RES-077

Co-occurrence of Intracellular *Pseudomonas aeruginosa* DNA in *Acanthamoeba* Isolates from Keratitis Patients Diagnosed at Parasitology and Medical Entomology Department, Faculty of Medicine, Universiti Kebangsaan Malaysia

Anisah Nordin & Noraina Ab Rahim
Department of Parasitology & Medical Entomology, Faculty of Medicine, Universiti Kebangsaan Malaysia
Corresponding author: anisah.nordin@ukm.edu.my

Summary

- *Acanthamoeba* act as a Trojan Horse, sheltering the bacteria from external stresses thus establishing an **endosymbiotic relationship.**
- The presence of *P. aeruginosa* DNA within **15 (65.2%)** *Acanthamoeba* isolates from AK patients suggest a **crucial correlation** between these two pathogens **in ocular infections.**
- This highlight the need for **dual-target diagnostic and therapeutic strategies in keratitis management.**

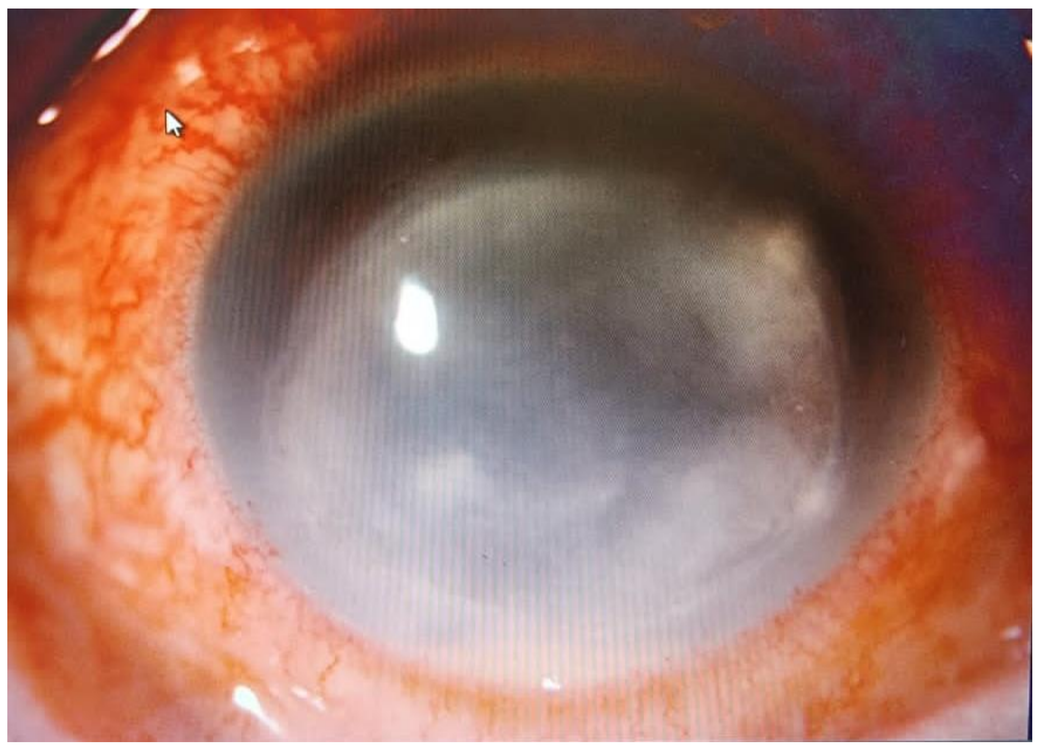

Fig A Clinical presentation of Acanthamoeba keratitis showing a dense corneal infiltrate and prominent surrounding inflammation.

Introduction

- Acanthamoeba keratitis (AK) is a severe, sight-threatening corneal infection caused by free-living amoebae, often misdiagnosed and quite difficult to treat.
- Concurrently, *Pseudomonas aeruginosa* is a leading cause of bacterial keratitis, notorious for its rapid progression, potent virulence factors, and increasing antimicrobial resistance.
- Emerging evidence suggests a complex interaction between *Acanthamoeba*, which can act as hosts and protect bacteria from environmental stresses, potentially enhancing their pathogenicity.

.Objective

This study aimed to investigate the molecular co-occurrence of *P. aeruginosa* within *Acanthamoeba* isolated from corneal scrapings of patients clinically diagnosed with keratitis.

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Method

23 *Acanthamoeba* isolates (AC1-AC23) from corneal scrapings of AK patients

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Species specific PCR
Target: ITS 23S-5S
Forward: 5'- ACCTCCTTTCTAAGGAGCACC-3'
Reverse: 5'-GATGCTCGCAACCACTATCCA-3

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BLAST analysis
Database comparison (GenBank)

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Species confirmation

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Results and discussions

Table 1 Distribution of intracellular *Pseudomonas aeruginosa* endosymbionts in Acanthamoeba keratitis isolates

Clinical isolates	Genotype	*Pseudomonas aeruginosa*	
		Yes	No
AC 1	T4	√	
AC 2	T4	√	
AC 3	T4	√	
AC 4	T5		√
AC 5	T4	√	
AC 6	T4	√	
AC 7	T4	√	
AC 8	T4		√
AC 9	T4		√
AC 10	T4		√
AC 11	T4	√	
AC 12	T4	√	
AC 13	T4		√
AC 14	T4	√	
AC 15	T3		√
AC 16	T4		√
AC 17	T4	√	
AC 18	T4		√
AC 19	T4	√	
AC 20	T4	√	
AC 21	T4	√	
AC 22	T4	√	
AC 23	T4	√	

- **15 (62.5%) isolate harbours *P. aeruginosa* DNA with percentage similarities of 91-100%**
- The predominance of T4 genotype and detection of *P. aeruginosa* suggests a potential synergistic role in worsening keratitis outcomes.
- Possible release of viable *P. aeruginosa* from the amoeba within the cornea may lead to a polymicrobial keratitis, which is extremely difficult to diagnose and treat, leading to worse visual outcomes and delayed diagnosis.

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Anisah Nordin
Dept Parasitology & Medical Entomology, Faculty of Medicine, UKM
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