

# ***In vitro* growth of uropathogenic *Escherichia coli* isolated from a snow leopard treated with homeopathic and isopathic remedies: a pilot study**

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## **ABSTRACT**

This paper reports the results of incubation of a strain of uropathogenic *Escherichia coli* (UPEC) isolated from a snow leopard - which had died of septicemia secondary to necro-hemorrhagic cystitis - with homeopathic and isopathic remedies. **Methods:** UPEC was isolated from heart blood and previously typified for virulence factors; it was incubated with homeopathic remedies *Cantharis vesicatoria* (urinary tract infection affinity), *Mercurius solubilis* (from symptoms analysis) and nosode prepared from the actual strain, all in dilution 12cH. **Results:** 2 patterns of bacterial growth were observed, associated to the quality of nutrients in the culture medium; in rich-nutrient medium, nosode of *E. coli* 12cH had a significant inhibitory effect; in poor-nutrient medium, *Merc* 12cH exerted significant inhibitory effect. **Conclusion:** results suggest that the previous conditions of prokaryote systems may influence the *in vitro* response to homeopathic and isopathic remedies.

**Keywords:** Urinary tract infection; Felines; Uropathogenic *Escherichia coli*; Homeopathy; Isopathy

## **Introduction**

Urinary tract infection (UTI) is one among the most frequent bacterial infections in both animals and human beings [1, 2]. Infection happens when uropathogens colonize the lower or upper urinary tract, giving rise to cystitis and/or pyelonephritis. *Escherichia coli* is by far the most common agent involved [3].

This study sought to investigate *in vitro* the action of homeopathic remedies on the growth of uropathogenic *E. coli* (UPEC) isolated from the blood of a snow leopard (*Panthera uncia*) which had died from sepsis secondary to necro-hemorrhagic cystitis.

## **Materials and methods**

**Sample:** The UPEC strain used in this study was previously isolated from the heart blood of a snow leopard (*Panthera uncia*) kept in captivity and which had died from sepsis secondary to necro-hemorrhagic cystitis. UPEC was previously typified regarding virulence factors: genes codifying adhesins (*fimH*, *sfa*), toxins (*cnf*, *hlyA*), siderophore

(*fyuA*) and a pathogenicity island (PAI I -CFT073) marker *malX* [4].

The primary (matrix) culture of *E. coli* was maintained at 4° C during all the study, in the same broth. Samples were obtained at 2 different growing times of matrix culture: 1 and 2 weeks. The samples were inoculated in LB broth and incubated for 2 hours at 37°C, then, standardized for tube 4 in MacFarland's scale and immediately diluted in scale 1:10<sup>6</sup> in normal saline.

**Homeopathic remedies:** Mother tinctures (MT) for the preparation of homeopathic remedies were obtained from Farmácia HN Cristiano, São Paulo. Remedies were prepared in dilution 12cH according to Brazilian Homeopathic Pharmacopoea 2<sup>nd</sup> edition [5]; the pharmacy is registered at the Brazilian Agency for Sanitary Vigilance (ANVISA). The last 3 dilutions were prepared in normal saline, to avoid osmotic interference in growth of bacteria. Remedies were prepared the same day they were used.

Remedies selected for this study were:

1- *Cantharis vesicatoria*: this remedy has particular affinity for the urinary system, experimentally producing intense and fast inflammation tending to necrosis [6]; furthermore, Fontes et al showed the effectiveness of this remedy in dilutions 6cH and 30cH in the treatment of rats experimentally infected with *E. coli* [7].

2- *Mercurius solubilis*: indicated by the analysis of the symptoms presented by the animal donor according to its clinical record (Table 1).

3- Nosode of *E. coli*: prepared from the isolated strain.

**Table 1.** Relevant symptoms selected from the clinical records of the donor, a snow leopard.

<b>Elimination symptoms:</b>	
1.	Lack of coordination
2.	Curved nails; Claudicating
<b>Secondary symptoms:</b> Purulent nasal discharge	
3.	Hemorrhage
4.	Dental calculi
5.	Skin ulcer
6.	Diarrhea
7.	Anemia
<b>Remedies that covered all listed symptoms and respective score according to repertory:</b>	
1.	Conium (score 10)
2.	Cuprum (score 7)
3.	Mercurius (score 14)
4.	Stramonium (score 8)

*In vitro treatment:* 100 µl of diluted sample were added to 100 µl of sterile normal saline, incubated in microtitration plate for 30 minutes at environmental temperature and, then, for further 30 minutes after addition of 50µl of *Canth* 12cH, *Merc* 12cH, nosode 12cH and saline (control). After incubation, 50 µl of each sample were plated by uniform distribution with Drigalsky loop into BHI agar and incubated for 24 hours at 37°C.

Another control (baseline) was made incubating bacteria from the same source (saline) directly into BHI agar, without any previous treatment.

At the end of the procedure, the number of colony-forming units (CFU) was assessed *blindly*. Thirty minutes was the time chosen for the first incubation because it is the normal cycle time of mitosis in *E. coli*.

*Statistical analysis:* Since Bartlett test indicated that data agreed parametric parameters, the chosen test was ANOVA followed by Tuckey-Krammer. p=0.05 was established as criterion of significance.

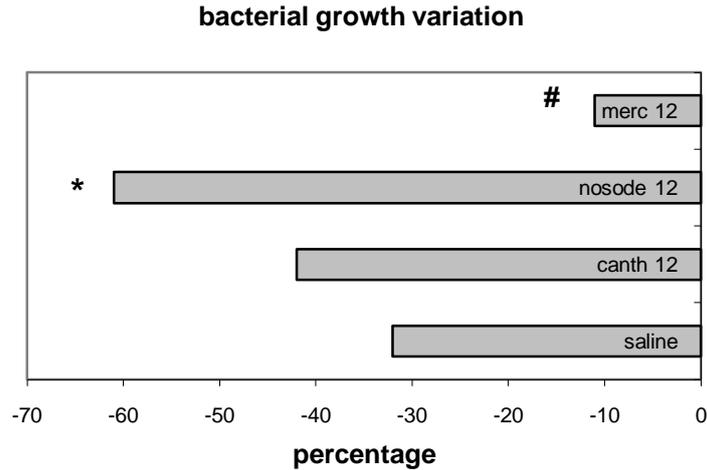
**Results**

Two patterns of response were identified: one (pattern A), exhibited by bacteria originated from 1-week old matrix colony (Figure 1); and the other (pattern B), exhibited by bacteria originated from 2-week old matrix colony (Figure 2).

Since no broth change of matrix colonies was performed during this time, the expected availability of nutrients at 2 weeks was smaller than at 1 week, therefore, the condition of bacteria before 2-hour incubation in LB broth is expected to differ, which indeed was actually verified.

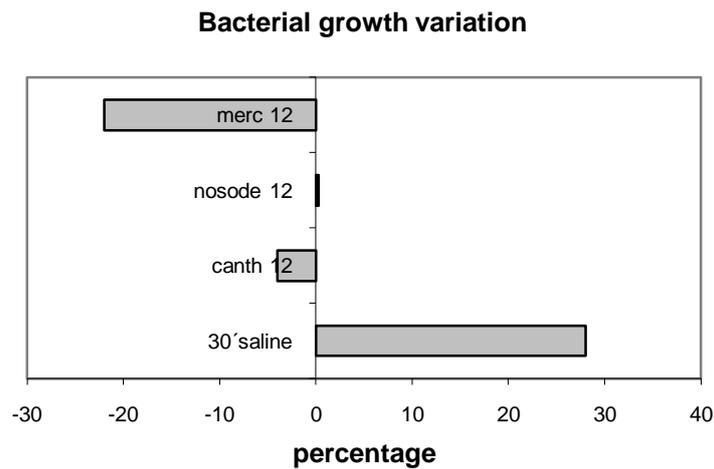
**Figure 1.** Number of Colony Forming Units of *E. coli* in relation to baseline: *pattern A*. The table expresses the detailed data. Figure expresses the percentage of growth variation of each group in relation to control (saline). \* p=0.05 in relation to baseline; # p=0.05 in relation to nosode, ANOVA / Tuckey Krammer.

<i>Pattern A</i>	Sample (baseline)	Sample 30 min in normal saline	<i>Canth</i> 12cH	<i>Merc</i> 12cH	Nosode 12cH
Mean	8	5.44	4.58	7.08	3.08
Standard deviation	2.78	1.66	2.35	3.52	2.90
Ratio		0.68	0.57	0.88	0.38
Variation %		-0.31	-0.42	-0.11	-0.61



**Figure 2.** Number of Colony Forming Units of *E. coli* in relation to baseline: *pattern B*. The table expresses the detailed data. Figure expresses the percentage of growth variation of each group in relation to control (saline). ANOVA, without significance.

<i>Pattern B</i>	Sample (baseline)	Sample 30 min in normal saline	<i>Canth 12cH</i>	<i>Merc 12cH</i>	<i>Nosode 12cH</i>
Mean	23.91	30.72	22.91	18.58	24
Standard deviation	10.05	13.43	11.39	8.43	13.46
Ratio		1.28	0.95	0.77	1.00
Variation %		0.28	-0.04	-0.22	0.003



**Discussion**

This study involved 4 series of experiments with 3 repetitions in each one; results indicate that *E. coli* obtained from a matrix colony raised in a nutrient-rich medium and exposed to nosode 12cH exhibited

significant lower growth in BHI agar by comparison to non treated samples and samples treated with *Merc 12cH* – to remind, the remedy hypothetically similar to the clinical image of the patient. On the other hand, bacteria obtained from matrix cultured

in a poor-nutrient medium exhibited non-significant lower growth ratio under the influence of *Merc* 12cH, by comparison to non treated samples.

Several studies involving *in vitro* models in high dilution research has been performed in the last years, some of them showing differences in membrane cell permeability under several homeopathic stimuli, including *Mercurius* 30c [8]. However, there are few papers in the literature about the *in vitro* action of high diluted substances in microorganism cultures [9]. Even though, there are some preliminary evidences that biochemical pattern changes can occur in these organisms after exposure to diluted metabolites [10].

The results of this pilot study allow one to suggest that survival of *E. coli* in a nutrient-poor medium might represent a putative selective effect of *Mercurius* on the best adapted bacteria. New studies are needed to understand this phenomenon better.

These preliminary results suggest that variations in the previous conditions of the studied biological system may elicit variations in the patterns of response to homeopathic and/or isotherapeutic remedies.

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