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Dose-Dependent Inhibition of Multidrug Resistant *Pseudomonas aeruginosa* in Vitro With Combination of Riboflavin and Ultraviolet-A Light

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Introduction

- **Bacterial ocular infections – potentially sight threatening**
- **Medical management – antibiotic therapy**
- **Frequent, indiscriminate antibiotic use**

- **Development of resistant strains – need for new therapies**

- Kunimoto et al : In vitro susceptibility of bacterial keratitis pathogens to ciprofloxacin-Emerging resistance: Ophthalmology1999;106:80 –85
- Goldstein et al : Emerging fluoroquinolone resistance in bacterial keratitis : Ophthalmology1999; 106 (7):1313–1318
- Afshari NA et al. Trends in resistance to ciprofloxacin, cefazolin, and gentamicin in the treatment of bacterial keratitis:J Ocul Pharmacol Ther. 2008 Apr;24(2):217-23.

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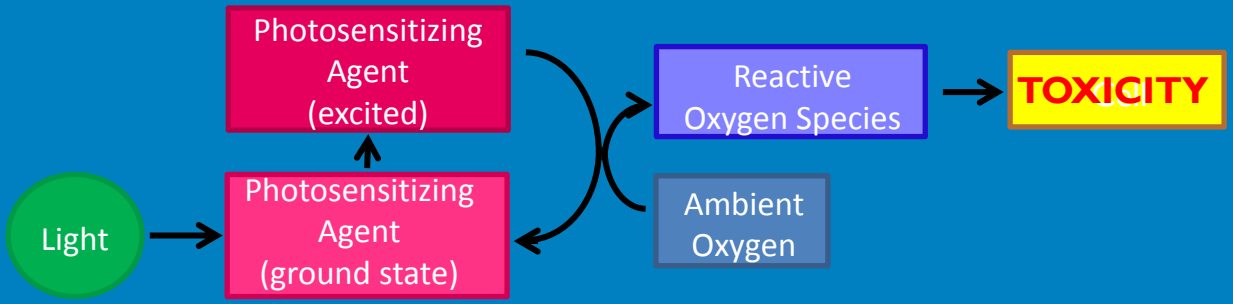
Photodynamic Therapy (PDT)

- **Discovered in 1900 by Oscar Raab[1]**
- **Employs 3 nontoxic elements to result in cellular toxicity:**
  1. **Photosensitizing agent (PS)**
  2. **Light**
  3. **Oxygen**



1.Raab, O. Uber die Wirkung fluoreszierender Stoffe auf Infusorien. Zeitung Biol. 39, 524–526 (1900)

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Need for Study

- *P. aeruginosa* is a highly virulent gram-negative organism well known for its resistance to first- and second-line antibiotics in systemic and nosocomial infections.

- Reports of antibiotic resistance to this organism in ocular infections are now emerging. At our Institute these emerging strains have been found to be resistant to all antibiotics except Colistin, Imipenem, Piperacillin, Cefotaxim.

- Therefore, there exists a definite need to look for new therapies for such exigencies.

Rajat Jain et al : Clinical Outcomes of Corneal Graft Infections Caused by Multi–Drug Resistant *Pseudomonas aeruginosa*, Cornea 2014;33:22–26

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Purpose

To evaluate the effect of increasing doses of riboflavin and ultraviolet a light (UVA) combination on a strain of multi drug resistant *Pseudomonas aeruginosa* (PA) in vitro.

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Methods

Two strains of *Pseudomonas Aeruginosa* (PA)
– a quality controlled ATCC-27853 PA sensitive to most antibiotics
– and a proven multi drug resistant PA (MDR-PA)
were inoculated onto plates of Mueller Hinton agar as lawn cultures .

- **Riboflvin (in 0.1% dextran) was then instilled over these plates**
- **These plates were then exposed to a varying period of exposure to UV-A light (30, 60 and 90 minutes)**
- **All plates were incubated at 37°C**
- **Zone of inhibition of growth was observed after 18 hours.**
- **Digital images of each plate were analyzed using image analysis software.** (ImageJ 1.45s; National Institutes of Health, USA; http://imagej.nih.gov/ij)

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Results

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- The plate seeded with drug sensitive PA and exposed to combination of riboflavin and UVA showed a clear zone of inhibition of growth in the centre corresponding to the area of exposure, which was measured to be 53.91 mm^2.

- The plate seeded with MDR-PA and treated with single exposure of riboflavin and UVA for 30 minutes showed a faint, barely discernible zone of clearance in the centre.

- The plates seeded with MDR-PA and subjected to two and three exposures of riboflavin and UVA for 30 minutes each displayed well demarcated zones of clearing measuring 61.67 mm^2 and 99.25 mm^2 respectively.

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Results

Control plate of Drug Sensitive *Pseudomonas aeruginosa* (PA) showing zone of inhibition to both ciprofloxacin and Imipenem discs.

Control plate of Multi Drug Resistant *Pseudomonas aeruginosa* (MDR-PA) showing a small zone of inhibition to Imipenem disc but none to ciprofloxacin.

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Drug sensitive *Pseudomonas aeruginosa* (PA)

No zone of inhibition of growth when exposed to UV light alone.

No zone of inhibition of growth when exposed to Riboflavin alone

Good zone of inhibition (53.91 mm^2 when exposed to both Riboflavin and UV light

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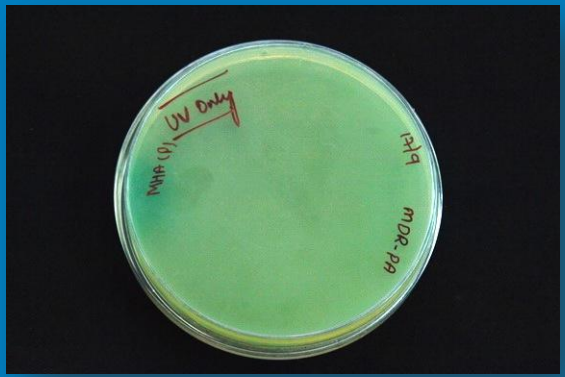
Multi Drug Resistant *Pseudomonas aeruginosa* (MDR-PA)



No zone of inhibition of growth when exposed to UV light alone.

No zone of inhibition of growth when exposed to Riboflavin alone

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Multi Drug Resistant *Pseudomonas aeruginosa* (MDR-PA)

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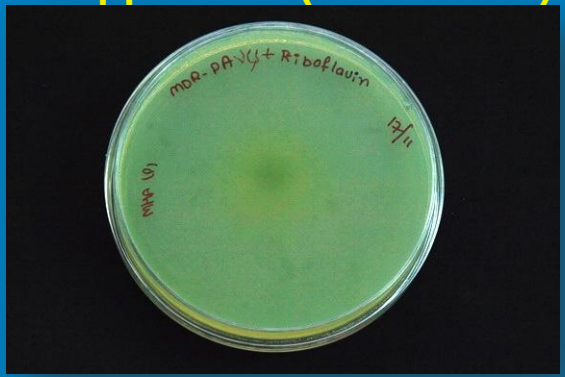
Application(Ribo+UVx1)



No significant zone of inhibition of growth

Application(Ribo+UVx2)

Zone of inhibition of growth : 61.67 mm^2

Application(Ribo+UVx3)

Zone of inhibition : 99.25 mm^2

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Conclusion

- There is a definite dose dependent antimicrobial effect of this treatment on multi drug resistant bacteria (PA).

- Elucidation of optimum treatment protocols, customized according to the sensitivity of the causative organism may help define the role of riboflavin and UVA combination treatment in ocular infections due to drug resistant organisms.