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REVIEW

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

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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-Armanet 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).

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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 *Paracaedibacter 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	King et al. (1988) 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) Moreno-Mesonero et al. (2016)
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 Increase in invasiveness	Rowbotham (1980) Cirillo et al. (1994)
Legionella sp.	Not described	Michel et al. (1997)
Listeria monocytogenes	Intracellular multiplication	Ly and Müller (1990)
Mobiluncus curtisii	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 saccharophilia	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)

Table 1 (continued)

Organism	Changes	References
Fungi		
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 brasiliensis	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)

Acanthamoeba as predator

Amoebae consume bacteria, mostly non-pathogens, as food source to fulfil 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

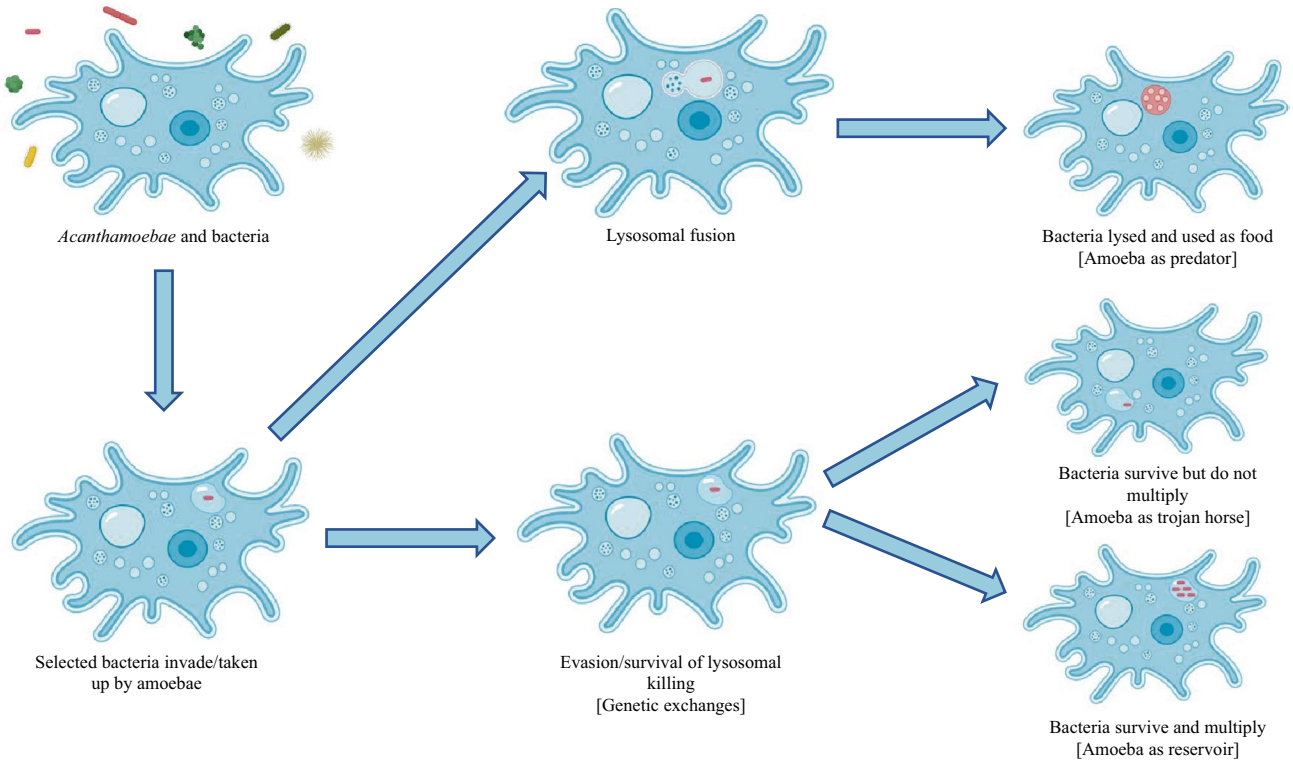

Fig. 1 Interaction of *Acanthamoeba* with bacteria. The figure describes how *Acanthamoeba* and bacteria interact, which may lead to the amoebae acting as predator, Trojan horse or reservoir. On the left-hand side, *Acanthamoeba* in a bacteria-rich environment following which selected bacteria are taken 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

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; Wetzel 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 phagocytotic 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 phagocytotic 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* (Jousset and Bonkowski 2010), which may enhance its predatorial 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 predatorial 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; Rubeniņa 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; Yousuf 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 phagocytosis 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 brasiliensis*, 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", *Acanthamoebae* 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 (Siddiqui 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 (Siddiqui 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 (Siddiqui 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 targetted 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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