arthropod > 10^2) were identified to the species level using 16S rRNA gene sequencing. The DNA extraction of the bacterial colonies was performed using QIAamp DNA Mini Kit (Qiagen, Venlo, Netherlands) following the protocol for bacteria. The 16S rRNA gene was amplified using primers and the HotStarTaq Master Mix Kit (Qiagen, Venlo, Netherlands). The polymerase chain reaction (PCR)-formed amplicons were purified using QIAquick PCR Purification Kit (Qiagen, Venlo, Netherlands) and sequenced by a commercial service (Eurofins Genomics, Ebersberg, Germany). The resulting sequences were analysed using the software FinchTv (version 1.4.0) and Blast.

Analysis of the arthropods' microbiome with 16S rRNA gene metabarcoding

DNA extraction, library construction, and sequencing. The bacterial communities associated with the captured arthropods were studied by sequencing the V1 region of the 16S rRNA gene. From the remaining arthropods' external washings and macerates, the total DNA was extracted using the DNeasy PowerWater kit (Qiagen, Venlo, Netherlands) using 0.2 μm filter funnels. The V1 region of the bacterial 16S rRNA gene was amplified using the universal primers 16S_27 and 16S_338, and the Q5 High-Fidelity Master Mix (New England BioLabs, Ipswich, USA). Following PCR amplification and purification steps, a second round of PCR using Q5 High-Fidelity Master Mix (New England BioLabs, Ipswich, USA) and 1μl of the combined customised forward and reverse primers with unique barcodes took place. The PCR products underwent a double purification using Agencourt AMPure XP beads (Beckman Coulter Inc., Brea, USA) following the manufacturer's instructions and the consistency of the amplification and absence of primer dimers were checked on a 2% agarose gel. Afterward, the amplicons were multiplexed, the final library was checked on the tape station (High Sensitivity D1000 ScreenTape, Agilent Technologies, Santa Clara, USA) and sequenced using Illumina MiSeq platform (Illumina, San Diego, CA, USA) with a 250bp paired-end sequencing kit.

RESULTS

Arthropod collection

There were 23 Arthropod Orders collected from homes (Figure 1.) during 11 months of collection (note that the analysis of the final month of collections is pending, due for full publication in a peer-reviewed journal). Of the 2,125, arthropods sampled from 19 households, Diptera were the most abundant, representing 30.1% of the total, followed by Zygentoma (16.2%), Araneae (13.9%) and Collembola (8%) (Figure 1).

Listed in Table 1 are a selection of the more widespread arthropod families found to be inhabiting homes. Over half the homes featured Sciaridae (dark-winged fungus gnats), Pholcidae (cellar spiders), Psocoptera (booklice), Psychodidae (owl midges), Lepismatidae (silverfish) and Oecophoridae (concealer moths).

Analysis of the arthropods' culturome

*Isolation of bacteria***.** There were 324 culturable bacterial and yeast isolates recovered from the arthropods analysed. Of the isolates, 48.5% were Gram-positive cocci, followed by Gram-positive rods (24.1%) and Gram-negative rods (13.6%), Gram-negative cocci (5.6%), the remaining represented by filamentous bacteria (3.7%), yeasts (2.5%) and Gram-negative coccobacilli (2.2%) (Figure 2).

Identification of bacteria. Culturable bacterial colonies, identified to species level using 16S rRNA gene sequencing, represented a diverse range of human skin flora, human oral flora, pathogens and opportunistic pathogens, encompassing Enterobacteriaceae, Staphylococci, Streptococci, Micrococci, spore-forming Bacilli and Clostridia (Table 2).

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Analysis of the arthropods' microbiome with 16S rRNA gene metabarcoding

The following bacterial families / groups that accounted for more than 5% of the reads were identified, as preliminary results awaiting further analysis, across a range of arthropod families / groups: Xanthomonadaceae, Pseudomonadaceae, Moraxellaceae, Orbaceae, Enterobacteriaceae, Burkholderiales, Comamonadaceae, Sphingomonadaceae, Rickettsiaceae, Anaplasmataceae, Rhodobacteraceae, Rhizobiales, Methylobacteriaceae, Peptostreptococcaceae, Streptococcaceae, Enterococcaceae, Staphylococcaceae, Bacillaceae, Flavobacteriaceae, Porphyromonadaceae, Pseudonocardiaceae, Propionibacteriaceae, Frankiales, Corynebacteriaceae, Micrococcaceae. The arthropod families / groups contributing to these preliminary results were Lepismatidae (silverfish), Pholcidae (cellar spiders), Agelenidae (house spiders), Thomisidae (crab spiders), Calliphoridae (bluebottle flies), Coccinellidae (ladybirds), Chironomidae, (non-biting midges), Culicidae (mosquitoes), Vespidae (wasps), Lepidoptera (moths).

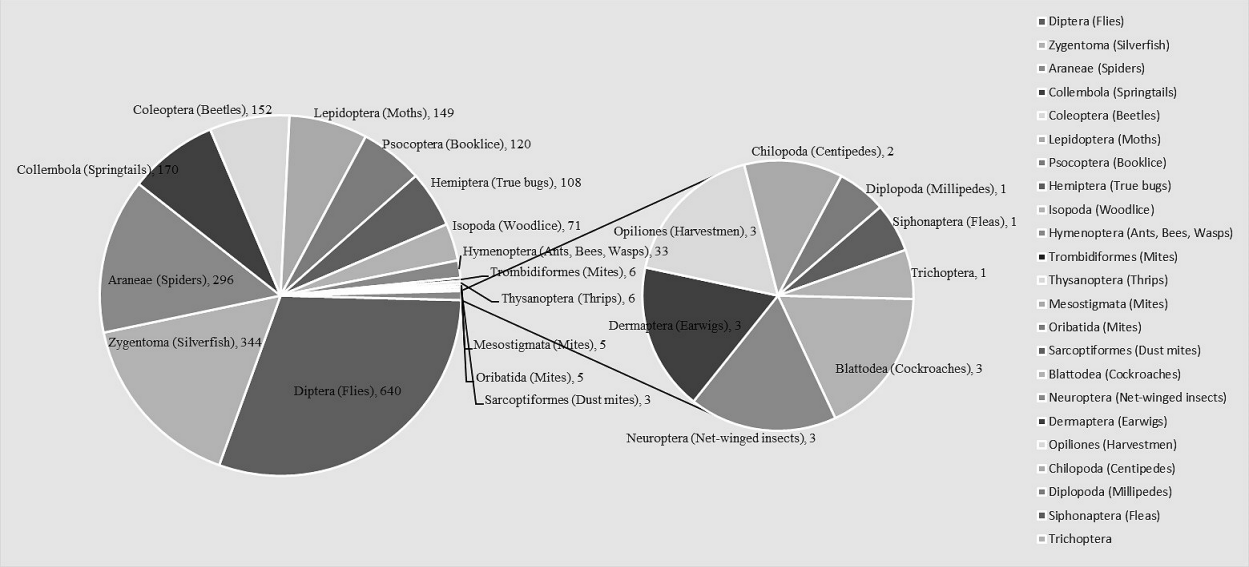

Figure 1. Abundance (number) of arthropods, by Order, collected from homes

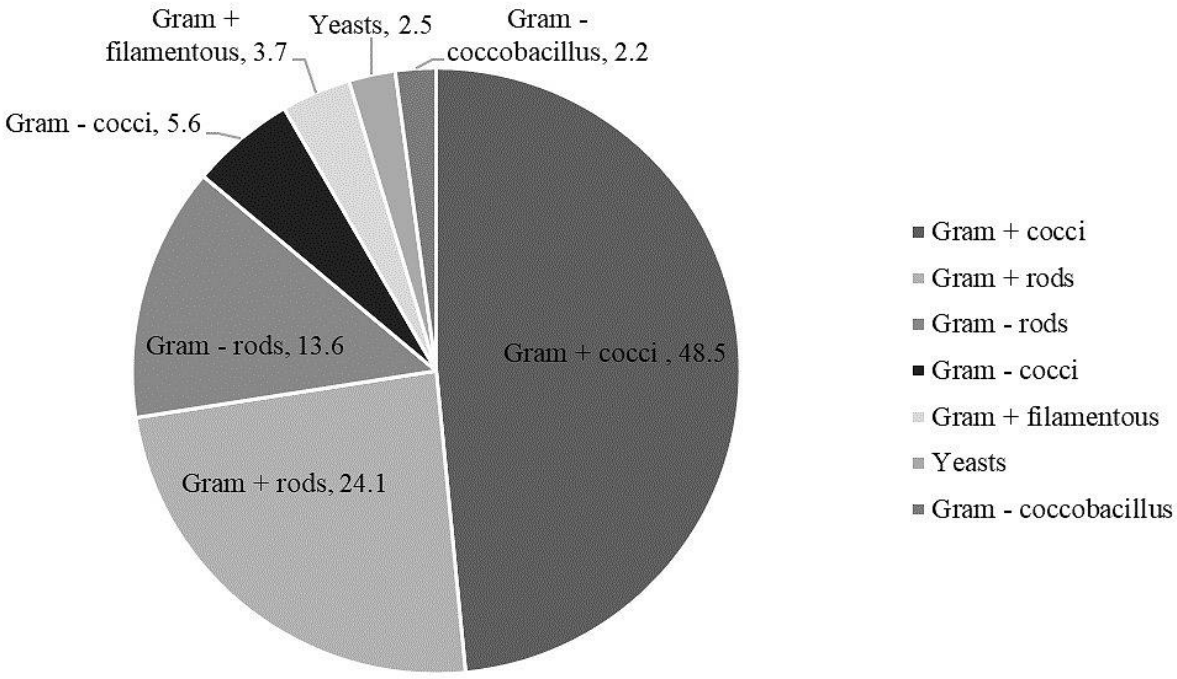

Figure 2. Percentage of culturable bacterial isolate groups and yeasts from collected Arthropods.

Table 1. Most widespread arthropod families in households

Arthropod family identified	Percentage (%) of households inhabited
Sciaridae (dark-winged fungus gnats)	75
Pholcidae (cellar spiders)	70
Psocoptera (booklice)	70
Psychodidae (owl midges)	60
Lepismatidae (silverfish)	55
Oecophoridae (concealer moths)	55
Aphididae (aphids)	50
Latridiidae (plaster beetles)	45
Cecidomyiidae (gall midges)	45

Table 2. Bacteria isolated from the five arthropod families analysed and identified with 16S rRNA gene sequencing.

Coccinellidae	Calliphoridae	Lepismatidae	Pholcidae	Vespidae
Cedecea sp.	*Enterococcus* spp.	*Acinetobacter junii*	*Micrococcus luteus*	*Enterococcus* spp.
Stenotrophomonas rhizophila	*Pseudomonas* sp.	*Acinetobacter jhonsonii*	*Paracoccus yeei*	*Bacillus cereus*
Acinetobacter radioresistans	*Dysgomonas* sp.	*Staphylococcus* spp.	*Staphylococcus* spp.	*Bacillus licheniformis*
Serratia liquefaciens	*Streptomyces* sp.	*Kocuria rhizophila*	*Bacillus cereus*	*Carnobacterium divergens*
Serratia marcescens	*Bacillus pumilus*	*Paracoccus yeei*	*Bacillus pumilus*	*Streptomyces* sp.
Enterococcus sp.	*Enterococcus rivorum*	*Bacillus cereus*	*Bacillus humi*	
Micrococcus luteus	*Vagococcus teuberi*	*Streptomyces* sp. *Brachybacterium*	*Bacillus licheniformis*	
Clostridium perfringens	*Erwinia perscinia*	*paraconglomeratum*	*Neisseria* sp.	
Paracoccus yeei	*Staphylococcus equorum*	*Brevibacterium frigoritolerans*	*Corynebacterium suicordis*	
Bacillus humi	*Clostridium perfringens*	*Bacillus velezensis*	*Streptococcus pneumoniae / mitis*	
Streptomyces sp.	*Staphylococcus hominis*	*Bacillus humi*		
Micrococcus yunnanensis	*Clostridium sordellii*	*Carnobacterium divergens*		
		Enterococcus faecalis		
		Veillonella parvula		
		Streptococcus oralis/pneumoniae		
		Rothia aeria		

Key: bacteria with pathogenic potential underlined. = human skin flora = human oral flora

DISCUSSION

The study shows that arthropods are indeed numerous in UK homes with 2,125 being collected, over just an 11-month period, from only 19 households. Diptera were the most abundant arthropods collected, representing 30.1% of the total, a finding that corresponds with similar observations in UK hospitals (Boiocchi et al., 2019) and reinforces the need for flying insect management. The silverfish Zygentoma represented 16.2% of arthropods sampled, corresponding with reports of grey silverfish *Ctenolepisma longicaudata* (Escherich) spreading in Europe (Kulma et al., 2018). Although the Zygentoma collected, *Lepisma saccharina* (L) and *C. longicaudata,* can be considered pest species it emerged from this study that they were numerous in only one household. Like studies by Bertone et al., (2016) and Madden et al., (2016), this work found that 'casual intruders', and those arthropods less frequently considered as pests, (spiders Araneae and springtails Collembola are examples from this study) were in fact surprisingly numerous in homes.

There were 324 culturable bacterial isolates recovered from the arthropods analysed, showing that insects and spiders indoors are carriers of numerous bacteria. The carriage by arthropods of opportunistic pathogens such as *Staphylococcus* spp, *Acinetobacter* spp and *Serratia* spp was recorded. This perhaps indicates that post-pest control disinfection may warrant further discussion in order to deal with bacteria known to be deposited by insects in homes. Over half the homes featured 'small flies' / 'drain flies' Sciaridae and Psychodidae, therefore reinvigorating interest regarding such insects, which were previously highlighted as an emerging problem or even new vectors in the hospital environment, due to their carriage of pathogenic microorganisms in the clinical setting (Davies et al., 2017).

Of the bacteria isolated from five key arthropod families, and further identified with 16S rRNA gene sequencing**,** 40 isolates are known to have pathogenic potential. It is also noted that arthropods in homes carry environmental bacteria and bacterial species associated with human flora. These observations represent a potential for infection risk and respectively the close association of arthropods with the human living environment.

Analysis of the microbiome of 10 arthropod families / groups from homes, with 16S rRNA gene metabarcoding, revealed that 25 bacterial families / groups accounted for more than 5% of the reads. As the preliminary results of this aspect of the study are developed it is anticipated that valuable conclusions will be made. One observation was the finding of Anaplasmataceae and Rickettsiaceae, the insect symbionts and pathogens, in specimens from UK homes. Some specific observations can be made such as noting that over half of UK homes harbour silverfish, many of which are the grey silverfish *C. longicaudata*, that were found to be harbouring nine types of bacteria with pathogenic potential. Of the silverfish collected, 76.2% were captured from a single household. This shows that although silverfish were detected widely, in more than half of the homes surveyed, they were present in large numbers in only one household. Similarly, 87.7% of all Collembola were sampled from one household.

The observation regarding silverfish leads to a recommendation for pest control industry to become more aware of the specific monitoring and control options available for these insects, and to perhaps prioritise such options, versus traditional crawling insect monitors and broad-spectrum treatments. Regarding the carriage indoors of potential pathogens by a predominance of 'casual intruders', that typically enter buildings from the outdoor environment, this serves as a reminder of the importance of adequate proofing to protect properties from pest ingress. This then leads to further discussion about what traditionally constitutes a 'pest' considering new data regarding predominant species and their carriage of microbial pathogens in homes.

As the final pieces of data are collected, and further analysed, a clearer picture regarding an appreciation of arthropods in UK homes and their associated bacterial flora will be provided. Furthermore, there is an opportunity for pest management reference texts and training resources to be updated to consider the findings that casual intruders are surprisingly more numerous in homes, and present different microbial risks, than the traditional pests. The findings of this work will also present an opportunity for the pest control industry to identify areas for product development, and updated treatment strategies and priorities, based on the arthropod species and associated microbial risks in homes. The collected findings continue to emphasize the importance of pest control as a component of infection control and protection of public health in indoor premises.

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