MINI-REVIEW

Acanthamoeba in Southeast Asia – Overview and Challenges

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Abstract: Acanthamoeba, one of free-living amoebeae (FLA), remains a high risk of direct contact with this protozoan parasite which is ubiquitous in nature and man-made environment. This pathogenic FLA can cause sight-threatening amoebic keratitis (AK) and fatal granulomatous amoebic encephalitis (GAE) though these cases may not commonly be reported in our clinical settings. Acanthamoeba has been detected from different environmental sources namely; soil, water, hot-spring, swimming pool, air-conditioner, or contact lens storage cases. The identification of Acanthamoeba is based on morphological appearance and molecular techniques using PCR and DNA sequencing for clinico-epidemiological purposes. Recent treatments have long been ineffective against Acanthamoeba cyst, novel anti-Acanthamoeba agents have therefore been extensively investigated. There are efforts to utilize synthetic chemicals, lead compounds from medicinal plant extracts, and animal products to combat Acanthamoeba infection. Applied nanotechnology, an advanced technology, has shown to enhance the anti-Acanthamoeba activity in the encapsulated nanoparticles leading to new therapeutic options. This review attempts to provide an overview of the available data and studies on the occurrence of pathogenic Acanthamoeba among the Association of Southeast Asian Nations (ASEAN) members with the aim of identifying some potential contributing factors such as distribution, demographic profile of the patients, possible source of the parasite, mode of transmission and treatment. Further, this review attempts to provide future direction for prevention and control of the Acanthamoeba infection.

Key words: Acanthamoeba, clinico-epidemiology, medicinal plant, molecular, nanotechnology, Southeast Asia

INTRODUCTION

Acanthamoeba spp. is one of pathogenic free-living amoebeae (FLA) along with Naegleria fowleri, Balamuthia mandrillaris, and Sappinia sp. which are potential to cause rare infection in central nervous system. These protozoan parasites are mostly found in natural soil and water bodies and immunocompro-

mised patients as the main target [1]. Recently, Acanthamoeba spp. are recognized as increasing threat against contact lens wearers and healthy individuals also take some risks on amoebic keratitis (AK) [2]. Understanding on Acanthamoeba infections is therefore crucial but still limited in ASEAN countries even though studies on anti-Acanthamoeba agent do exist. Herein, an overview of Acanthamoeba was put in a nutshell as well as challenges on recent issues to encounter against this amoeba in our regional ASEAN countries including Brunei Darussalam, Cambodia, Indonesia, Lao People’s Democratic Republic (PDR), Malaysia, Myanmar, the Philippines, Singapore, Thailand, and Vietnam.
ORIGIN OF ACANTHAMOEBA

*Acanthamoeba* spp. is a centrosome-bearing, single-celled, flattened naked amoeba in Order Acanthopodida, Class Centramoebida, Phylum Discosea, Amoebozoa clade in Amorphean domain of Eukaryotic organisms [3]. Term “Acanth” in Greek means spike representing prominent sub-pseudopodia while “amoeba” means alteration like their appearance. The bacteria-phagocytosing protozoa is one of clinical FLA ubiquitous in nature soil and water bodies as well as man-made environment as a secondary decomposer. Ubiquity is implied by presence of antibodies in healthy individuals [4]. *Acanthamoeba* sp. was first recognized as contaminant of *Cryptococcus pararoseus* culture by Castellani in 1930 and named as *Hartmannella castellanii* and then a year later, *Acanthamoeba* spp. because of its double-walled cyst with irregular ectocyst appearance which is different from round and smooth cyst wall of *Hartmannella* spp. [5].

**BRIEF BIOLOGY OF ACANTHAMOEBA**

*Acanthamoeba* spp. appears in 2 forms of life cycle: trophozoite (25-40 µm) and cyst (13-20 µm). Trophozoite is an infective stage with amoeboid locomotion whilst cyst is a dormant stage against harsh environment such as temperature and pH imbalance, malnutrition, or presence of anti-Acanthamoeba agents [6]. One third of strength of cyst wall might come from polymer of glycosidic linkages between saccharides while another 2/3 are protein and other components, respectively [7]. Furthermore, the protist acts as potential reservoir or vector of human-pathogenic bacteria, fungi, or viruses while endosymbiont and Acanthamoeba-resistant organisms also are identified [8-10]. Recently, more than 25 species were recorded in NCBI taxonomy database and 20 genotypes were published which T4 is a major genotype associated with human infections [9,11]. For cultivation, xenic culture is obtained by using non-nutrient (Page’s amoeba saline) or PYG (peptone 0.05%, yeast extract 0.05%, glucose 0.1%) agar coated with living or killed bacteria (e.g., *Escherichia coli*) at 25-28°C in the dark for 2-3 days for trophozoite proliferation and 1-2 weeks for encystment while PYG (peptone 2%, yeast extract 0.5%, glucose 0.5%) agar was used for axenic culture [12]. Culture in PYG medium at 4°C would be convenient method for long-term preservation at least 1-4 years [13].

**EPIDEMIOLOGY OF ACANTHAMOEBA IN ASEANS**

FLA, especially *Acanthamoeba* spp., occur worldwide and have a variety of habitats. Many studies have recorded the wide distribution in soil and water, with differing range of thermal tolerance (Table 1). They have been isolated in untreated natural freshwaters, like lakes, ponds, hot springs and waterfalls [14-17]; and brackish, seawaters, and ocean sediments [18]. They were also isolated from treated waters like domestic water systems, swimming pools, hydrotherapy pools, remedial spas, tap water and drinking water [14,16,19,20]. Unconventional water sources like sewage and aquaria were not spared with the presence of amoebas [18].

Aside from water, *Acanthamoeba* spp. were also present in different types of soils such as agricultural, garden and mining [21-23]. Acanthamoeba genotypes of infected cats and dogs were matched with dry soil and dust. [24]. Acanthamoeba-infected individuals can also be a source of the isolates of organism through sinuses, brain and corneal and skin specimens [22,25-27] and even in necrotic tissues [18].

The presence of *Acanthamoeba* spp. has impacted for the last decades because of the increasing cases of a rare condition AK, a severe infection of the eye cornea associated with intense pain. This has been observed in contact lens wearer population [28]. It is believed that the cause of infection is due to the exposure of the eye to the Acanthamoeba-contaminated contact lens solutions. Acanthamoeba isolated from contact lens storage cases were confirmed [29]. Further, the usual spread of the contaminant is due to poor hygiene and maintenance of the lens; and exposure to contaminated water (swimming pool or other recreational waters) while wearing contact lenses.

However, the disease has also been reported in non-contact lens wearers [18,26,27]. This further affirms the possible contamination through direct contact to contaminated water and soil. The wide dispersal of *Acanthamoeba* onto the environment is due to the wind dispersal of its resistant form, the cysts. Likely that indoor ventilation system, blowing fan, air diffuser and other furniture contaminated with Acanthamoeba can be a cause of spreading indoor [30]. Thus, individuals who are not contact lens wearers but have been constantly exposed to dust particles and soil are also at high risk of infection [25]. It is also important to note that exposure to *Acanthamoeba* can be as simple as accidental splash of contaminated water to the face or bruised skin [14], making a fast and easy transmission.
### Table 1. Distribution of environmental Acanthamoeba spp. in Southeast Asia

| Country           | Type of samples          | No. of sample | Positive culture | Acanthamoeba spp. morphology | References                  |
|-------------------|--------------------------|---------------|------------------|-------------------------------|-----------------------------|
| Thailand          | Water                    |               | FLA              | Acanthamoeba spp.            |                             |
|                   | Water samples            | 95            | 51.58% (49/95)   | 18.95% (18/95)               | Nacapunchai et al. (2001) [23]|
|                   | Hot spring water         | 69            | 37.68% (25/69)   | 13% (9/69)                  | Lekkla et al. (2005) [17]   |
|                   | Freshwater pond and irrigation canals | 84 | ND               | 19.05% (16/84)              | Nuprasert et al. (2010) [59]|
|                   | Flood water              | 7             | 100% (7/7)       | 14.29% (1/7)                | Wannasan et al. (2013) [60] |
|                   | Freshwater pond in public parks | 300 | ND               | 35% (105/300)               | Buppan et al. (2018) [61]  |
|                   | Water-logged fields      | 2             | 100% (2/2)       | 100% (2/2)                  |                             |
|                   | Ditches                  | 4             | 100% (4/4)       | NF                           |                             |
|                   | Paddy fields             | 6             | 100% (6/6)       | 16.67% (1/6)                |                             |
|                   | Fish farms               | 10            | 50% (5/10)       | 10% (1/10)                  |                             |
|                   | Large pond               | 6             | 50% (3/6)        | NF                           |                             |
|                   | Natural water            | 63            | ND               | 15.87% (10/63)              | Thammaratana et al. (2016) [15]|
|                   | Air                      |               | FLA              | Acanthamoeba spp.            |                             |
|                   | Outdoor air              | 103           | ND               | 41.7% (43/103)              | Yaicharoen et al. (2007) [63]|
|                   | Indoor air               | 64            | ND               | 18.1% (37/64)               |                             |
|                   | Soil                     |               | FLA              | Acanthamoeba spp.            |                             |
|                   | Soil swab samples        | 120           | 69.17% (83/120)  | 33.33% (40/120)             | Nacapunchai et al. (2001) [23]|
|                   | Water-logged fields      | 2             | 100% (2/2)       | 50% (1/2)                   | Wannasan et al. (2009) [62] |
|                   | Ditches                  | 4             | 75% (3/4)        | 50% (2/4)                   |                             |
|                   | Paddy fields             | 6             | 100% (6/6)       | NF                           |                             |
|                   | Fish farms               | 10            | 50% (5/10)       | NF                           |                             |
|                   | Large pond               | 6             | 66.7% (4/6)      | 16.67% (2/6)                |                             |
| Malaysia          | Water                    |               | FLA              | Acanthamoeba spp.            |                             |
|                   | Domestic tap water       | 42            | ND               | 2.4% (1/42)                 | Anisah et al. (2003) [64]   |
|                   | Swimming pools in Kuala Lumpur | 840 | 54.4% (457/840) | 46.19% (388/840)           | Int et al. (2010) [32]     |
|                   | Recreational anthropogenic lake A | 7 | ND | 100% (7/7) | Onichandran et al. (2013) [16] |
|                   | Recreational anthropogenic lake B | 6 | ND | 100% (6/6) |                             |
|                   | Tap water                | 181           | 29.8% (54/181)   | 24.9% (45/181)              | Gabriel et al. (2019) [65]  |
|                   | Recreational places      | 57            | 66.7% (38/57)    | 70.2% (40/57)               |                             |
|                   | Water dispenser units    | 3             | 33.3% (1/3)      | 66.7% (2/3)                 |                             |
|                   | Filtered water           | 4             | 75% (3/4)        | NF                           |                             |
|                   | Drain water              | 1             | 100% (1/1)       | NF                           |                             |
|                   | Paddy fields             | 4             | 50% (2/4)        | 100% (4/4)                  |                             |
|                   | Drinking water treatment | 61            | 90.2% (55/61)    | 18.03% (7/11)               | Richard et al. (2016) [20]  |
|                   | Water samples            | 15            | ND               | 100% (15/15)                | Basher et al. (2018) [24]   |
|                   | Swabs (rocks and stones) | 15            | ND               | 73.33% (7/11)               |                             |

(Continued to the next page)
| Country | Type of samples | No. of sample | Positive culture | Acanthamoeba spp. morphology | References |
|---------|----------------|--------------|------------------|-----------------------------|------------|
| Soil | Wet soil | 15 | ND | 100% (15/15) | ND ND ND |
| | Children playgrounds (Dry soil) | 15 | ND | 100% (15/15) | ND ND ND |
| Other | Indoors wall surface | 20 | ND | 100% (20/20) | ND ND ND |
| | Outdoor wall surface | 20 | ND | 100% (20/20) | ND ND ND |
| | Air conditioners in KM | 87 | ND | 23% (20/87) | NF 71.43% (15/21) 28.57% (6/21) Chan et al. (2011) [66] |
| Vietnam | River | 10 | ND | 30% (3/10) | ND ND ND |
| | Swimming pools | 4 | ND | 50% (2/4) | ND ND ND |
| | Pond | 3 | ND | 66.67% (2/3) | ND ND ND |
| | Lake | 6 | ND | 33.33% (2/6) | ND ND ND |
| | Tap water | 3 | ND | 33.33% (1/3) | ND ND ND |
| | Rain/tap tank | 2 | ND | NF | ND ND ND |
| | Water dispenser | 2 | ND | 50% (1/2) | ND ND ND |
| | Well | 1 | ND | 100% (1/1) | ND ND ND |
| | Spring | 1 | ND | NF | ND ND ND |
| | Mineral | 1 | ND | NF | ND ND ND |
| | Water | 3 | ND | 100% (3/3) | ND ND ND |
| Vietnam | Soil | 10 | ND | 100% (10/10) | ND ND ND |
| | Soil | 4 | ND | 100% (4/4) | ND ND ND |
| Others (Lao PDR, Myanmar, and Singapore) | Soil | 1 | 359 small sub unit rDNA Sequences of Amoebae | 5.95% | ND ND ND |
| | Mining soil | 1 | | 4.76% | ND ND ND |
| | Treated water in Lao PDR | 9 | 11.11% (1/9) | NF | ND ND ND |
| | Untreated water in Lao PDR | 22 | 4.55% (1/22) | 4.55% (1/22) | ND ND ND |
| | Treated water in Yangon | 11 | 18.18% (2/11) | NF | ND ND ND |
| | Untreated water in Yangon | 31 | 16.13% (5/31) | 9.68% (3/31) | ND ND ND |
| | Treated water in Singapore | 6 | NF | NF | ND ND ND |
| | Untreated water in Singapore | 15 | NF | NF | ND ND ND |

ND, Not detected; NF, Not found.
Ironically, with the many studies proving the presence of *Acanthamoeba* in different environmental media (soil, water and air), the dearth of information in Southeast Asian (ASEAN) countries is quite a concern, considering that the varying climatic conditions of the region is a favorable habitat for this organism which has an unusual geographic distribution [31].

The ASEAN countries’ tropical condition, favorite tourist destinations during summer, consists of beaches, falls, and lakes are among the popular areas where more people involve with these outdoor activities. The congestion can increase risk of contamination with *Acanthamoeba* especially when the environment is dry during summer and dust particles can be easily spread. Likewise, resorts with swimming pools are occupied the entire summer with local and foreign tourists. Since resorts gain profit only during this time of the year, owners tend to maximize the use of the swimming pools which may compromise the proper cleanup of the swimming facility. This poses the risk to the swimmers, adding to the fact that *Acanthamoeba* can also be resistant to disinfectants [26,32].

The detection of *Acanthamoeba* in soil, water and air in other countries in ASEAN (Fig. 1), confirms that a continual contamination of the environment persists, and this poses a risk
to people dependent on the soil and water for domestic activities, agricultural and farming occupation, and even for recreation. The lack of information in some countries (Cambodia and Brunei) does not mean the absence of Acanthamoeba-contaminated environment. Albeit, this may result to the inability of one country to control the spread of possible diseases associated with Acanthamoeba considering that this amoeba may also harbor pathogenic bacteria or fungi.

**CLINICAL SIGNIFICANCE AND DIAGNOSIS**

Potential pathogenicity of Acanthamoeba was first observed in monkey kidney cell in vitro as well as intracerebral/intraspinal inoculation in monkeys and intravenous/intranasal inoculation in mice [33,34]. First patient was recognized as GAE in 1972 and a year later, AK [35,36]. Acanthamoeba spp. are therefore considered as rare potential pathogen causing cutaneous lesions, sinusitis, AK, GAE, and disseminated form in human and prefer individuals with underlying diseases or immunocompromised host but AK was frequently reported in immunocompetent patients especially, contact lens wearers [37].

For AK, poor sanitation of contact lens wearer is a potential risk and corneal trauma seem required before trophozoite infection as well as eye secretion after contact lens wore might be preferred by Acanthamoeba [38,39]. Onset of AK is days to weeks with symptoms of tormenting eye pain, redness, photophobia, stromal infiltration leading to sight-threatening condition which are similar and misdiagnosed to Herpes simplex, bacterial or fungal keratitis [39,40]. AK is confirmed by presence of trophozoite with large nucleolus and contractile vacuoles as well as pseudopodia and transparent protrusions of Acanthamoeba from corneal scrapings or biopsies under direct microscopy with several stains. Encystment on non-nutrient agar (NNA) and nucleic acid amplification testing are further investigated for species identification and genotyping, respectively. Taxonomic identification mainly investigated by cyst morphology under microscope [41] and a hypervariable sequence part of 18S small subunit rDNA gene called ASA.S1 by Acanthamoeba-specific primers: JDP1 and JDP2 (amplicon size 467 bps for Neff strain of A. castellanii accession number M13435.1) [42]. Extended or almost complete of 18S rDNA amplicon size provide better solution for genotyping [11,42]. Pathogen broad-spectrum and most effective anti-Acanthamoeba agents against two forms, 0.02% polyhexamethylene biguanide (PHMB) or chlorhexidine, still need antibacterial, antifungal, or aromatic diamidines combination because of resistance of cyst form and PHMB is toxic to human corneal cells [40].

For GAE, a very rare condition, is opportunistic and fatal infection with onset of weeks to months mostly in immunocompromised patients, especially HIV/AIDS patients through skin breaks, respiratory tract, and olfactory epithelium. GAE patient will encounter with neurological signs such as confusion, headache, and stiff neck as well as psychological change, e.g. irritability generally like other brain infections due to effect of edema, necrosis, and hemorrhages in infected part of brain [43]. To confirm GAE, microscopy and culture from CSF remain gold standard methods used after neuroimaging detection of brain lesions while indirect immunofluorescence on tissue and multiplex real-time PCR assay are available [44]. Late/missed diagnosis, blood-brain barrier crossing of antimicrobial, drug side effects, drug combination are still an issue on GAE treatment and only few patients were cured [45,46].

There are many reports on Acanthamoeba infection in ASEAN countries (Table 2). Most infections are AK with contact lens while cases of GAE is rare. Notably, Acanthamoeba can be involved with gastric ulcer and sinusitis and found from nasal swab from healthy individuals and corneal swab from infected animal (Table 2). Undeniably, exposure to soil and contaminated water are potential risk but underlying disease might be another one factor for the infections. Misdiagnosis and delay in diagnosis are common among patients leading to permanent vision blurriness because of injured cornea or deeper layers for AK and death for GAE. These problems are still insolvable till date. Rapid and accurate prognosis is therefore an urgent need for Acanthamoeba infection.

**CURRENT CHALLENGES AND FUTURE PERSPECTIVES**

Contact with Acanthamoeba spp. is common. Immunocompromised patients should realize this risk and avoid exposure to, especially, natural soil and water bodies even though it is a rare disease but GAE is fatal and AK is vision-threatening [6]. Moreover, no specifically therapeutic course is available for Acanthamoeba spp. infections, in case of GAE. However, commercial drugs for AK are highly toxic due to prolonged treatment duration as well as diagnosis and combination of treatments depends on medical expertise of physician and availability of resources [6,47]. The statement diagnostic is challenge that a new molecular technology can be used in Acan-
### Table 2. Examples of *Acanthamoeba* infection cases in Southeast Asia

| Ethnicity/Gender | Age (yr) | Clinical sample | Diagnostic method | Condition (Genotype) | Potential history of patients | Treatment | Status after treatment | Status after references |
|------------------|---------|----------------|-------------------|----------------------|-----------------------------|-----------|-----------------------|-------------------------|
| Singaporean male | 28      | Corneal scraping | Microscopy and culture | AK with *Pseudomonas aeruginosa* | Hit with polyvinyl/chloride pipe, *Before diagnosis*: cefazolin and gentamicin; *After diagnosis*: topical cycloplegics; topical 0.1% hexamidine, 0.02% chlorohexidine, and transplantation | Vision blurriness | Alive with altered mental status | Lim et al. (2018) [68] |
| Adults and juveniles (48/200 felines and 8/25 canines (56/225 naturally-infected animal) in Malaysia) | - | Corneal swabs | Microscopy, culture, and partial 18S rDNA sequencing | AK (T4) | Dry soil and dust (strain-matched partial 18S rDNA sequence) | - | - | Basher et al. (2018) [24] |
| Indonesian female | 32      | Corneal scraping | Microscopy and culture | AK | Monthly disposable soft contact lens wearer for 1 year with tap water to rinse contact lens and case in many occasions | Improved vision blurriness | Alive with altered mental status | Muslim et al. (2018) [19] |
| Thai female | 58      | Brain abscess | CT scan, Microscopy, and PCR on partial 18S rDNA sequencing | GAE | Farmer with pulmonary tuberculosis history, Raynaud’s phenomenon, mild myositis, and high antinuclear antibody (speckle type) | Metronidazole and Prednisolone | Loss of follow-up | Wera-Asawapati et al. (2017) [22] |
| Indonesian male | 2       | Cerebrospinal fluid | CT scan and microscopy | GAE | Drowning survivor | Intravenous ceftazidime, metronidazole, fluconazole and rifampicin | Alive with altered mental status | Gunawan et al. (2016) [69] |
| Filipino male | 76      | Corneal scraping | Microscopy, culture, and partial 18S rDNA sequencing | AK (T4) | Non-contact lens wearer | Chlorhexidine | Corneal scar | Buerano et al. (2014) [27] |
| 12/180 Filipinos | - | Nasal swab | Microscopy, culture, and partial 18S rDNA sequencing | -(T5, 54, T11) | Street sweeper (4/44), Garbage collector (2/37), Garbage sorter (0/16), Landscaper (1/37), Bioreactor laborer (0/4), foremen and supervisors (0/3), and students (1/70) | - | - | Cruz and Rivera (2014) [25] |
| 22 cases in Siriraj hospital, Thailand (1996-2006) | 48.3±14.5 for 8 non-contact lens wearers, 30.6±15.3 for 12 contact lens wearers | Corneal scraping | Microscopy and culture | AK | Contact lens wearer with lack of hygiene | Chlorhexidine, polyhexamethylenguanidine or propamidine | Improved vision blurriness and loss of follow-up for some patients | Wanachiwanswin et al. (2012) [70] |

(Continued to the next page)
| Ethnicity/Gender | Age (yr) | Clinical sample | Diagnostic method | Condition (Genotype) | Potential history of patients | Treatment | Status after treatment | References |
|------------------|---------|----------------|------------------|--------------------|-----------------------------|-----------|-----------------------|------------|
| 9/103 infective keratitis patients with eye surgery | - | - | - | AK | Polymyxin B, chlorhexidine, propamidine isethionate, and transplantation. | Improved vision blurriness | Anshu et al. (2009) [71] |
| 22 Chinese, 8 Malay, 5 Indian, 7 others (2005-2007 in Singapore) | <20 years-old=13, 21-40 years-old=25, 41-60 years-old=4 | Corneal scraping, biopsy, and keratoplasty specimen | Microscopy and culture | AK | Suboptimal hygiene practices | 0.02% topical polyhexamethylene biguanide, 0.02% chlorohexidine, 0.1% hexamidine, 0.1% propamidine isethionate, and transplantation. | Vision blurriness | Por et al. (2009) [72] |
| 3 Filipinos | | Corneal scraping | Microscopy | AK | Non-contact lens wearer | 0.1% topical diclofenac sodium and atropine drops. | Vision blurriness in 2/3 patients | Agahan et al. (2009) [73] |
| 3 AK patients/127 microbial keratitis eyes (2001-2004) in Ramathibodi Hospital, Thailand | Mean age 40±22 for all 127 microbial keratitis patients | Corneal scraping | Microscopy and culture | AK | Contact lens wearers | | | Sirikul et al. (2009) [74] |
| Chinese female | 13 | Corneal scraping | Microscopy and culture | AK | Rigid gas-permeable contact lens wearer | Before diagnosis; Acanthamoebic agents: 0.02% topical polyhexamethylene biguanide, 0.02% chlorohexidine, 0.1% hexamidine, and transplantation. After surgery: 0.1% topical dexamethasone phosphate, 0.5% levofloxacin, same Acanthamoebic agents, and topical preservative-free steroids. | Improved vision blurriness | Parthasarathy and Tan (2007) [75] |
| Thai female | - | Biopsy and autopsy | Microscopy | GAE | Swimming in a dam | Caldwell-Luc operation, Intravenous amphotericin B, oral ketoconazole, and amoxicillin/clavulanic acid | Cured | Sripanth (2005) [76] |
| Thai male | 36 | Nasal exudate | Microscopy and culture | Ameobia co-infection sinusitis (Nagehria sp. and Acanthamoeba sp.) | Diving in a natural pond | | | Sukthana et al. (2005) [77] |
| Singaporean female | 39 | Corneal scraping | Microscopy and culture | AK | Contact lens wearer with multipurpose disinfectant solution | Miscellaneous: Occulentum Acyclovir, Guttia Chloramphenicol, and 0.12% Guttia Prednisolone; After diagnosis: 0.1% gutt propamidine isethionate, 0.02% gutt polyhexamethylene biguanide, and laser In Situ keratomileusis (LASIK) for Myopia | Improved vision blurriness and nearsightedness | Lim and Wei (2004) [78] |
| Malaysian male | 28 | Corneal scraping | Microscopy and culture | AK | Construction worker eye washed with water from opemink after sand and dust strucked in the eye | Topical Propamidine isethionate, Chlorohexidine 0.02% and fortified Gentamycin | | Kamel et al. (2003) [79] |

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Table 2. Continued

| Ethnicity/Gender | Age (yr) | Clinical sample | diagnostic method | Condition (Genotype) | Potential history of patients | Treatment | Status after treatment | References |
|------------------|---------|-----------------|-------------------|----------------------|------------------------------|-----------|-----------------------|------------|
| Chinese male     | 24      | Corneal scraping | Microscopy and culture | AK                   | Non-disposable soft contact lens wearer and no contact lens when swim in lake/pool | Before diagnosis: gutt spersadexoline; After diagnosis: 0.1% gutt propamidine isethionate, and gutt tobramycin | Stromal scar | Cheng et al. (2000) [79] |
| Malay male       | 26      | Corneal scraping | Microscopy and culture | AK                   | Non-disposable soft contact lens wearer | Before diagnosis: tetracycline ointment and neosporin eyedrops; After diagnosis: 0.1% gutt propamidine isethionate | Stromal scar | Cheng et al. (2000) [79] |
| Thai female      | 58      | Corneal scraping | Microscopy, culture and mtDNA-RFLP | AK                   | Left eye injured by straw fragment and dirt cleaned off from her face using water in a jar near her home after digging in the garden on the outskirts | Before diagnosis: antimicrobial eye drops and ointment; 1% trifluorothymidine eye drops and acyclovir eye ointment; After diagnosis: ketoconazole eye drops, neosporin, polymyxin, neomycin, gramicidin, propamidine isethionate eye drops, dibromopropamidine isethionate eye ointment, and transplantation. | Recurrence necessitating evisceration | Jongwutiwes et al. (2000) [80] |
| Thai male        | 30      | Corneal scraping | Microscopy, culture, and mtDNA-RFLP | AK                   | Splashing fish pond water to left eye injured by tiny piece of bamboo | Before diagnosis: miconazole and neosporin eye drops; After diagnosis: propamidine isethionate eye drops, and dibromopropamidine isethionate eye ointment | Vision blurriness | Jongwutiwes et al. (2000) [80] |
| Thai female      | 57      | Corneal scraping | Microscopy, culture, indirect immunofluorescence testing, and isoenzyme analysis | AK                   | Pond water for washing | Before diagnosis: spersapolymyxin eyedrops, cefazolin and gentamicin subconjunctival injection, topical neomycin sulfate, polymyxin B, and gramicidin; After diagnosis: 0.006% chlorhexidine hydrochloride with antidiarrhea for recurrence | Improved vision blurriness with cataract | Kosrirukvongs et al. (1999) [81] |
| Thai male        | 36      | Corneal scraping | Microscopy, culture, indirect immunofluorescence testing, and isoenzyme analysis | AK                   | Dust | Before diagnosis: topical neomycin sulfate, polymyxin B, and gramicidin; After diagnosis: 0.006% chlorhexidine solution | Loss of follow-up but no recurrence | Kosrirukvongs et al. (1990) [81] |
| Thai female      | 33      | Corneal scraping | Microscopy, culture, indirect immunofluorescence testing, and isoenzyme analysis | AK                   | Daily-wear soft contact lenses | Before diagnosis: fortified cefazolin, gentamicin, neomycin, topical tobramycin, topical neomycin sulfate, polymyxin B, and gramicidin; After diagnosis: 0.006% chlorhexidine solution | Improved vision blurriness | Kosrirukvongs et al. (1990) [81] |
| Thai male        | 74      | Corneal scraping | Microscopy, culture, indirect immunofluorescence testing, and isoenzyme analysis | AK                   | Plant root exposure | Before diagnosis: antibiotics and plant root, topical neomycin sulfate, polymyxin B, and gramicidin; After diagnosis: 0.006% chlorhexidine solution and 1% topical clotrimazole eyedrops Note: non-compliance | Enucleation | Kosrirukvongs et al. (1990) [81] |
| Ethnicity/Gender | Age (yr) | Clinical sample | Diagnostic method | Condition (Genotype) | Potential history of patients | Treatment | Status after treatment | References |
|------------------|---------|-----------------|-------------------|----------------------|-----------------------------|-----------|-----------------------|------------|
| Thai female      | 65      | Corneal scraping | Microscopy, culture, indirect immunofluorescence testing, and isoenzyme analysis | AK | Unknown | Before diagnosis: topical neomycin sulfate, polymixin B, and gramicidin; After diagnosis: cefazolin and gentamicin eye drops for *P. aeruginosa* as well as chlorhexidine for *Acanthamoeba* sp. | Vision blurriness with cataract before diagnosis; topical neomycin sulfate, polymyxin B, and gramicidin as well as chlorhexidine for *Acanthamoeba* sp. after diagnosis; topical neomycin sulfate, polymyxin B, and gramicidin as well as chlorhexidine for *Acanthamoeba* sp. | Kosrirukwongs et al. (1990) [81] |
| Malaysian female | 40      | Corneal scraping | Microscopy | AK with *P. aeruginosa* and *E. coli* | Contact lens wearer | Before diagnosis: Zovirax®; After diagnosis: gentamycin and homatropin eye drops, neosporin, miconazole eyedrops and Brolene® (0.1% Propamidine isethionate) | - | Kamel and Norazah (1995) [82] |
| Thai female      | 26      | Brain autopsy   | Microscopy and indirect immunofluorescence test | GAE | Worker | - | Death | Sangruchi et al. (1994) [83] |
| Thai male        | 20      | Brain autopsy   | Microscopy and indirect immunofluorescence test | GAE | Farmer | - | Death | Sangruchi et al. (1994) [83] |
| Thai female      | 42      | Biopsy         | Radiography and microscopy | Proliferated gastric ulcer with gastric acanthamoebiasis and sepsis from operative site with *E. coli* and *K. pneumoniae* | Immunocompetent patients | Venesection and rapid fluid replacement, antibiotics, gastrojejunostomy, and parenteral ampicillin, gentamicin, and metronidazole | Death | Thamprasert et al. (1993) [84] |

**AK, Acanthamoeba keratitis; GAE, Granulomatous amoebic encephalitis; -, Not mentioned in the published paper.**
Table 3. Anti-Acanthamoeba agents and nanoparticles in ASEAN studies

| Chemicals                                           | Nanotechnology | Anti-Acanthamoeba activity against | References |
|-----------------------------------------------------|----------------|------------------------------------|------------|
| Cyclic samarium complexes                           |                | IC₅₀ = 6.5 µg/ml against Acanthamoeba keratitis isolate | Kusrini et al. (2018; Indonesia) [85] |
| [Sm(Pic)₂(18C₆)] (Pic)                               |                | IC₅₀ = 0.7 µg/ml against Acanthamoeba keratitis isolate | Kusrini et al. (2018; Indonesia) [85] |
| Acyclic samarium complexes                           |                | IC₅₀ = 7 µg/ml against Acanthamoeba keratitis isolate | Kusrini et al. (2016; Indonesia) [86] |
| Terbium complex [Tb(NO₃)₃(OH₂)₉](18C₆)               |                | IC₅₀ = 2.6 µg/ml against Acanthamoeba keratitis isolate | Kusrini et al. (2016; Indonesia) [86] |
| Tb(NO₃)₃.6H₂O in CH₃CN                                |                | IC₅₀ = 1.2 µg/ml against Acanthamoeba keratitis isolate | Kusrini et al. (2016; Indonesia) [86] |
| 18C₆ in CH₃CN                                       |                | IC₅₀ = 0.7 µg/ml against Acanthamoeba keratitis isolate | Kusrini et al. (2018; Indonesia) [85] |
| Phosphanegold (I) thiolates                         |                | No effect on viability, growth, cellular differentiation, and extracellular proteolytic activities against A. castellanii (ATCC50492) | Siddiqui et al. (2017; Malaysia) [87] |
| 3% DMSO                                             |                | Encystation induction and excystation inhibition against A. castellanii (ATCC50492) | Siddiqui et al. (2016; Malaysia) [88] |
| Carbonyl Thiourea derivatives                       |                | IC₅₀ = 2.39-8.77 µg/ml against A. castellanii (CCAP 1501/2A) and 3.74-9.30 µg/ml against A. polyphaga (CCAP 1501/3A) | Ibrahim et al. (2014; Malaysia) [89] |
| Commercial fusaric acid                             |                | No effect on viability, growth, cellular differentiation, and extracellular proteolytic activities against A. castellanii (ATCC50492) | Boonman et al. (2012; Thailand) [90] |
| Betadine® solution                                   | SCC = 0.04% dilution after 24 hr against Acanthamoeba keratitis isolate | - | Roongruangchai et al. (2011; Thailand) [91] |
| Virkon® solution                                     | SCC = 0.25% dilution after 24 hr against Acanthamoeba keratitis isolate | - | Roongruangchai et al. (2010; Thailand) [92] |
| Plant products                                       |                |                                    |            |
| Hesperidin, commercial flavonoid from Citrus sp.    | Silver nanoparticles stabilized by gum acacia | Encystation and excystation inhibition against A. castellanii (ATCC50492) | Anwar et al. (2019; Malaysia) [93] |
| Naringin, commercial flavonoid from Citrus sp.      | Gold nanoparticles stabilized by gum tragacanth | Encystation and excystation inhibition against A. castellanii (ATCC50492) | Anwar et al. (2019; Malaysia) [93] |
| Periglaucine A from Pericampylus glaucus            | (DL-lactide-co-glycolide) Poly | C₅₀/Cₐ₅₀ = 100 against A. triangularis from environmental water sample | Mahboob et al. (2018; Malaysia) [94] |
| Betulinic acid from Pericampylus glaucus            | (DL-lactide-co-glycolide) Poly | C₅₀/Cₐ₅₀ = 10 against A. triangularis from environmental water sample | Mahboob et al. (2018; Malaysia) [94] |
| Periglaucine A from Pericampylus glaucus            | (DL-lactide-co-glycolide) Poly | C₅₀/Cₐ₅₀ = 8.5 against A. triangularis from environmental water sample | Mahboob et al. (2017; Malaysia) [95] |
| Betulinic acid from Pericampylus glaucus            | (DL-lactide-co-glycolide) Poly | C₅₀/Cₐ₅₀ = 3.75 against A. triangularis from environmental water sample | Mahboob et al. (2017; Malaysia) [95] |

(Continued to the next page)
### Table 3. Anti-Acanthamoeba agents and their effects

| Anti-Acanthamoeba agents | Nanotechnology | Anti-Acanthamoeba activity against | References |
|--------------------------|----------------|-----------------------------------|------------|
| **Cysts** | **Trophozoites** | | |
| Cinnamic acid from *Cinnamomum cassia* | Gold nanoparticles | Encystation inhibition against *A. castellanii* (ATCC 50492) | Significantly enhanced anti-Acanthamoeba activity against *A. castellanii* (ATCC 50492) when compared with cinnamic acid alone | Anwar et al. (2018; Malaysia) [96] |
| Ethyl acetate, water, butanol fractions from *Lonicera japonica* | - | - | Significant anti-Acanthamoeba effect against *A. triangularis* trophozoites by ethyl acetate (most potent fraction) and cyst: trophozoites ratio reduction by commercial chlorogenic acid (major constituent in *L. japonica*) | Mahboob et al. (2016; Malaysia) [97] |
| *Pouzolzia indica* methanolic extract fraction 2 | - | MCC = 1: 4 dilution after 24 hr against Acanthamoeba keratitis isolate | - | Roongruangchai et al. (2011; Thailand) [91] |
| *Pouzolzia indica* methanolic extract fraction 3 | - | MCC = 1: 8 dilution after 24 hr against Acanthamoeba keratitis isolate | - | Roongruangchai et al. (2010; Thailand) [92] |
| Supernatants from bacteria isolated from cockroach gut: *Serratia marcescens* and *Escherichia coli* from Madagascar cockroach; two *Klebsiella* spp., *Citrobacter* sp., *Bacillus* sp., *Streptococcus* sp. from Dubia cockroach | - | - | Significant anti-Acanthamoeba effect against *A. castellanii* (ATCC 50492) | Akbar et al. (2019; Malaysia) [98] |
| Effective microorganisms (EM™) | - | Undiluted, 1:2, 1:4, 1:6 dilution of EM resulted in lower than 40% viable cysts | - | Sampaotong et al. (2016; Thailand) [99] |
| Fusaric acid from *Fusarium fujikuroi* species complex Tlau3 isolated from *Thunbergia laurifolia* | - | - | IC<sub>50</sub> = 0.31 μm against Acanthamoeba keratitis isolate | Boonman et al. (2012; Thailand) [90] |
| Dehydrofusaric acid from *Fusarium fujikuroi* species complex Tlau3 isolated from *Thunbergia laurifolia* | - | - | IC<sub>50</sub> = 0.34 μm against Acanthamoeba keratitis isolate | Boonman et al. (2012; Thailand) [90] |
| Drugs | | | |
| Nystatin, Fluconazole, and Amphotericin B | Gold nanoparticles | - | Enhanced anti-Acanthamoeba activity at 10 μM (Amphotericin B > Fluconazole > Nystatin) against *A. castellanii* (ATCC 50492) | Anwar et al. (2019; Malaysia) [100] |
| Nystatin, Fluconazole, and Amphotericin B | Silver nanoparticles | - | Enhanced anti-Acanthamoeba activity at 10 μM against *A. castellanii* (ATCC 50492) | Anwar et al. (2018; Malaysia) [101] |
| Diazepam (Valium), Phenobarbitone (Luminal), and Phenytoin (Dilantin) | And their silver nanoparticles | Anti-Encystation activity (Diazepam and Phenobarbitone activity enhanced with silver nanoparticles and anti-cyst activity (Phenobarbitone and Phenytoin activity enhanced with silver nanoparticles) against *A. castellanii* (ATCC 50492) | Anti-Acanthamoeba activity observed and enhanced activity with silver nanoparticles against *A. castellanii* (ATCC 50492) | Anwar et al. (2018; Malaysia) [102] |
| Diclofenac sodium and Indomethacin (NSAIDs) | - | Encystation inhibition of A. castellanii (ATCC 50492) | Growth affected but not viability of *A. castellanii* | Siddiqui et al. (2016; Malaysia) [103] |

(Continued to the next page)
**Table 3. Continued**

| Anti-Acanthamoeba agents | Nanotechnology | Cysts | Trophozoites | References |
|--------------------------|----------------|-------|--------------|------------|
| Acetaminophen (NSAIDs)   | -              | No effects on encystation inhibition of A. castellanii (ATCC 50492) | No effects on growth of A. castellanii (ATCC 50492) | Siddiqui et al. (2016; Malaysia) [103] |
| Bortezomib (proteasome inhibitor) | -              | Encystation inhibition against A. castellanii | Static effect on growth but not viability of A. castellanii (ATCC 50492) | Siddiqui et al. (2016; Malaysia) [104] |
| Lactacystin and active form as clasto-lactacystin β-lactone (proteasome inhibitors) | -              | Encystation inhibition and encystation inhibition | No effects on growth and viability of A. castellanii (ATCC 50492) | Siddiqui et al. (2016; Malaysia) [104] |
| Artesunate (Antimalaria) | -              | Presence of cytocidal effect on Acanthamoeba polyphaga-like amoebae were isolated from natural water courses at concentrations of 500-700 μg/ml | Dose-dependent growth inhibition (5-700 μg/ml) against Acanthamoeba polyphaga-like amoebae were isolated from natural water courses | Nacapunchai et al. (2003; Thailand) [105] |
| Metronidazole            | -              | No effects (5-1,000 μg/ml) | No effects (5-1,000 μg/ml) | Nacapunchai et al. (2003; Thailand) [105] |

**Animal products**

|                     |                 | Anti-Acanthamoeba activity against A. castellanii (ATCC 50492) | Anti-Acanthamoeba activity against A. castellanii (ATCC 50492) | References |
|---------------------|-----------------|---------------------------------------------------------------|---------------------------------------------------------------|------------|
| Crocodile (Crocodylus palustris) serum | -              | I_{50} = 0.615-0.876 μg/ml against clinical A. castellanii | I_{50} = 0.615-0.876 μg/ml against clinical A. castellanii | Siddiqui et al. (2017; Malaysia) [106] |
| Sea sponge crude methanol extracts (Aaptos aaptos) from different localities | -              | Anti-Acanthamoeba activity against A. castellanii | Anti-Acanthamoeba activity against A. castellanii | Nakissah et al. (2012; Malaysia) [107] |

IC, Inhibition concentration; CC, Cytotoxicity concentration; MCC, Minimal cysticidal concentration; -, Not mentioned in the published paper.
doi.ac.th/pharm/search.asp: March 13, 2019). It is therefore noteworthy to strongly recommend for more research works that should be further explored on the plants-based medicinal therapy for severe or deadly infections with *Acanthamoeba* spp.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest related to this study.

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