War of the microbial world: *Acanthamoeba* spp. interactions with microorganisms

Mohammad Ridwane Mungroo1 · Ruqaiyyah Siddiqui2 · Naveed Ahmed Khan1,3

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Abstract
*Acanthamoeba* is known to interact with a plethora of microorganisms such as bacteria, fungi and viruses. In these interactions, the amoebae can be predatory in nature, transmission vehicle or an incubator. Amoebae consume microorganisms, especially bacteria, as food source to fulfil their nutritional needs by taking up bacteria through phagocytosis and lysing them in phagolysosomes and hence play an eminent role in the regulation of bacterial density in the nature and accountable for eradication of around 60% of the bacterial population in the environment. *Acanthamoeba* can also act as a “Trojan horse” for microbial transmission in the environment. Additionally, *Acanthamoeba* may serve as an incubator-like reservoir for microorganisms, including those that are pathogenic to humans, where the microorganisms use amoebae’s defences to resist harsh environment and evade host defences and drugs, whilst growing in numbers inside the amoebae. Furthermore, amoebae can also be used as a “genetic melting pot” where exchange of genes as well as adaptation of microorganisms, leading to higher pathogenicity, may arise. Here, we describe bacteria, fungi and viruses that are known to interact with *Acanthamoeba* spp.

Introduction
*Acanthamoeba castellanii* is known to interact with bacteria and viruses as well as fungi (Table 1) (Mella et al. 2016; Siddiqui and Khan 2012). The ability of bacteria to infect and lyse *Acanthamoeba* has been recorded since 1954, whilst the ability of bacteria to act as an endosymbiont was reported in 1975, and the potential of *Acanthamoeba* as a reservoir for pathogenic bacteria was suggested in 1978 (Drozanski 1956; Prasad and Gupta 1978; Proca-Ciobanu et al. 1975). Behaviour of the endosymbionts may vary depending on conditions, such as temperature, pH, osmolarity and availability of food (Scheid 2014). It is also known that amoebae can serve as genetic “melting pot”, that allows exchange of genes (Moreira and Brochier-Armant 2008; Grillot-Courvalin et al. 1998). Intracellular growth of bacteria in amoebae is suggested to be essential for adaptation of bacteria to higher eukaryotic cells, leading to increased pathogenicity of the bacteria and resistance to destruction by macrophages (Harb et al. 2000; Molmeret et al. 2005; Albert-Weissenberger et al. 2007; Greub and Raoul 2004; Thomas et al. 2006). The community of bacteria associated with amoebae is very large and diverse and can be classified in 155 different taxa (Delafont et al. 2016). Whilst it is known that *Acanthamoeba* can feed on bacteria, little is known about the mechanisms through which the amoebae locate the bacteria. Comparing it with an amoeba that uses chemotaxis, *Dictyostelium*, it was revealed that *Acanthamoeba* do not use chemotaxis as the primary mechanism to find bacterial food sources (Kuburich et al. 2016). Interestingly, in an experiment comparing the ability of ciliates and *Acanthamoeba*, it was revealed that the competition was asymmetric in favour of the ciliates, but *Acanthamoeba* had better long-term negative effect, both in the open-water phase as well as in biofilm, on bacterial biomass (Zhang et al. 2014). A large number of bacterial and viral species that have been shown to interact with *Acanthamoeba* (Balczun and Scheid 2017). Here, we describe some of the interactions of *Acanthamoeba* with microorganisms (Fig. 1).
Table 1  Microorganism that interacts with Acanthamoeba. Several bacteria, fungi and viruses that interact with Acanthamoeba as well as changes that occur following the interaction are described

| Organism                          | Changes                                      | References                                      |
|-----------------------------------|----------------------------------------------|------------------------------------------------|
| **Bacteria**                      |                                              |                                                 |
| *Amoebophilus asiaticus*          | Intracellular multiplication                  | Horn et al. (2001)                              |
| *Arcobacter butzleri*             | Not described                                | Medina et al. (2014)                            |
| *Bacillus anthracis*              | Intracellular multiplication                  | Dey et al. (2012)                               |
| *Burkholderia cepacia*            | Intracellular growth                          | Landers et al. (2000)                           |
| *Burkholderia pickettii*          | Intracellular multiplication                  | Michel and Hauröder (1997)                      |
| *Campylobacter jejuni*            | Increase in bacterial resistance to disinfection | Snelling et al. (2005)                          |
| *Candidatus Paracaeidibacter symbiosus* | Not described                              | Horn et al. (1999)                              |
| *Chlamydia pneumoniae*            | Intracellular growth                          | Essig et al. (1997)                             |
| *Chlamydiales*                    | Increase in amoebae pathogenicity             | Fritsche et al. (1998)                          |
| *Citrobacter freundii*            | Increase in bacterial resistance to chlorine  | King et al. (1988)                              |
| *Coxiella burnetii*               | Not described                                | La Scola and Raoult (2001)                      |
| *Cytophaga johnsonae*             | Intracellular multiplication                  | Hoffmann and Michel (2001)                      |
| *Enterobacter agglomerans*        | Increase in bacterial resistance to chlorine  | King et al. (1988)                              |
| *Enterobacter cloacae*            | Increase in bacterial resistance to chlorine  | King et al. (1988)                              |
| *Escherichia coli*                | Intracellular growth and increase in bacterial resistance to chlorine and antibiotics | Barker et al. (1999)                            |
| *Flavobacterium indologines*      | Intracellular multiplication                  | Hoffmann and Michel (2001)                      |
| *Flavobacterium johnsoniae*       | Not described                                | Horn et al. (2001)                              |
| *Flavobacterium succinicans*      | Not described                                | Horn et al. (2001)                              |
| *Francisella tularensis*          | Intracellular growth                          | Abd et al. (2003)                               |
| *Helicobacter pylori*             | Intracellular growth                          | Winiecka-Krusnell et al. (2002)                 |
| *Klebsiella oxytoca*              | Increase in bacterial resistance to chlorine  | King et al. (1988)                              |
| *Klebsiella pneumoniae*           | Increase in bacterial resistance to chlorine  | King et al. (1988)                              |
| *Legionella pneumophila*          | Intracellular growth                          | Rowbotham (1980)                                |
|                                   | Increase in invasiveness                      | Cirillo et al. (1994)                           |
| *Legionella sp.*                  | Not described                                | Michel et al. (1997)                            |
| *Listeria monocytogenes*          | Intracellular multiplication                  | Ly and Müller (1990)                            |
| *Mobiluncus curtisi*              | Intracellular multiplication                  | Tomov et al. (1999)                             |
| *Mycobacterium avium*             | Intracellular multiplication, increase in bacterial virulence | Cirillo et al. (1997)                            |
| *Mycobacterium xenopi*            | Intracellular multiplication                  | Drancourt et al. (2007)                         |
| *Porphyromonas gingivalis*        | Intracellular multiplication                  | Wagner et al. (2006)                            |
| *Prevotella intermedia*           | Intracellular multiplication                  | Wagner et al. (2006)                            |
| *Procabacter acanthamoebae*       | Not described                                | Horn et al. (2002)                              |
| *Pseudomonas saccharophila*       | Intracellular multiplication                  | Hoffmann and Michel (2001)                      |
| *Pseudomonas stutzeri*            | Intracellular multiplication                  | Hoffmann and Michel (2001)                      |
| *Rickettsiales*                   | Increase in amoebae pathogenicity             | Fritsche et al. (1998)                          |
| *Staphylococcus aureus*           | Intracellular multiplication                  | Huws et al. (2008)                              |
| *Salmonella enterica*             | Intracellular multiplication                  | Tezcan-Merdol et al. (2004)                     |
| *Salmonella typhimurium*          | Intracellular multiplication                  | Gaze et al. (2003)                              |
| *Simkania negevensis*             | Intracellular growth                          | Kahane et al. (2001)                            |
| *Stenotrophomonas maltophilia*    | Intracellular multiplication                  | (Hoffmann and Michel 2001; Corsaro et al. 2013) |
| *Streptococcus pneumoniae*        | Intracellular multiplication                  | Siddiqui et al. (2017)                          |
| *Streptococcus pyogenes*          | Intracellular multiplication                  | Siddiqui et al. (2017)                          |
| *Waddlia chondrophila*            | Intracellular growth                          | Michel et al. (2004)                            |
| *Yersinia enterocolitica*         | Intracellular survival                        | Lambrecht et al. (2013)                         |
Acanthamoeba as predator

Amoebae consume bacteria, mostly non-pathogens, as food source to fulfill their nutritional needs by taking up bacteria through phagocytosis and lysing them in phagolysosomes (Khan and Siddiqui 2014). Due to their consumption of bacteria, amoebae play an eminent role in the regulation of bacterial density in the nature by being accountable for eradication of around 60% of the bacterial population in the environment (Rosenberg et al. 2009). Whilst both

![Diagram of Acanthamoeba and bacteria interaction](image)

**Fig. 1** Interaction of *Acanthamoeba* with bacteria. The figure describes how *Acanthamoeba* and bacteria interact, which may lead to the amoebae acting as predator, Trojan horse or reservoir. On the left-hand side, *Acanthamoeba* in a bacteria-rich environment following which selected bacteria are taken up by the amoeba can be observed. In the middle section, the two main options following intake of bacteria are depicted, namely, digestion or survival of the bacteria. On the right-hand side, bacteria surviving internalization into *Acanthamoeba*, namely, using the amoeba as a transmission vehicle or replication site, can be observed.

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**Table 1 (continued)**

| Organism                  | Changes                                      | References                                      |
|---------------------------|----------------------------------------------|-------------------------------------------------|
| *Candida albicans*        | Increase in fungal virulence                 | Gonçalves et al. (2019)                         |
| *Cryptococcus neoformans* | Intracellular replication, increase in fungal virulence | (Steenbergen et al. 2001; Gonçalves et al. 2019) |
| *Cryptosporidium parvum*  | Not described                                | Scheid and Schwarzenberger (2011)               |
| *Paracoccidioides brasiliiensis* | Increase in fungal virulence              | Gonçalves et al. (2019)                         |
| *Sporothrix brasiliensis* | Increase in fungal virulence                 | Gonçalves et al. (2019)                         |
| Virus                     |                                              |                                                 |
| Adenoviruses              | No multiplication observed                   | Scheid and Schwarzenberger (2012)               |
| Coxsackieviruses          | Not described                                | Mattana et al. (2006)                           |
| Marseillevirus            | Not described                                | Boyer et al. (2009)                             |
| Mimivirus                 | Not described                                | Arslan et al. (2011)                            |
| Pandoraviruses            | Not described                                | Philippe et al. (2013)                          |
| *Mollivirus sibericum*    | Not described                                | Legendre et al. (2015)                          |
neuropathogenic *Escherichia coli* K1 and the non-invasive *E. coli* K-12 have been shown to be present intracellularly in amoebae, K1 survived whilst K-12 were lysed by the amoebae, indicating K-12 as bacterial prey (Alsam et al. 2006). Even as a bacterial predator, it has been shown that *Acanthamoeba* is selective in its phagocytic uptake, since a study on the effects of *Acanthamoeba* on the composition of bacterial communities revealed that *Betaproteobacteria* and *Firmicutes* showed the most decrease in numbers, whilst *Betaproteobacterial* ammonia oxidizers and *Gammaproteobacteria* numbers did not change, and an increase was observed in the number of *Actinobacteria*, *Nitrospira*, *Verrucomicrobia* and *Planctomycetes* (Rosenberg et al. 2009). The selective intake of bacteria by *Acanthamoeba* suggests underlying mechanisms and conditions that allow selection of bacteria. As such, it has been reported that the intake of particles by *Acanthamoeba* is dependent on size, where latex beads of sizes between 1.035 and 2.68 µm were taken up whilst bead of sizes between 0.126 to 0.557 µm accumulated at the surface of the amoebae (Korn and Weisman 1967). The uptake of beads was also shown to be higher in larger cells as compared to smaller cells and was affected by the culture age and agitation of cell culture (Avery et al. 1995). Another reason for selective uptake of bacteria might be their ability to achieve high motility and escape from amoebae grazing (Jürgens and Matz 2002). It has been indicated that the amoebic vacuoles containing food are retained, and those containing non-nutrient particles are exocytosed, upon presentation of new particles, and phagocytic vesicles that are retained have been reported to fuse with each other as well as digestive vesicles (Bowers and Olszewski 1983; Wetzl and Korn 1969). It is known that surface elements, such as lipopolysaccharide, peptidoglycan, capsule and β-(1–4)-N-acetylMuramic acid, play an important role in the selection of bacteria by amoebae, and it has been reported that the phagocytosis of *Acanthamoeba* might be mediated by tyrosine kinase (Alsam et al. 2005, 2006; Humann and Lenz 2009). It has also been reported that actin polymerization and cytoskeletal rearrangement are processes that are involved in the phagocytic uptake of bacteria by *Acanthamoeba* where killing and degradation of bacteria are caused by acidification and phagosomal–lysosomal fusion (Akya et al. 2009). Moreover, it has been noted that mannose-binding proteins and membrane-associated amoebae receptors are essential for the phagocytic intake of bacteria by *Acanthamoeba* and that tyrosine kinase-induced actin polymerization, as well as PI3K and Rhoa pathways, play important roles in the uptake (Medina et al. 2014). Furthermore, it was shown that the conditioned media from *Acanthamoeba* culture possess bactericidal activities against selected bacteria, since the medium eliminated all methicillin-resistant *Staphylococcus aureus* whilst showing limited effects against *Acinetobacter* sp., vancomycin-resistant *Enterococcus faecalis* and *Pseudomonas aeruginosa* (Iqbal et al. 2014). Also, it was recently reported that *Acanthamoeba* produces a metabolite that inhibits the growth of methicillin-resistant *Staphylococcus aureus* (de Souza et al. 2017). Interestingly, it has been shown that *Acanthamoeba* may interfere with bacterial toxin production, such as 2,4-diacetylphloroglucinol and monoacetyl–phloroglucinol in *Pseudomonas fluorescens* (Jouset and Bonkowski 2010), which may enhance its predatory capabilities. Interestingly, *Acanthamoeba* has the ability to produce protein toxins, such as the pore-forming acanthaporin, which possesses anti-bacterial activities against a variety of bacteria (Michalek et al. 2013) and hence may also promote its predatory capabilities.

Recently, a thermodimorphic fungi, *Paracoccidioides brasiliensis*, was reported to be efficiently ingested, killed and digested by *Acanthamoeba* leading to 90% decrease in fungal colony-forming units in 24 h, and electron microscopy revealed internalized fungal cell debris and cell wall debris inside amoeba vacuoles (Albuquerque et al. 2019). These findings indicate that the predatory characteristics of *Acanthamoeba* are not limited to bacteria but also affect fungi and viruses.

**Acanthamoeba as transmission vehicles**

*Acanthamoeba* can act as a “Trojan horse” for microorganisms which force their entry into amoebae, resist amoeba-mediated killing, and are hence protected by *Acanthamoeba*’s ability to resist extreme temperatures, pH and osmolarity (Khan and Siddiqui 2014). The resistance of *Acanthamoeba* cysts to chlorine is especially beneficial to the microorganisms since elimination of intracellular microorganisms from water supplies can prove challenging (Khan 2009). Additionally, *Acanthamoeba* cysts are air-borne and can travel through ventilation systems/ducts, as well as long distance such as during strong winds and dust storms, that can lead to bacterial transmission to the wider community. *Burkholderia cepacia* and *Chlamydophila pneumoniae* are examples of bacteria that have been shown to use *Acanthamoeba* as “Trojan horse”, whereby they remain viable in the amoebae without growing in numbers (Khan and Siddiqui 2014). This indicates that some bacteria possess mechanisms that allow them to protect themselves from *Acanthamoeba* killing. As such, the Dot/Icm type IV secretion system genes encode for protein, secreted by bacteria such as *Legionella*, that inhibit lysosome adhesion, phagosome maturation and acidification of vesicles, protecting bacteria from attack (Molmeret et al. 2005; Taylor et al. 2009; Rubenina et al. 2017).

Fungi have been shown to interact with *Acanthamoeba*, and the interaction has been shown to increase the virulence of the fungi that have been shown to survive due to expression of capsular polysaccharides and melanin (Steenbergen and Casadevall 2003; Chrisman 2011; Gonçalves et al. 2019). Survival of fungi within amoebae resulted in more virulent fungi since the “surviving” fungi
were more pathogenic when tested using in vivo models (Fuchs et al. 2010; Mylonakis 2008; Thomaz et al. 2013; Rizzo et al. 2017). Moreover, instance of fungi, namely, Cryptococcus neoformans, taken up by amoebae and then released without notable genetic or pharmacological changes have been recently reported (Watkins et al. 2018).

The cultivation of adenoviruses in the presence of Acanthamoeba was used as a model for studying their relationship, and it was revealed that Acanthamoeba is used as a carrier or a paratenic host by the virus, whereby the viruses did not proliferate or multiply inside the amoebae (Scheid and Schwarzenberger 2012). Acanthamoeba also serve as transmission vehicle for coxsackie B viruses, whereby the virus maintains its infectivity when internalized in the amoebae but does not replicate and is released during interaction between amoebae and human macrophages (Mattana et al. 2006).

In addition, the interactions of Cryptosporidium spp., a protozoan parasite that infects a wide range of vertebrate hosts, and Acanthamoeba was investigated. It was revealed that the amoebae likely act as s carriers of Cryptosporidium oocysts since the amoebae internalized several oocysts which did not show any changes in morphology (Gómez-Couso et al. 2007).

Acanthamoeba as reservoir

Acanthamoeba may also serve as an incubator-like reservoir for bacteria that are pathogenic to humans, where the bacteria can use the amoebae’s defences to resist harsh environment and evade host defences and drugs, whilst growing in numbers inside the amoebae (Khan and Siddiqui 2014; Scheid 2014; Lim et al. 2020). Moreover, the ability of bacteria to survive in amoebae cysts has also been reported, indicating that the cysts might protect bacteria from chemical disinfection (Khan 2006; Thomas et al. 2010; Youssf et al. 2013). In contrast to Acanthamoeba as a “Trojan horse” whereby bacteria remain viable in the amoebae without growing in numbers, when Acanthamoeba are used as reservoir, the bacteria multiply inside the amoebae. In addition to being used as a safe heaven, genetic transfer between bacteria whilst inside amoebae has been reported, indicating that the amoebae may lead to drug resistance due to transfer of genetic information related to multiple drug resistance between several bacterial species using the amoebae as a reservoir (McCuddin et al. 2006; Mella et al. 2016). Exchange of genetic material between intracellular bacteria lead to the development of virulence traits using amoebae as evolutionary crib to adapt for survival within macrophages (Harb et al. 2000; Huws et al. 2008; Kebbi-Beghdadi and Greub 2014). The genome of intra-amoeba microorganisms is significantly larger than that of their relatives, further confirming the role of Acanthamoeba as a gene melting pot, whilst also suggesting gene transfer from Acanthamoeba to their endosymbionts (Moliner et al. 2010). Moreover, several markers of horizontal gene transfer between genomes of intra-amoebal bacteria and between the bacterial genomes and the genomes of their amoebal hosts are additional evidence that Acanthamoeba functions as a gene melting pot (Moliner et al. 2010). One bacterium that has been extensively studied is Legionella pneumophila that has been shown to grow and multiply intracellularly of Acanthamoeba trophozoites and survive from harsh treatment, such as 50 mg/L free chlorine, inside Acanthamoeba cysts (Kilvington and Price 1990). It has also been shown that L. pneumophila uses the same genes to grow intracellularly in both human macrophages and amoebae since all the genes that were absolutely required for growth in human macrophages were also required for growth in Acanthamoeba (Segal and Shuman 1999). Moreover, a study comparing L. pneumophila grown in standard laboratory conditions to those grown intracellularly in Acanthamoeba revealed that bacteria grown in Acanthamoeba was 100-fold more invasive to macrophages via coiling phagoctosis at a higher frequency (Cirillo et al. 1994). Light and electron microscopy revealed differences in the structure and morphology between amoebae-grown and agar-grown L. pneumophila, and analysis of protein expression suggested that amoeba-grown L. pneumophila expressed new proteins leading to phenotypic differences (Cirillo et al. 1994).

Recently, it was shown that several types of fungi can survive as well as replicate intracellularly of Acanthamoeba, indicating that Acanthamoeba also acts as a reservoir for fungi, such as Cryptococcus spp., Histoplasma capsulatum, Blastomyces dermatitides and Sporothrix schenckii (Gonçalves et al. 2019). Mannose-binding proteins have been linked to the recognition of fungi by Acanthamoeba, whereby interaction of Acanthamoeba with H. capsulatum, C. neoformans, Candida albicans, Saccharomyces cerevisiae and Paracoccidioides brasiliensis was inhibited by mannose (Gonçalves et al. 2019). The importance of mannose-binding protein was shown to be specific to the uptake of fungi, as mannose inhibited uptake of fungi but not latex beads (Allen and Dawidowicz 1990). It was further shown that the fungi that were taken up by Acanthamoeba were used to infect Galleria mellonella, and it was revealed that they killed the larvae faster than control yeasts in all cases (Gonçalves et al. 2019).

The first known Mimivirus, Acanthamoeba polyphaga mimivirus, was isolated from an amoebal co-culture in a water sample collected from a cooling tower of a hospital in England, following which over a dozen viruses have been identified (Abrahão et al. 2014). This virus was shown to use Acanthamoeba as a viral factory whereby viral entry and viral membrane fusion are followed by release of viral seed into amoeba cytoplasm where, within few hours, the
viral factory grew and orchestrated the morphogenesis of the viral progeny which were subsequently released by cell lysis (Abrahão et al. 2014). Also, Faustoviruses are viruses that can use Acanthamoeba as virus factories where, 20 h after infection, complete viral particles are released (Reteno et al. 2015). More recently, a novel virus, Yaravirus brasilensis, was discovered inside Acanthamoeba castellanii and was found to use the amoebae as a viral factory to create new virus following which the virus causes lysis of the amoebae (Boratto et al. 2020).

**Miscellaneous interactions of Acanthamoeba**

Other than Acanthamoeba acting as predator, transmission vehicles and reservoirs, other types of interactions have also been described. One such case is when Acanthamoeba internalizes Aspergillus fumigatus conidia, where part of the ingested fungi escapes the vacuole, resulting in fungal intracellular germination and subsequently causing structural changes in amoebae that causes amoebal permeabilization (Van Waeyenberghe et al. 2013). In other cases, it has been shown that fungi such as Histoplasma capsulatum, can be phagocytosed by Acanthamoeba and convert into filamentous form, causing the death of the amoebae, whilst more virulent phenotypes of the fungi are selected or induced (Steenbergen et al. 2004).

Moreover, viruses are known to survive inside Acanthamoeba, without noticeable changes in infectivity even after 6 months, whilst the viral deoxyribonucleic acid (DNA) of numerous viruses has been detected inside the amoebae, indicating that Acanthamoeba plays a role as vector, reservoir or carrier for the viruses (Lorenzo-Morales et al. 2007; Mattana et al. 2006; Scheid and Schwarzenberger 2012). Additionally, “giant viruses” Mimiviridae were initially discovered as an intracellular endocytobiont of Acanthamoeba further indicating that there is association of great importance between Acanthamoeba and viruses (La Scola et al. 2003). Later virus of the Iridoviridae family was identified as endocytobiont of Acanthamoeba and led to the discovery of new giant viruses in amoebae (Aherfi et al. 2016; Colson et al. 2012, 2013). Another group of viruses, known as Pandoraviruses, was identified from Acanthamoeba, and it was revealed that the viruses were unique, since they did not share genetic or morphological similarities to known viruses (Philippe et al. 2013; Scheid 2015, 2016; Scheid et al. 2008). There is also evidence of horizontal gene transfer between viruses and Acanthamoeba where 10% of protein encoded by the virus is homologous to Acanthamoeba protein (Legendre et al. 2015).

Interestingly, Pseudomonas chlororaphis strain PA23 is a bacterium that can produce compounds including pyrrolnitrin, phenazine and hydrogen cyanide that have been recently shown to possess both toxic and repellent effects on Acanthamoeba (Ghergab et al. 2021). Co-culture of P. chlororaphis and amoebae led to changes in gene expression and secondary metabolite production of bacteria, suggesting that they can sense the presence of Acanthamoeba and adjust its physiology in response (Ghergab et al. 2021).

Acanthamoeba is known to interact with bacteria, viruses as well as fungi (Mella et al. 2016). A recent study on humans with stromal keratitis has shown that Acanthamoeba infections occurred in around 10% of cases and co-infections with fungi occurred in around 5% of cases whilst co-infections with bacteria were also noted (Raghavan et al. 2019). Depending on conditions and pathogenicity of the microorganisms and the amoebae, the interactions vary leading to lysis of the microorganisms or amoebae, intake of microorganisms without any changes, incubation and growth of the microorganisms within the amoebae or increase in virulence of the microorganisms. Acanthamoeba cysts have been shown to be resistant to extreme conditions including freezing, pH 2.0, γ and ultraviolet irradiation, moist heat, heavy metals, desiccation for over 20 years and storage at 4 °C for over 20 years (Sriram et al. 2008). Acanthamoeba cyst has also been shown to be resistant to biocides, chlorination and antibiotics (Marciano-Cabral and Cabral 2003). Hence, whilst acting as a reservoir or a “Trojan horse”, Acanthamoeba can hence protect the endocytobionts against virtually most extreme conditions that they would encounter in the environment as well as treatments such as antibiotics and biocides. Hence, the role of Acanthamoeba as a sanctuary for pathogens and a training ground for their virulence, whilst assisting their transmission to susceptible hosts, is of huge concern to public health. As such, it has been recently suggested that amoebae might also harbour coronaviruses, responsible for COVID-19 pandemic (Siddiqi and Khan 2020a, b). The ability of Acanthamoeba to work as reservoirs or “Trojan horses” renders the connection between exposures with a pathogen, and the resulting infection more complicated as the amoebae might allow the pathogen to evade several of the host responses and defences and even allow the pathogen entry into the central nervous system of the host (Siddiqi and Khan 2020a, b). SARS-CoV-2 as an endosymbiont of Acanthamoeba might also allow it to persist in the environment, especially in the air-conditioning systems (Siddiqi and Khan 2020a). Moreover, Acanthamoeba cysts have been isolated from the brain tissue (Marciano-Cabral and Cabral 2003), indicating that some cysts might remain dormant in the host and hence may lead to recurrence of infection caused by pathogens released from the cysts. However, despite knowing that Acanthamoeba plays an essential role in the survival, evolution and transmission of a vast repertoire of microorganisms, many of which are pathogenic to humans, studies on the mechanisms behind the interaction of the amoebae with the endocytobionts...
remain scarce. Understanding the mechanisms of action involved in the uptake and release of microorganisms might allow researchers to use Acanthamoeba for the culture of obligate intracellular microorganisms that cannot be cultured in axenic media. Furthermore, if the mechanisms are revealed, it might be possible to introduce targeted antimicrobials into the amoebae to kill the endocytobionts. Recently, a study investigated the effects of Acanthamoeba in a complex microbial community in an experimental aquatic model, using 16S rRNA sequencing, and it was revealed that the amoebae did not drastically change the bacterial community but, by reducing certain oxygen-consuming bacteria, such as Cyanobacteria, affected the dissolved oxygen concentrations (Tsai et al. 2020). This indicates that due to the complex ecological processes, it is still too complex to understand the exact mechanisms of interaction between Acanthamoeba and microorganisms in a complex microbial community. Despite all the studies performed on the interaction of Acanthamoeba with microorganisms, important factors in the interactions remain unelucidated. The selection process of food by both the amoebae and the microorganisms, in other words, in the presence of Acanthamoeba, varying species of microorganisms and other food sources (such as axenic media and nutrient agar/broth) questions such as (i) what microorganisms would be taken up and protected by the amoebae, (ii) would the amoebae take up the microorganisms species by species or several species at one time, (iii) what is the mechanism behind the selection process of food source by the amoebae, (iv) what microorganisms would be used as food source by the amoebae despite the axenic media, (v) what microorganisms would force their way into the amoebae despite being in a nutritionally rich environment, (vi) what microorganisms would use the amoebae as food source despite the presence of other food sources, and (vii) what microorganisms would escape or be released from the amoebae due to the availability of nutritious environment and the mechanisms involved are areas for further investigations.

Conclusion

In general, interaction of pathogens with Acanthamoeba might cause an evolutionary pressure leading to increase in virulence, whilst, in other cases, microorganisms can be used as food source by the amoebae or the amoebae can be used as a “Trojan horse” or reservoir by the microorganisms. Understanding the molecular basis of the interactions between microorganisms and Acanthamoeba, such as receptors used by the amoeba and change in gene expressions, would give insight on how pathogenicity could be modulated and drive knowledge on the role of amoebae in diseases caused by microorganisms distributed in the environment.

Author contribution NAK and RS conceived the concept. MRM reviewed literature under the supervision of RS. MRM and RS wrote the first draft. NAK corrected and finalized the manuscript.

Declarations

Conflict of interest The authors declare no competing interests.

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