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▣ MINI-REVIEW

Acanthamoeba in Southeast Asia – Overview and Challenges

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Abstract: *Acanthamoeba*, one of free-living amoebae (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 amobae (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 immunocompromised 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.

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ORIGIN OF *ACANTHAMOEBA*

Acanthamoeba spp. is a centrosome-bearing, single-celled, flattened naked amoeba in Order Acanthopodida, Class Centramoebia, Phylum Discosea, Amoebozoa clade in Amorphea 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
			FLA	*Acanthamoeba* spp.	Group I	Group II	Group III	
Thailand	Water							
	Water samples	95	51.58% (49/95)	18.95% (18/95)	ND	ND	ND	Nacapunchai et al. (2001) [23]
	Hot spring water	69	37.68% (28/69)	13% (9/69)	ND	ND	ND	Lekkla et al. (2005) [17]
	Freshwater pond and irrigation canals	84	ND	19.05% (16/84)	15.79% (3/19)	84.21% (16/19)	NF	Nuprasert et al. (2010) [59]
	Flood water	7	100% (7/7)	14.29% (1/7)	ND	ND	ND	Wannasan et al. (2013) [60]
	Freshwater pond in public parks	300	ND	35% (105/300)	23.36% (25/105)	73.83% (79/105)	2.8% (3/105)	Buppan et al. (2018) [61]
	Water-logged fields	2	100% (2/2)	100% (2/2)	ND	ND	ND	Wannasan et al. (2009) [62]
	Ditches	4	100% (4/4)	NF	ND	ND	ND	
	Paddy fields	6	100% (6/6)	16.67% (1/6)	ND	ND	ND	
	Fish farms	10	50% (5/10)	10% (1/10)	ND	ND	ND	
	Large pond	6	50% (3/6)	NF	ND	ND	ND	
	Natural water	63	ND	15.87% (10/63)	ND	ND	ND	Thammaratana et al. (2016) [15]
	Air							
	Outdoor air	103	ND	41.7% (43/103)	NF	16.5% (17/43)	15.5% (16/43)	Yaicharoen et al. (2007) [63]
	Indoor air	64	ND	18.1% (37/64)	NF	13.7% (28/37)	2.9% (6/37)	
	Soil							
	Soil swab samples	120	69.17% (83/120)	33.33% (40/120)	ND	ND	ND	Nacapunchai et al. (2001) [23]
	Water-logged fields	2	100% (2/2)	50% (1/2)	ND	ND	ND	Wannasan et al. (2009) [62]
	Ditches	4	75% (3/4)	50% (2/4)	ND	ND	ND	
	Paddy fields	6	100% (6/6)	NF	ND	ND	ND	
	Fish farms	10	50% (5/10)	NF	ND	ND	ND	
	Large pond	6	66.7% (4/6)	16.67% (2/6)	ND	ND	ND	
Malaysia	Water							
	Domestic tap water	42	ND	2.4% (1/42)	ND	ND	ND	Anisah et al. (2003) [64]
	Swimming pools in Kuala Lumpur	840	54.4% (457/840)	46.19% (388/840)	Positive	Positive	ND	Init et al. (2010) [32]
	Recreational anthropogenic lake A	7	ND	100% (7/7)	ND	ND	ND	Onichandran et al. (2013) [16]
	Recreational anthropogenic lake B	6	ND	100% (6/6)	ND	ND	ND	
	Tap water	181	29.8% (54/181)	24.9% (45/181)	ND	ND	ND	Gabriel et al. (2019) [65]
	Recreational places	57	66.7% (38/57)	70.2% (40/57)	ND	ND	ND	
	Water dispenser units	3	33.3% (1/3)	66.7% (2/3)	ND	ND	ND	
	Filtered water	4	75% (3/4)	NF	ND	ND	ND	
	Drain water	1	100% (1/1)	NF	ND	ND	ND	
	Paddy fields	4	50% (2/4)	100% (4/4)	ND	ND	ND	
	Drinking water treatment	61	90.2% (55/61)	18.03% (7/11)	ND	ND	ND	Richard et al. (2016) [20]
	Water samples	15	ND	100% (15/15)	ND	ND	ND	Basher et al. (2018) [24]
	Swabs (rocks and stones)	15	ND	73.33% (7/11)	ND	ND	ND	

(Continued to the next page)

Table 1. Continued

Country	Type of samples	No. of sample	Positive culture		*Acanthamoeba* spp. morphology			References
			FLA	*Acanthamoeba* spp.	Group I	Group II	Group III	
	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]
The Philippines	Water							
	River	10	ND	30% (3/10)	ND	ND	ND	Onichandran et al. (2014) [67]
	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	Rivera and Adao (2008) [29]
	Soil							
	Soil	10	ND	100% (10/10)	ND	ND	ND	Rivera and Adao (2008) [29]
	Soil	4	ND	100% (4/4)	ND	ND	ND	Cruz and Rivera (2014) [25]
	Other							
	Contact lens storage cases	4	ND	100% (4/4)	ND	ND	ND	Rivera and Adao (2008) [29]
Vietnam	Soil							
	Garden soil	1	359 small sub unit rDNA Sequences of Amoebae	5.95%	ND	ND	ND	Denet et al. (2017) [21]
	Mining soil	1		4.76%	ND	ND	ND	
Others (Lao PDR, Myanmar, and Singapore)	Treated water in Lao PDR	9	11.11% (1/9)	NF	ND	ND	ND	Majid et al. (2017) [14]
	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.

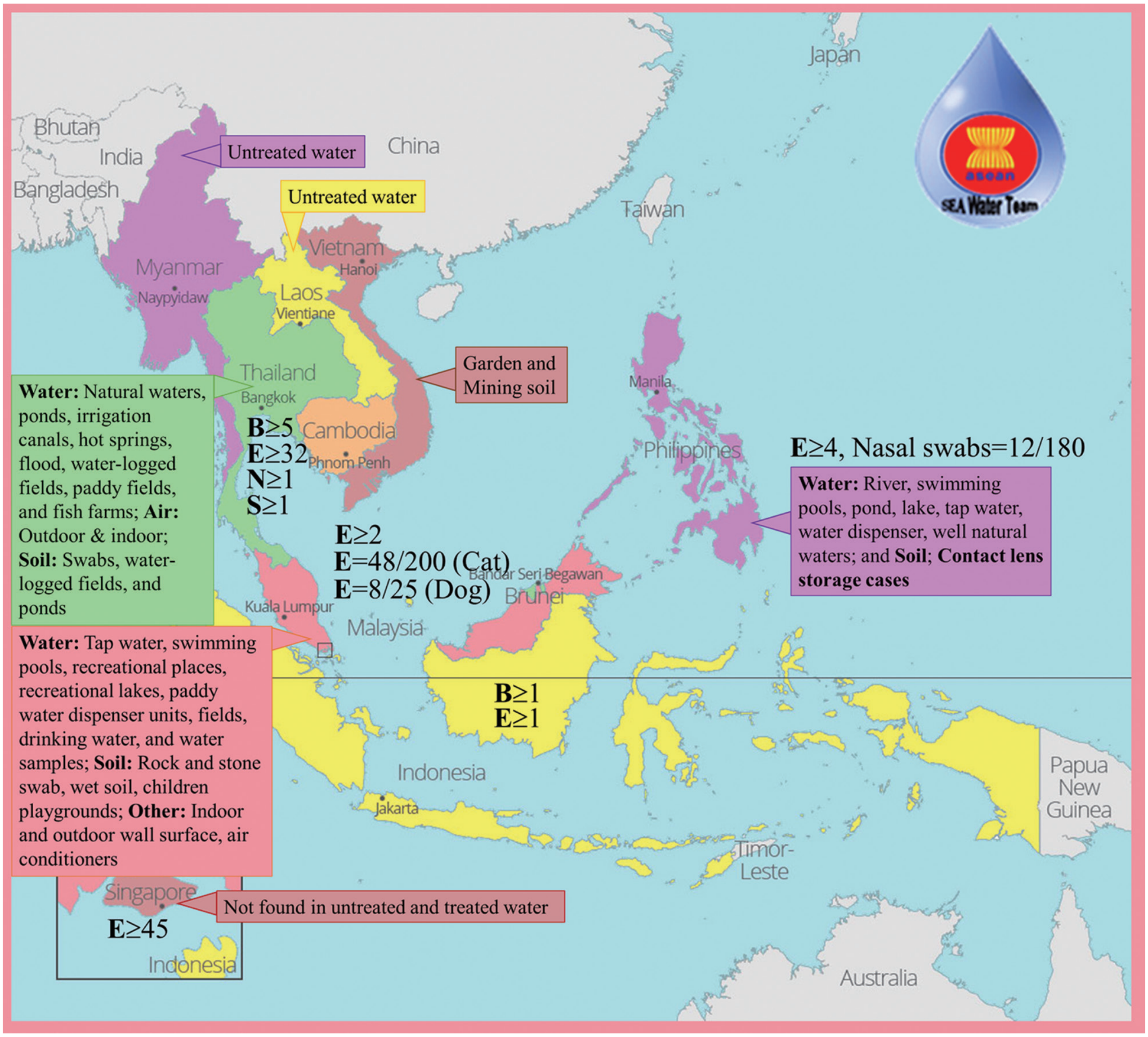

Fig. 1. Epidemiology and clinical cases of *Acanthamoeba* infection in Southeast Asia. B: Granulomatous amoebic encephalitis; E: *Acanthamoeba* keratitis; N: *Acanthamoeba* sinusitis; and S: Gastric acanthamoebiasis.

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 *Acanthopodia* 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	References
Singaporean male	28	Corneal scraping	Microscopy and culture	AK with *Pseudomonas aeruginosa*	Hit with polyvinylchloride pipe, topical steroids	*Before diagnosis*: cefazolin and gentamicin; *After diagnosis*: topical cycloplegics; topical 0.1% hexamidine, 0.02% chlorohexidine, and transplantation	Vision blurriness	Lim et al. (2018) [68]
48/200 felines and 8/25 canines (56/225 naturally-infected animal) in Malaysia	Adults and juveniles	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 occassions	*Before diagnosis*: Steroid eye drops, Moxifloxacin eye drops, natamycin eye drops, polymyxin-neomycin-gramicidin eye drops (Polygran®), and voriconazole eye drops *After diagnosis*: propamidine isethionate (Brolene®) and Polygran®	Improved vision blurriness	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	Wara-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 (1/6), 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, polyhexamethylene biguanide or propamidine	Improved vision blurriness and loss of follow-up for some patients	Wanachiwanawin et al. (2012) [70]

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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
9/103 infective keratitis patients with eye surgery	-	-	-	AK	-	Polyhexamethylbiguanide, chlorohexidine, propamidine dexamethasone, hexamidine, 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 keratoplasy specimen	Microscopy and culture	AK	Suboptimal hygiene practices	0.02% topical polyhexamethylene niguanide, 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. (2008) [74]
Chinese female	13	Corneal scraping	Microscopy and culture	AK	Rigid gas-permeable contact lens wearer	*Before diagnosis*; Acantamoebic agents: 0.02% topical polyhexamide methylene 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	-	Death	Siripanth (2005) [76]
Thai male	36	Nasal exudate	Microscopy and culture	Amoeba co-infection sinusitis (*Naegleria* sp. and *Acanthamoeba* sp.)	Diving in a natural pond	Caldwell-Luc operation, Intravenous amphotericin B, oral ketoconazole, and amoxycillin/clavulanic acid	Cured	Sukthana et al. (2005) [77]
Singaporean female	39	Corneal scraping	Microscopy and culture	AK	Contact lens wearer with multipurpose disinfectant solution	*Misdiagnosis*: Occulentum Acyclovir, Guttae Choramphenicol, and 0.12% Guttae Prednisolone; *After diagnosis*: 0.1% gutt propamidine isethionate, 0.02% gutt polyhexamethyline 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 open tank after sand and dust strucked in the eye	Topical Propamidine isethionate, Chlorhexidine 0.02% and fortified Gentamycin	Improved vision blurriness but loss of follow-up	Kamel et al. (2005) [26]

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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 antidlaucoma 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]

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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
Thai female	65	Corneal scraping	Microscopy, culture, indirect immunofluorescence testing, and isoenzyme analysis	AK	Unknown	*Before diagnosis*: topical neomycin sulfate, polymyxin B, and gramicidin; *After diagnosis*: cefazolin and gentamicin eye drops for *P. aeruginosa* as well as chlorhexidine for *Acanthamoeba* sp.	Vision blurriness with cataract	Kosrirukvongs et al. (1990) [81]
Malaysian female	40	Corneal scraping	Microscopy	AK with *P. auruginosa* 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

Anti-*Acanthamoeba* agents	Nanotechnology	Anti-*Acanthamoeba* activity against: Cysts	Anti-*Acanthamoeba* activity against: Trophozoites	References
Chemicals				
Cyclic samarium complexes [Sm(Pic)2(18C6)] (Pic)	-	-	IC_{50} = 6.5 µg/ml against *Acanthamoeba* keratitis isolate	Kusrini et al. (2018; Indonesia) [85]
Acyclic samarium complexes [Sm(Pic)2(18C6)] (Pic)	-	-	IC_{50} = 0.7 µg/ml against *Acanthamoeba* keratitis isolate	Kusrini et al. (2018; Indonesia) [85]
Terbium complex $[Tb(NO_3)_3(OH_2)_3](18C6)$	-	-	IC_{50} = 7 µg/ml against *Acanthamoeba* keratitis isolate	Kusrini et al. (2016; Indonesia) [86]
$Tb(NO_3)_3.6H_2O$ in CH_3CN	-	-	IC_{50} = 2.6 µg/ml against *Acanthamoeba* keratitis isolate	Kusrini et al. (2016; Indonesia) [86]
18C6 in CH_3CN	-	-	IC_{50} = 1.2 µg/ml against *Acanthamoeba* keratitis isolate	Kusrini et al. (2016; Indonesia) [86]
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	-	-	IC_{50} = 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	-	-	IC_{50} = 0.33, 0.33, 0.66 µM against *Acanthamoeba* keratitis isolate and 2 soil isolates, respectively	Boonman et al. (2012; Thailand) [90]
Betadine® solution	-	MCC = 0.04% dilution after 24 hr against *Acanthamoeba* keratitis isolate	-	Roongruangchai et al. (2011; Thailand) [91]
Virkon® solution	-	MCC = 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* (ATCC 50492)	100% abolished amoeba viability of 5×10^5 *A. castellanii* (ATCC 50492) at 50 µg/ml	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* (ATCC 50492)	Significanly abolished amoeba viability of 5×10^5 *A. castellanii* (ATCC 50492) at 50 µg/ml	Anwar et al. (2019; Malaysia) [93]
Periglaucine A from *Pericampylus glaucus*	Poly (DL-lactide-co-glycolide)	CC_{50}/IC_{50} = 100 against *A. triangularis* from environmental water sample	CC_{50}/IC_{50} = 25 against *A. triangularis* from environmental water sample	Mahboob et al. (2018; Malaysia) [94]
Betulinic acid from *Pericampylus glaucus*	Poly (DL-lactide-co-glycolide)	CC_{50}/IC_{50} = 10 against *A. triangularis* from environmental water sample	CC_{50}/IC_{50} = 5 against *A. triangularis* from environmental water sample	Mahboob et al. (2018; Malaysia) [94]
Periglaucine A from *Pericampylus glaucus*	-	CC_{50}/IC_{50} = 8.5 against *A. triangularis* from environmental water sample	CC_{50}/IC_{50} = 170 against *A. triangularis* from environmental water sample	Mahboob et al. (2017; Malaysia) [95]
Betulinic acid from *Pericampylus glaucus*	-	CC_{50}/IC_{50} = 3.75 against *A. triangularis* from environmental water sample	CC_{50}/IC_{50} = 1.5 against *A. triangularis* from environmental water sample	Mahboob et al. (2017; Malaysia) [95]

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Table 3. Continued

Anti-*Acanthamoeba* agents	Nanotechnology	Anti-*Acanthamoeba* activity against		References
		Cysts	Trophozoites	
Cinnamic acid from *Cinnamomum cassia*	Gold nanopaticles	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 environmental *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]
Microorganism products				
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. (2018; 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_{50} = 0.31 µm against *Acanthamoeba* keratitis isolate	Boonman et al. (2012; Thailand) [90]
Dehydrofuseric acid from *Fusarium fujikuroi* species complex Tlau3 isolated from *Thunbergia laurifolia*	-	-	IC_{50} = 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 (Amphotericin B and Nystatin but not Fluconazole) 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* (ATCC 50492)	Siddiqui et al. (2016; Malaysia) [103]

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Table 3. Continued

Anti-*Acanthamoeba* agents	Nanotechnology	Anti-*Acanthamoeba* activity against		References
		Cysts	Trophozoites	
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* (ATCC 50492)	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 excystation inhibition against *A. castellanii* (ATCC 50492)	No effects on growth and viability of *A. castellanii* (ATCC 50492)	Siddiqui et al. (2016; Malaysia) [104]
Artesunate (Antimalaria)	-	Presence of cytostatic 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				
Crocodile (*Crocodylus palustris*) serum	-	-	Anti-*Acanthamoeba* activity against *A. castellanii* (ATCC 50492)	Siddiqui et al. (2017; Malaysia) [106]
Sea sponge crude methanol extracts (*Aaptos aaptos*) from different localities	-	-	IC_{50} = 0.615-0.876 μg/ml against clinical *A. castellanii*	Nakisah et al. (2012; Malaysia) [107]

IC, Inhibition concentration; CC, Cytotoxicity concentration; MCC, Minimal cystcidal concentation; -, Not mentioned in the published paper.

thamoeba detection and monitoring system to understand these amoebic infections and diagnostic approaches. So far, the gold standard of *Acanthamoeba* laboratory testing has been cultured on NNA overlaid with *E. coli* and PYG medium for axenic culture. A modern technique has been applied as far as the laboratory diagnosis is concerned. This can provide a better routine diagnosis especially using molecular-based intervention such as PCR and MALDI-TOF/MS [5]. It is important because mistaken or late diagnosis has been usually reported due to poor prognosis leading to worsening clinical symptoms and subsequently under postmortem diagnosis [48,49]. Moreover, *Acanthamoeba* spp. are potential Trojan horse of human-pathogenic viruses, infectious bacteria, and fungi which might be one way of disease spread and gene transfer [10]. Early detection technique is needed as well as physician should be aware of *Acanthamoeba* infection through patient interview and history taking [50,51]. Unfortunately, most patients also come up with lesion in brain for GAE which most of the cases are too late to be cured whilst AK are mainly associated with contact lens wearer and immunocompetent patients are basically affected [52-54].

Hygiene and proper contact lens usage is a critical point of care which ophthalmologist should pass on knowledge of appropriate usage of contact lens [55]. Most disinfectant solutions for contact lens are ineffective against *Acanthamoeba* cyst which is rich with cellulose structure [56]. Effort on novel anti-*Acanthamoeba* agents therefore focus on cyst form or other potential target sites [7]. Biology of *Acanthamoeba* spp. should be studied to guide action of desired anti-*Acanthamoeba* agents which have been identified [57]. In ASEAN nations, Anti-*Acanthamoeba* activity has been investigated among human-made chemicals, plant extracts, microbial metabolites, anti-*Acanthamoeba* side effect of drugs, and animal products which nanoparticles are attractive antimicrobial agent delivery technology to enhance the activity of these anti-*Acanthamoeba* agents (Table 3). However, these anti-*Acanthamoeba* agents were tested only in vitro. Blood-brain barrier is another challenge for anti-*Acanthamoeba* agents to pass through for the treatment of GAE [58]. There is a long road lying ahead for in vivo experiment and clinical application in ASEAN nations. In fact, Southeast Asia (ASEAN) is a gigantic resource of medicinal plants and bioactive agents. Interestingly, the only PHARM database is available at the Faculty of Pharmacy, Mahidol University, Thailand in which more than 1,000 collections of Thai medicinal plants were recorded (http://www.medplant.mahidol.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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