

PRELIMINARY DATA ON *ESCHERICHIA COLI*, *YERSINIA ENTEROCOLITICA* AND OTHER BACTERIAL SPECIES IN THE INTESTINAL MICROBIOTA OF WILD RODENTS FROM RILA MOUNTAIN

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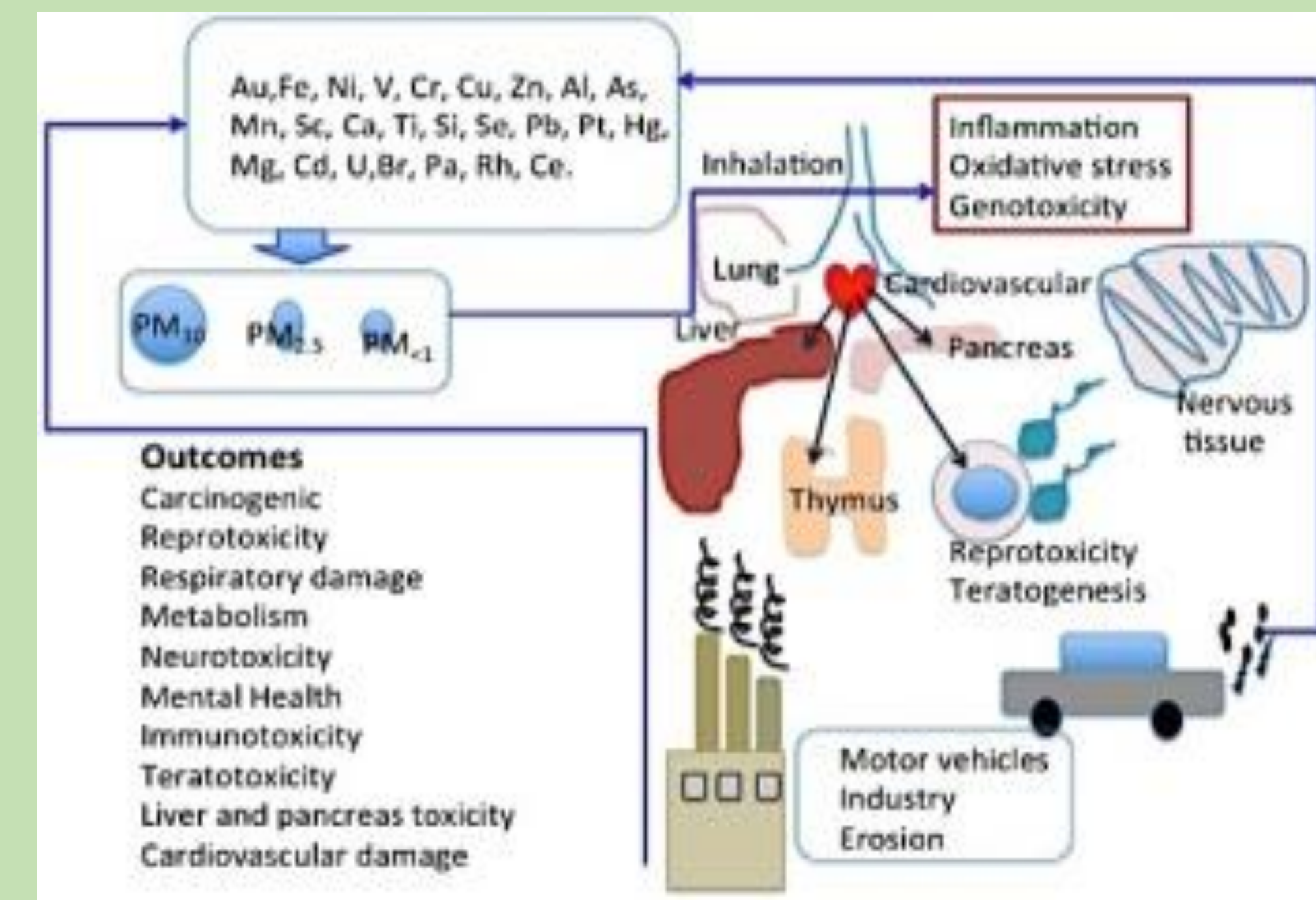
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Introduction

- Wild rodents are carriers of foodborne pathogenic bacteria like *Escherichia coli* and *Salmonella* spp.
- Rodents could contribute to the spread of antimicrobial (antibiotic) resistance (AMR) if their intestinal bacteria carry genes for AMR and are able to exchange them with other bacteria through horizontal gene transfer.
- Small mammals like rodents have a significant biological reaction to the human-caused environmental changes which could influence their gut microbiota.

All of this determines the importance of the research of the intestinal microflora of wild small rodents.



Small mammals like rodents were also selected due to:

large population number

wide distribution area

Materials and Methods

Animal collections

- summer of 2019
- Rila Mountain (locality Skakavtsite, 1500 m a.s.l.)
- live-bait and snap traps
- All collected animals were dissected to remove the intestinal tract and fecal matter was taken from the rectum.

2 ♂ forest voles

7 yellow-necked mice

(*Clethrionomys glareolus*)

(*Apodemus flavicollis*)

(2 ♀ and 5 ♂)

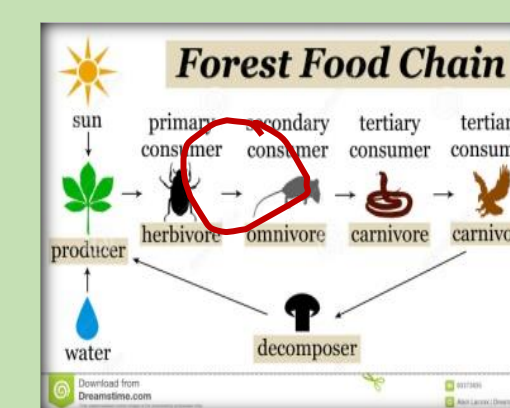
omnivorous



herbivorous



Area of Beli iskar vilage



Results

- 52 pure cultures were isolated from single bacterial colonies.
- They were identified morphologically or biochemically as *E.coli*, *Yersinia enterocolitica*, *Y. kristensenii*, *Hafnia alvei*, *Serratia marcescens*, *Serratia liquefaciens*, *Pantoea agglomerans*, *Klebsiella pneumoniae* spp. *ozaenae*, *Enterobacter cloacae*, *Bacillus thuringiensis*, *Enterococcus faecium* and *E.faecalis*.



Isolates identified as *Y. enterocolitica* by BD Phoenix™ (isolates 13, 14 and 16)

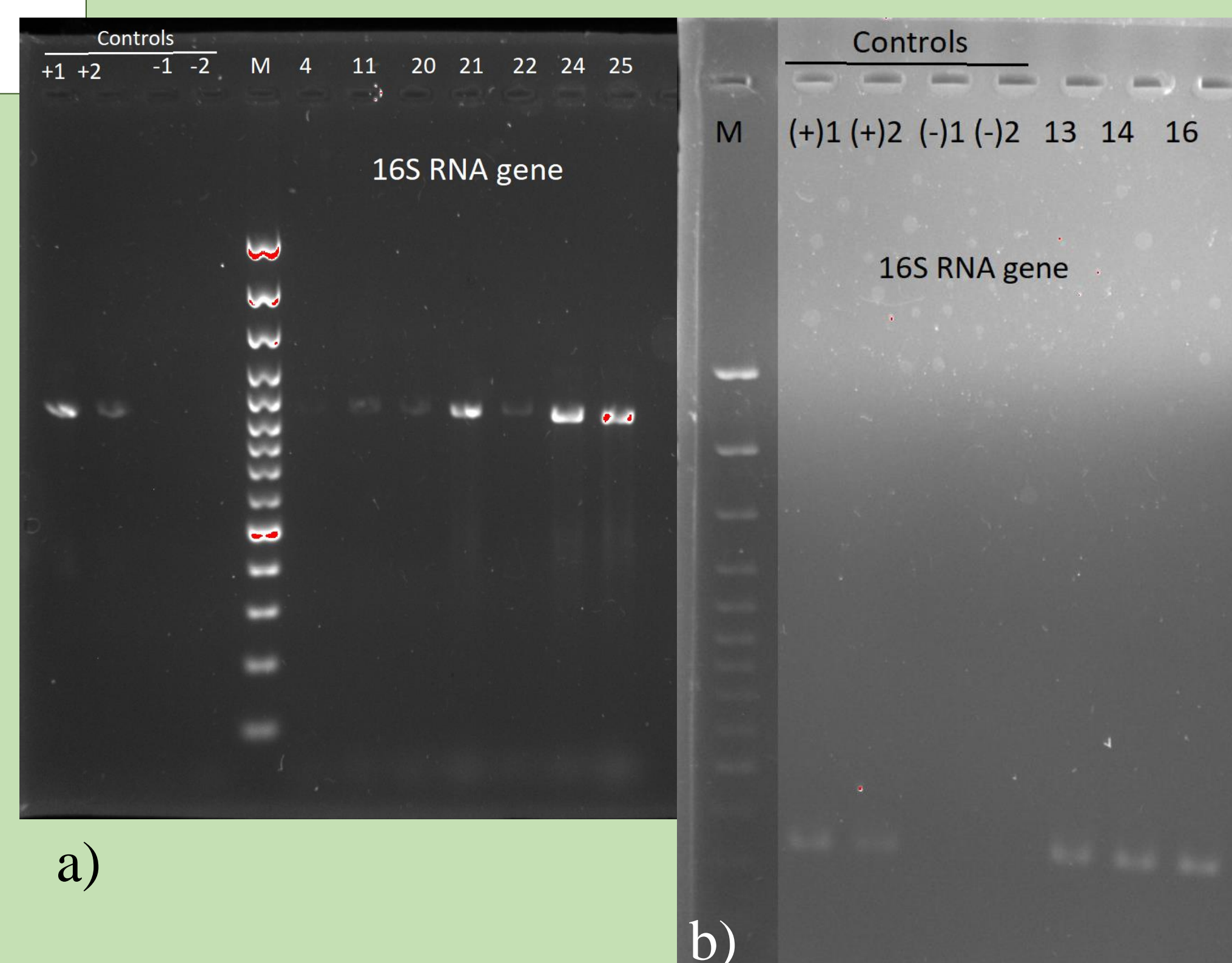
- The antimicrobial susceptibility and resistance of 16 bacterial species was elucidated by BD Phoenix™
- The strains most resistant to antibiotics turned out to be *E. faecalis*, *Y. enterocolitica* and *H. alvei*

An example of Antimicrobial susceptibility of an isolate (*Y. enterocolitica*, sample 13)

Antimicrobial (antibiotic)	MIC (µg/mL)	
Amikacin	≤4	S
Amoxicillin-	>8/2	R
Clavulanate (f)		
Ampicillin	>8	R
Aztreonam	≤1	S
Cefazolin	>4	R
Cefotaxime	≤0.5	S
Ceftazidime	1	S
Cefuroxime	8	
Cephalexin	16	R
Ciprofloxacin	≤0.125	S
Colistin	≤1	X
Fosfomicin w/G6P	32	S
Gentamicin	2	S
Meropenem	≤1	S
Nitrofurantoin	32	
Piperacillin-	≤4/4	S
Tazobactam		
Tobramycin	≤1	S
Trimethoprim	≤1	S
Trimethoprim-	≤1/19	S
Sulfamethoxazole		

MIC – Minimal inhibitory concentration
R – Resistant
S – Susceptible
X – Cannot give an interpretation

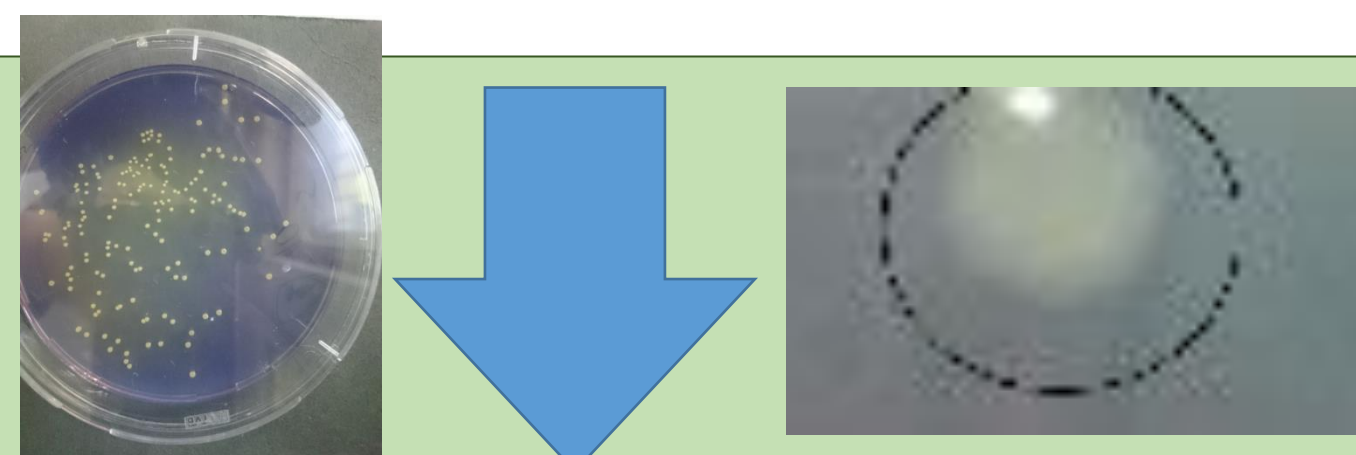
7 of the colonies were confirmed as *E.coli* and 3 of them were confirmed as *Y. enterocolitica* by PCR.



PCR confirmation of 7 strains of *E. coli* (a) and 3 strains of *Y. enterocolitica* (b)

The *ail* pathogenicity and virulence gene was not found in the yersinias using traditional and ddPCR.

Classic microbiological methods - the method of serial dilutions, bacterial culturing on differential media, Gram stain, microscopic slide examination by light microscopy, etc.



Identification of bacteria
Biochemical: BD Phoenix™
Automated Identification and Susceptibility Testing – used also for antimicrobial resistance elucidation



Identification of bacteria
Molecular-biology methods: conventional PCR, digital droplet PCR

Conclusions

52 bacterial species were isolated from single colonies from the gut microflora of small wild rodents in Bulgaria. Opportunistic pathogens were identified: 7 and 3 of them were confirmed as *E. coli* and *Y. enterocolitica*, resp. by PCR. This study is a platform for future biomonitoring, ecological and ecotoxicological research and for studying the spread of (non-)pathogenic bacteria and zoonoses among wild rodents and the fauna in general in Bulgaria.

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