

**MEDICAL UNIVERSITY OF WARSAW
FACULTY OF HEALTH SCIENCES**

**Evaluation of anti-amoebic activity *in vitro*
of selected physical and chemical agents
on detected in Poland *Acanthamoeba* strains,
factors of increasing threats for public health**

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Dissertation for the doctoral degree in health sciences



Warsaw 2019



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Key words:

Acanthamoeba strains: environmental, corneal pathogenic strains, T4 genotype, *Acanthamoeba* keratitis, *in vivo* confocal microscopy, *in vitro* cultures, temperature, contact lens solutions, Octenisept, Aerodesin@2000, Medicarine, chlorhexidine digluconate, povidone iodine, toyocamycin effects

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Abstract

Introduction

Different *Acanthamoeba* species are ubiquitous and widely distributed in natural and man-made environments worldwide, including Poland. The amoebae occur in soil, fresh-, sea-, chlorinated-, mineral- and tap -water, recreational water systems, air, dust, fruits, vegetable and animal bodies. The protists have also been isolated from hospital environments: from surgical instruments, dialysis units, dental irrigation units, air conditioning and ventilation systems. These organisms can be found in various human cavities, tissues, skin surfaces, oral cavities, paranasal sinuses, lungs and brain. The amphizoic amoebae generate a serious health threat due to their pathogenic potential as facultative parasites. These organisms are the causative agents of vision-threatening human disease *Acanthamoeba* keratitis (AK), mainly reported in contact lens wearers. Apart from contact lens wear, important risk factors of emerging, vision-threatening AK are: damage to the corneal epithelial cells, eye surgery and exposure to water in which *Acanthamoeba* trophozoites and cysts occur. *Acanthamoeba* trophozoites, in particular the cysts, are highly resistant to chemicals, disinfectants and drugs. This resistance results in increased difficulty and unsuccessful therapeutic management. Various chemicals with possible activity against *Acanthamoeba* strains - environmental and clinical - were tested and are still examined.

The purpose and aim of research

The aim of this study was to examine and evaluate anti-amoebic *in vitro* effect of selected physical and chemical factors on detected in Poland *Acanthamoeba* strains from severe incidents of the vision- threatening AK in comparison to environmental *A. castellanii* Neff strain.

Material and Methods

Corneal isolates from AK were initially diagnosed by clinicians with non-invasive methods: slit-lamp and *in vivo* confocal microscopy. Next, they were examined in Department of Medical Biology with contrast phase light microscope and identified using molecular techniques (PCR) based on genotype associations the 18S rRNA

gene sequence. The material from corneal isolates was *in vitro* cultivated; samples of cultivated *Acanthamoeba* strains were experimentally exposed to different chemicals.

Chemicals applied and evaluated

- Octenisept antiseptics with broad anti-microbial activity, (commonly used for disinfection, also around-treatment);

- AERODESIN® 2000 (the alcohol-based agent) and Medicarine; disinfectants used in health facilities and laboratories;

- Povidone iodine, a preparation used in ophthalmology for conjunctivitis;

- Toyocamycin with anti-*Trichomonas* sp. and *Toxoplasma gondii* activity, not used before against amoebas;

- Chlorhexidine digluconate with proven amoebostatic properties used *in vivo* as a chemotherapeutic agent in AK.

- selected contact lens care solutions conjugated with nanoparticles in terms of preventive approach to public health risk generated by *Acanthamoeba* strains.

- to increase the effectiveness of these fluids against *Acanthamoeba* trophozoites.

After exposure of *Acanthamoeba* culture samples to disinfectants - the overall number of surviving amoebae, a percentage of cysts and, also - the ability of amoebae to *in vitro* multiply - were determined. Differences in comparison to respective control cultures were assessed.

Effect of applied physical factors and chemical substances, depending on the type, concentration and duration of action were investigated by the systematic monitoring of the population dynamics of amoeba according to the phases of the culture.

Results

Comparative assessment of the results showed differences in population dynamics of *Acanthamoeba* strains as appearing by the action of physical and chemical factors.

- All substances applied caused the effect of anti-amoebal, depending on the type, concentration and duration of exposure.

- After a few days of exposure to chemotherapeutic drugs corneal *Acanthamoeba* strains *in vitro* were more resistant to these substances than the environmental standard strain.

- *In vitro*, all chemicals had some amoebostatic effect on the population of protozoans. These findings were based on decreased populations of the protozoan and the appearance of sporadically dividing amoebae.

- Furthermore, comparative assessment of the results showed that several agents changed the relationship between *Acanthamoeba* trophozoites and cysts.

- The differentiation of anti-amoebic effectiveness of individual compounds, manifesting in relation to the same pathogenic amoeba strain as altered ratios of trophic forms and cysts, was revealed after prolonged application.
- Povidone iodine and tojokamycin, not used in AK therapy, proved to be effective in comparison to chlorhexidine, used in AK therapy; however, unlike chlorhexidine, they did not induce encystation.
- Octanisept, an antiseptic with recognized bactericidal effectiveness, demonstrated *in vitro* the amebo-static effect by reducing the population of *A. polyphaga*.
- Both substances, Aerodesin® 2000 and Medicarine, used for rapid disinfection of devices and surfaces, showed an amebo-static and cysticidal effect however, they varied the time needed to reveal the cysticidal properties; the alcohol-based preparation was faster than the chlorine release.
- Contact lens care solutions, despite their disinfectional activity declared by manufacturer, did not reveal any amoebastatic effect. Promising anti-amoebic effect was observed for Solo Care Aqua with silver nanoparticles.
- A comparison of the *in vitro* effects of 20°C and 37°C showed an increased dynamics of the corneal strains population after transfer to 37°C, whereas the *A. castellanii* Neff strain was significantly lower at this temperature than at 20°C.

The results presented here demonstrate, from a practical perspective, anti-amoebic effects which are relevant to reducing the threat to public health.

Conclusions

Multifaceted, interdisciplinary research, carried out within the presented topic, using parasitological, microbiological methods, *in vitro* cultures, light and electron microscopy, molecular biology techniques allowed to extend the current knowledge on physico-chemical conditions of dispersion in amphibian amoebas in the human environment, as well as on the topic pertaining to inhibition of the encysting process, which is decisive in the occurrence of remission and relapse in the course of *Acanthamoeba* keratitis.

- The anti-amoebic effect of povidone iodide - an antiseptic with a broad anti-bacterial and anti-viral effect - demonstrated effectiveness against corneal strains and it should be taken into account for further research on its therapeutic potential in AK.
- The analysis of data obtained from monitoring *in vitro* cultures showed a correlation between increased population dynamics and more severe clinical course of AK; this confirms the usefulness of the results of *in vitro* monitoring, demonstrated in the work from previous studies, as a useful tool in assessing the course of treatment.
- With short-term use of Aerodesin® 2000 and Medicarine disinfectants, their amebo-static effect is certain; however, achieving the effect of cysticide will not be possible with prolonged use, in practice, the presence of potentially invasive cysts and the risk of infection cannot be excluded.

- In the light of the results obtained, it would be reasonable to recommend a longer time of using these substances for quick disinfection, eg in laboratories, to use their cysticidal effect.
- The dynamic growth and longer lifetime at higher temperature of *Acanthamoeba* strains, evaluated in these studies, in correlation with their pathogenicity, justifies the treatment of this indicator as an indirect marker useful in the prospective assessment of amoebic pathogenicity.
- The need to improve diagnostics and therapy for *Acanthamoeba* keratitis is a current public health problem, requiring further research.
- At the same time, it is justified to increase the awareness of hazards generated by amphibian amoebas of the genus *Acanthamoeba*, especially necessary in high risk groups.