

The Survival of Mycobacteria in Pure Human Urine

**Pedro O. Orumwense, Eila Torvinen and Helvi
Heinonen-Tanski**

**Department of Environmental Science, University
of Eastern Finland**



**UNIVERSITY OF
EASTERN FINLAND**

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Introduction

- Human urine is a valuable source of micronutrients and macronutrients necessary for plant growth
- Advantages of urine recycling in agricultural application
 - produces higher or similar yields of food crops when compared to chemical fertilizers
 - can help in alleviating some global problems such as food insecurity, eutrophication, global phosphorus depletion, poverty and hunger
- Disadvantage(s) of urine reuse
 - can be associated with hygienic risk and occupation hazards

Introduction

- Tuberculosis, caused by *Mycobacterium tuberculosis* complex, causes **8.8 million cases** and **1.3 million deaths** annually (WHO 2011)
- Tuberculosis in the kidney and other human tissues **may result in the excretion of mycobacteria via human urine**
- Also many other pathogenic mycobacterial species (MOTT) cause a wide range of human and animal diseases worldwide and may be excreted in urine
- The reuse of human urine in crop fertilization could **introduce a new transmission route for mycobacterial infections**
- The presence of pathogenic mycobacteria in human urine could be a potential source of infection for individuals involved in the application work.

Aims of Study

- to examine the survival of different Mycobacterial strains in fresh and stored urine at temperatures of 15°C and 30°C to mimic the two different climatic conditions
- to investigate the effect of urine pH on the survival of mycobacteria

Materials and Methods

- **Test organisms**

- rapid growers *Mycobacterium aurum* DSM 43999 and *Mycobacterium fortuitum* ATCC 6841.
- slow growers *Mycobacterium avium* ATCC 15769 and a clinical *Mycobacterium bovis* BCG.

- **Urine samples**

- stored urine > 6 months old collected from urine separating eco-toilets in the area of Tampere, Finland
- Fresh urine: < 1 day old obtained from healthy students in Kuopio.

- **Preparation of samples**

- The urine samples were inoculated with 10^8 cfu/ml of the mycobacterial strain
- Inoculated samples were stored at 15 and $30 \pm 0.5^\circ\text{C}$
- Three replicates per temperature treatments (Fig 1)

Materials and Methods



(a) Fresh urine incubated at $15 \pm 0.5^\circ\text{C}$



(b) Fresh urine incubated at $30 \pm 0.5^\circ\text{C}$



(c) Stored urine incubated at $15 \pm 0.5^\circ\text{C}$



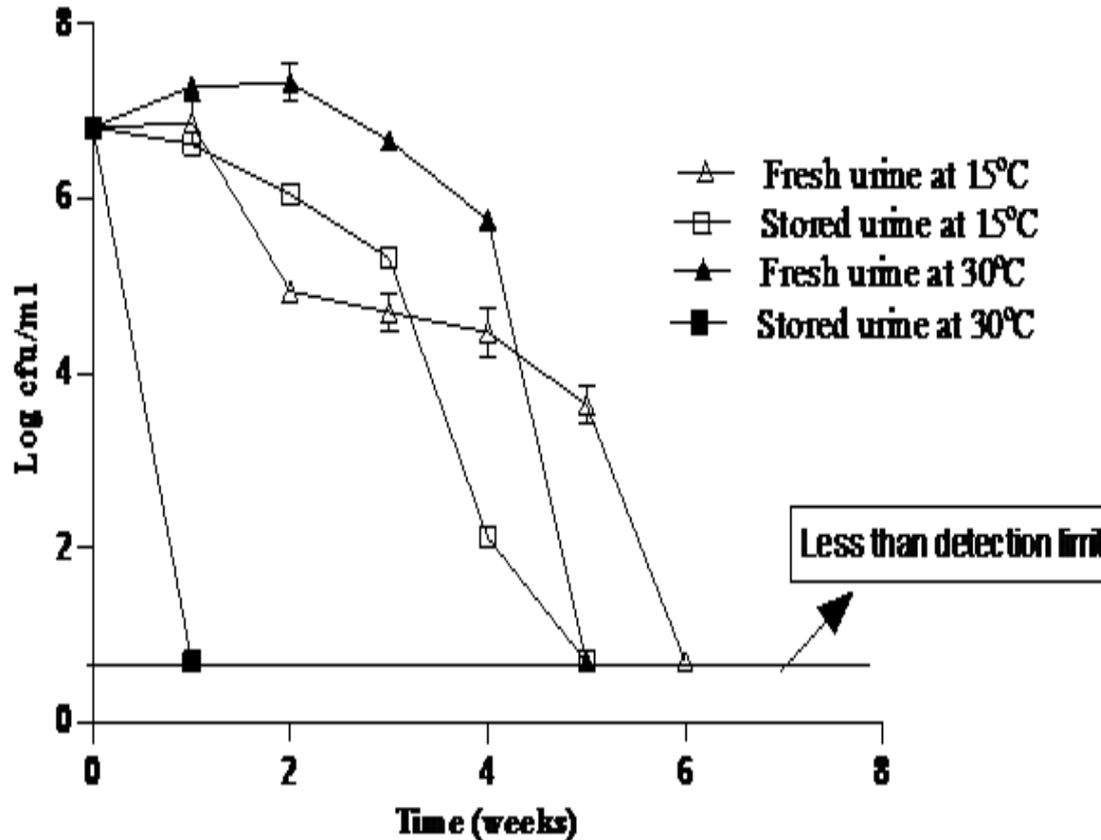
(d) Stored urine incubated at $30 \pm 0.5^\circ\text{C}$

Materials and Methods

- pH measurement
 - Weekly from bottles of fresh urine and bottles of stored urine kept at temperatures of 15 and $30 \pm 0.5^\circ\text{C}$
- Culture of mycobacteria
 - Dilution series (10^{-3} to 10^{-6}) from the inoculated urine samples were plated onto TSA or M7H11 + OADC
 - Incubation at $30 \pm 0.5^\circ\text{C}$ or $36 \pm 0.5^\circ\text{C}$ depending on the mycobacterium strain
 - Follow-up weekly up to 8 – 9 weeks
 - If two consecutive follow up analyses gave negative results no further experimental analyses will be performed
 - The experiments of the test organisms were performed in parallel runs

Results

Survival of *M. fortuitum*

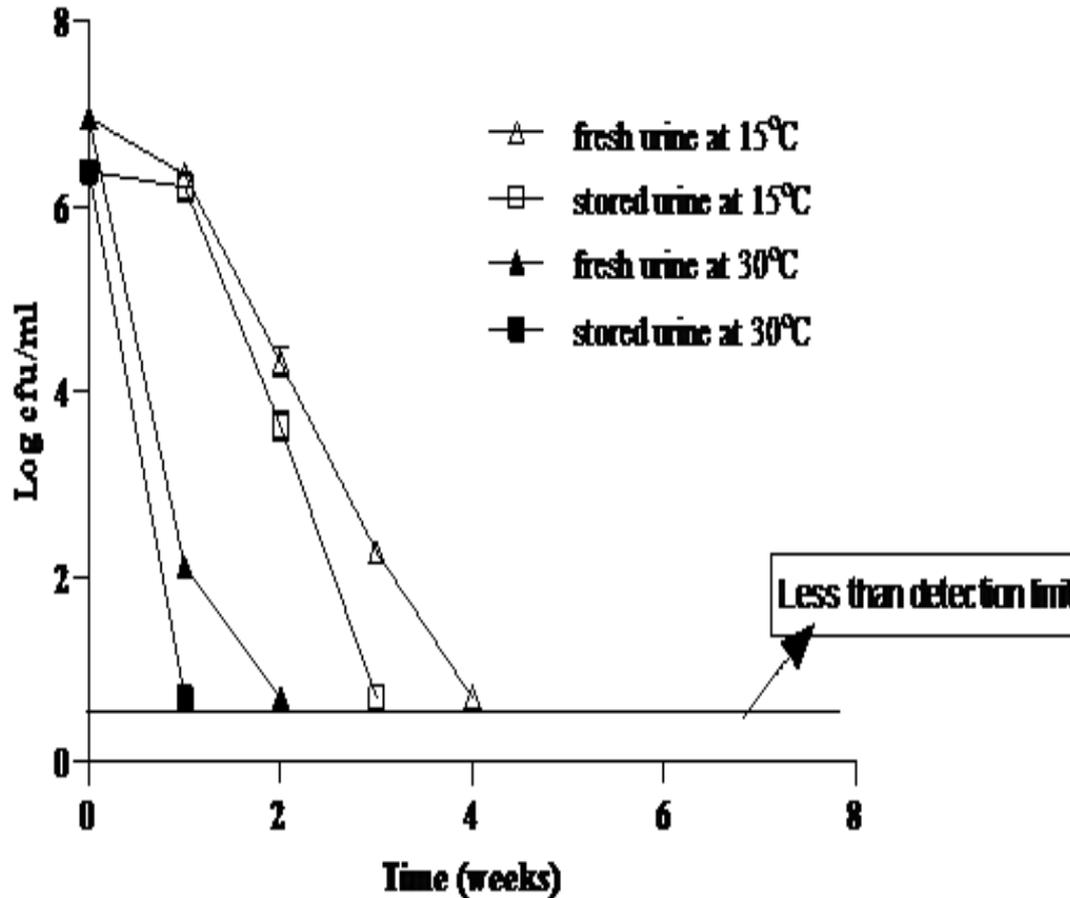


M. fortuitum survived for up to 4 weeks in fresh urine at 30°C and survived up to 5 weeks in fresh urine at 15°C

In stored urine at 30°C, *M. fortuitum* was inactivated rapidly within 1 week and survived up to 4 weeks in stored urine at 15°C

Results

Survival of *M. aurum*

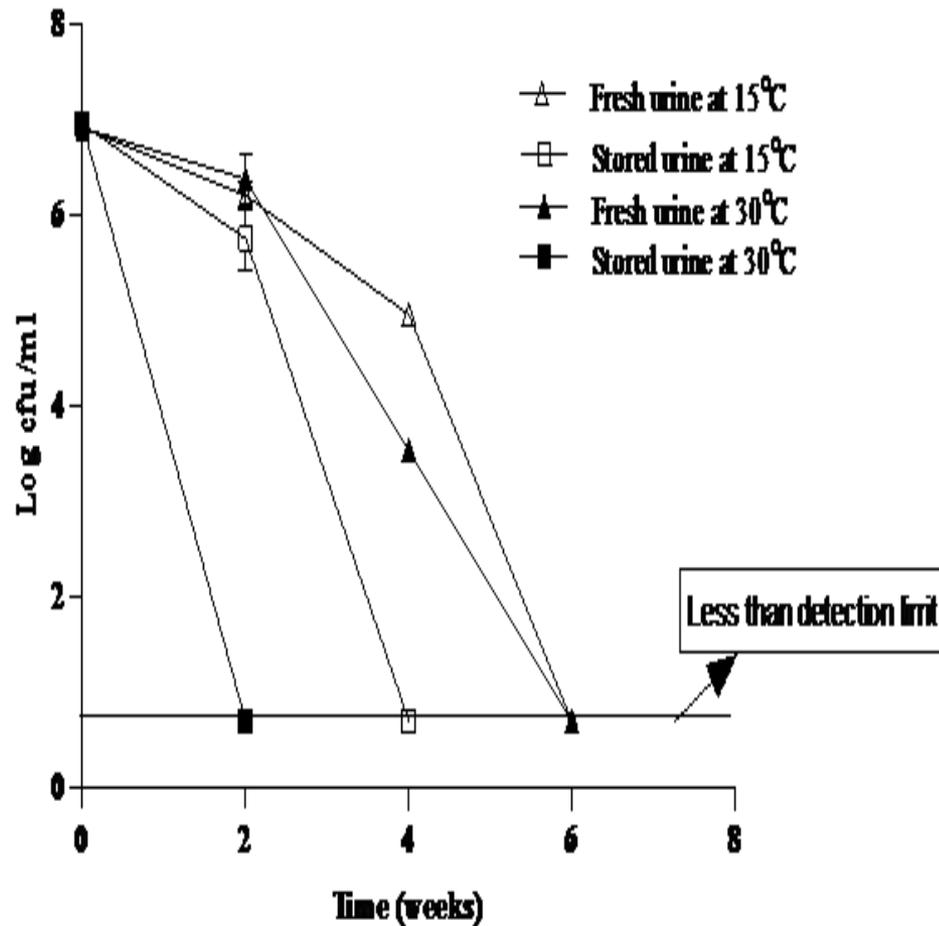


M. aurum declined rapidly at 30 °C, within 1 week in stored urine and 2 weeks in fresh urine.

At 15 °C, the organism survived up to 3 weeks in fresh urine and 2 weeks in stored urine

Results

Survival of *M. avium*

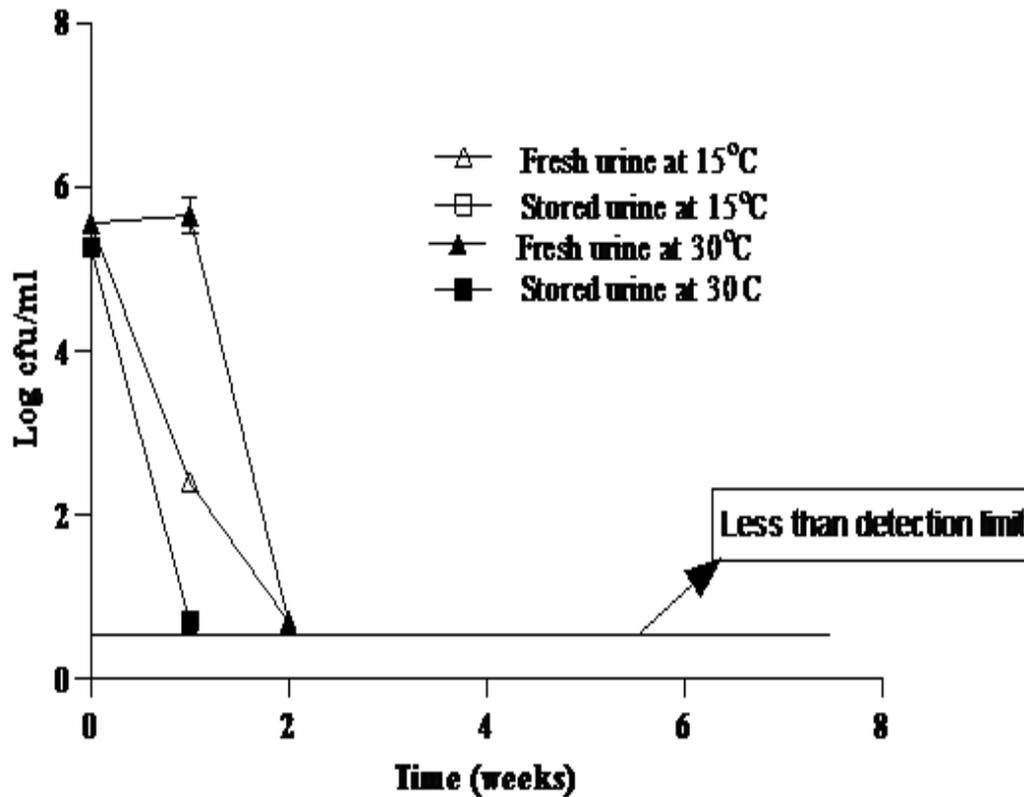


This organism reduced rapidly, less than 1 week, in the stored urine samples at 30°C and survived up to 2 weeks in the stored urine at 15°C.

In fresh urine samples kept at temperature 15 and 30°C, this organism had a better survival up to 4 weeks before rapid decline of their colony

Results

Survival of *M. bovis*



M. bovis survived in maximum up to 2 weeks in any urine samples

Results

The K value ($\log \text{ cfu/ml week}^{-1}$) of the exponential decay of mycobacteria survival per week in fresh and stored human urine at storage temperatures 15°C and 30°C over the study period

Test organism	K- values			
	Fresh urine at 15°C	Stored urine at 15°C	Fresh urine at 30°C	Stored urine at 30°C
<i>M. fortuitum</i>	-0.18	-0.24	-0.17	-2.28
<i>M. aurum</i>	-0.37	-0.40	-1.18	-1.04
<i>M. avium</i>	-0.18	-0.30	-0.23	-0.56
<i>M. bovis</i>	-0.91	-2.02	-0.49	-2.02

Results

The cfu/ml of *M. fortuitum* in pure human at different temperatures with relation to pH of the urine samples. The pH results of the other three experiments (*M. aurum*, *M. avium*, *M. bovis*) followed the same trend.

Week	Fresh urine at 15°C		Stored urine at 15°C		Fresh urine at 30°C		Stored urine at 30°C	
	pH	cfu/ml	pH	cfu/ml	pH	cfu/ml	pH	cfu/ml
0	6.95	6.6 x 10 ⁶	8.97	6.1 x 10 ⁶	6.95	6.6 x 10 ⁶	8.97	6.1 x 10 ⁶
1	7.12	7.2 x 10 ⁶	8.97	4.2 x 10 ⁶	7.12	1.9 x 10 ⁷	9.03	< l.d.1
2	7.66	8.5 x 10 ⁴	8.97	1.2 x 10 ⁶	7.65	2.1 x 10 ⁷	9.19	< l.d.1
3	8.18	4.9 x 10 ⁴	8.97	2.1 x 10 ⁵	8.35	4.5 x 10 ⁶	9.27	
4	8.35	2.9 x 10 ⁴	8.97	1.3 x 10 ²	8.60	5.6 x 10 ⁵	9.32	
5	8.87	4.3 x 10 ³	9.10	< l.d.1	8.97	< l.d.1	9.32	
6	9.03	< l.d.1	9.10	< l.d.1	9.27	< l.d.1	9.32	

cfu/ml = colony forming units per milliliters l.d.1 = less than the detection limit

Discussion

- All test organisms studied had a low survival rate in stored human urine
 - They all survived < 1 week at 30°C
- Survival was better in fresh urine and there slightly better at 15 than at 30°C
 - at 30°C from 2 to 4 weeks
 - at 15°C from 2 to 5 weeks
- The longest survival times were detected with *M. fortuitum* and *M. avium* → no difference in the survival between the rapid- and slow-growing species of mycobacteria
- The shortest survival was detected with *M. bovis*
 - a close relative to *M. tuberculosis* known to have a narrow pH range of growth

Discussion

- There was a weekly increase in pH level of the fresh urine up to pH value of 9.0 within 6-8 weeks
 - This may be an explanation to the decline of mycobacteria
- The storage temperature at 30°C enhanced rapid increase in the pH of urine compared to 15°C
 - This may be an explanation why all the test organisms had poorer survival at higher temperature

Conclusions

- For safe agricultural application, it is advisable to store human urine for more than five weeks before using it for plant fertilization in order to prevent a new exposure route for pathogenic mycobacteria
- High temperatures in tropical countries should guarantee rapid destruction of pathogenic mycobacteria in stored urine and make it's use applicable in developing countries

REFERENCES

World Health Organisation (WHO) (2011). Global Tuberculosis Control: WHO report 2011 ISBN 978 92 4 156438 0. Available at http://www.who.int/tb/publications/global_report/2011/en/index.html.

THANK YOU