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Colicins of the *Escherichia coli* uropathogenic strain collection

Kolicini zbirke uropatogenih bakterij *Escherichia coli*

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Abstract. 110 uropathogenic *Escherichia coli* (UPEC) strains were screened for colicin production and 42 (38%) of the tested UPEC strains were found to be colicinogenic. The ColA, ColB, ColD, ColE2, ColE3, ColE4, ColE5, ColE6, ColE7, ColIa, ColIb, ColK, ColN, MccB17, ColS4, MccC7 and ColE6-J colicin producer strains from Pugsley's collection of colicinogenic strains were lysed by all colicinogenic UPEC strains, the ColM and ColE1 producer strains by 93% of the UPEC colicinogenic strains and the ColV producer strain by only 81% of the UPEC colicinogenic strains. 67% of the colicinogenic UPEC strains were able to lyse all 20 used colicin producer strains and 33% of the colicinogenic UPEC strains were able to lyse 19 Pugsley's strains. Hence, a majority (67%) of the studied UPEC strains encode and produce either more than one colicin, or a colicin not tested. Colicins of UPEC strains producing only one colicin were identified; 8 strains (19% of the colicinogenic strains) produced only ColV, 3 strains (7%) ColM and 3 strains (7%) ColE1. Plasmids were found in 88% of the colicinogenic strains. 11 DL strains were found to harbour conjugative plasmids encoding antibiotic resistance(s) and colicinogenicity. Further, 19% of the haemolytic UPEC strains and 44% of non-haemolytic strains were also colicinogenic, 28% of the *cnf* encoding strains and 41% of the strains not encoding *cnf* were colicinogenic, while 40% of *ibeA* encoding strains and 38% of strains not encoding *ibeA* were colicinogenic.

Key words: colicin, uropathogenic *Escherichia coli*, UPEC, plasmid, haemolysin, *hly*, cytotoxic necrotising factor, *cnf*, invasin, *ibeA*.

Izvleček. 110 uropatogenih sevov bakterije *Escherichia coli* (UPEC) smo s pomočjo 20 kolicinogenih sevov iz Pugsleyeve zbirke testirali za produkcijo kolicinov. 42 testiranih sevov (38%) je bilo kolicinogenih. Vsi kolicinogeni sevi UPEC so povzročili propad producentskih sevov kolicinov ColA, ColB, ColD, ColE2, ColE3, ColE4, ColE5, ColE6, ColE7, ColIa, ColIb, ColK, ColN, MccB17, ColS4, MccC7 in ColE6-J iz Pugsleyeve zbirke. 93% kolicinogenih sevov UPEC je povzročilo propad ColM in ColE1 producentskega seva in 81% kolicinogenih sevov UPEC je povzročilo propad producentskega seva kolicina ColV. 67% kolicinogenih sevov UPEC je povzročilo propad vseh 20 kolicinogenih sevov Pugsleyeve zbirke in 33% kolicinogenih sevov UPEC je povzročilo propad 19 sevov iz Pugsleyeve zbirke. Večina kolicinogenih sevov (67%) je sintetizirala vsaj dva kolicina, ali pa kolicin, ki ni bil testiran. Kolicine sevov UPEC, ki sintetizirajo samo 1 kolicin, smo prepoznali; 8 sevov UPEC (19%) je sintetiziralo samo ColV, 3 sevi (7%) so sintetizirali samo ColM in 3 sevi (7%) so sintetizirali samo ColE1. V 88% kolicinogenih sevov smo našli plazmide. V 11 sevih DL smo našli konjugativne plazmide z zapisi za odpornost proti antibiotikom in za sintezo kolicinov. 19% hemolitičnih sevov UPEC in 44% nehemolitičnih sevov UPEC je bilo kolicinogenih; 28% sevov z zapisom *cnf* in 41% sevov brez

zapisa *cnf* je bilo tudi kolicinogenih, medtem ko je bilo 40% z zapisom *ibeA* in 38% brez tega zapisa tudi kolicinogenih.

Ključne besede: kolicin, uropatogena *Escherichia coli*, UPEC, plazmid, hemolizin, *hly*, citotoksičen nekrotizirajoč dejavnik, *cnf*, invazin, *ibeA*.

Introduction

Colicins are bacteriocins produced by *Escherichia coli* (*E. coli*) strains. As other bacteriocins, produced by different types of *Eubacteria* and *Archaebacteria* (Riley & Gordon 1999), colicins are extracellular bacterial toxic proteins, that are active against the same species, or closely related species of the producer cell (Daw & Falkiner 1996). The mechanism of action of these compounds involves adsorption to specific receptors located on the external surface of sensitive bacteria followed by killing via one of three primary mechanisms: the formation of channels in the cytoplasmic membrane, the degradation of cellular DNA or the inhibition of protein synthesis (Riley & Gordon 1999). Because of their narrow range of activity, it has been proposed that the primary role of bacteriocins is to mediate intraspecific, or population level, interactions (Riley 1998).

However, bacteriocins have been also implicated in virulence determination, since many pathogenic strains harbour plasmid-encoded bacteriocins, for example ColV (Waters & Crosa 1991). A relatively high frequency of colicin producing strains is found in isolates of pathogenic *E. coli* (van der Wal & al. 1995), for example approximately 80% of enterohemorrhagic *E. coli* strains studied by Bradley and Howard were colicinogenic (Bradley & Howard 1991).

In the presented study a collection of 110 uropathogenic *E. coli* (UPEC) strains, isolated at the Institute of Microbiology and Immunology of the Medical Faculty of Ljubljana, Slovenia, was examined for the ability to produce colicins and lyse other *E. coli* strains of the Pugsley collection of colicinogenic strains. Further, the association of colicinogenicity and some established virulence factors was also analysed. The results of the study show, that almost 40% of the UPEC strains are colicinogenic and that all colicinogenic strains are very efficient in lysing *E. coli* strains of the Pugsley collection. Further, a non-proportional distribution of the ability to produce colicins among the *hly* and *cnf* coding versus non-coding strains was determined. In 88% of the colicinogenic strains plasmids were found, 11 colicinogenic strains harboured conjugative plasmids encoding antibiotic resistances and colicin production.

Methods

Bacterial strains, plasmids and growth conditions

The 110 uropathogenic *Escherichia coli* DL strains used in this study were isolated from urine of patients with urinary tract infections at the Institute of Microbiology and Immunology of the Medical Faculty of Ljubljana. The strain AB1133 [*thr1 leuB6 proA2 his argE2 thi ara lacY galK2 xyl mtl rpsL* α^- *supE* (B. Bachmann)], which is sensitive to all colicins, was used to identify colicinogenic UPEC strains. The strains of Pugsley's collection of colicinogenic strains are listed in Tab. 1. For mating experiments the following strains were used: DH5α [Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169 *endA1 recA1 hsdR17 deoR thi-1 supE44 gyrA96 relA1* (BRL Life)], HB101 [*hsdR hsdM recA13 supE44 leuB6 lacZ proA2* (D. Ehrlich)], RU4404 [MM294::Tn*1725* Cm^r *thi endA hsdR* (R. Schmitt)], RU4406 [MM294::Tn*1732* Kn^r *thi endA hsdR* (R. Schmitt)] and TR51 [*ara*D139 Δ(*argF-lac*)U169 *rps*L150 *rel*A1 *flb*B5301 *pts*F25 *deo*C1 *cpxR*::*spc* Spc^r (T. J. Silhavy)]. Bacteria were grown in Luria-Bertani (LB) medium with aeration at 37°C or on LB plates without aeration. Ampicillin (Ap, 100 μg/ml), tetracycline (Tc, 10 μg/ml), kanamycin (Kn, 30 μg/ml), chloramphenicol (Cm, 50 μg/ml), spectinomycin (Spc, 20 μg/ml), streptomycin (Sm, 150 μg/ml), trimethoprim (Tp, 10 μg/ml) and nalidixic acid (Nal, 25 μg/ml) were added to the growth media, when appropriate.

Table 1: Bacterial strains of Pugsley's collection.
Tabela 1: Bakterijski sevi Pugsleyeve zbirke.

Strain	Relevant features
BZB2101	ColA producer
BZB2102	ColB producer
BZB2103	ColD producer
BZB2104	ColE1 producer
BZB2125	ColE2 producer
BZB2106	ColE3 producer
BZB2107	ColE4 producer
BZB2108	ColE5 producer
BZB2109	ColE6 producer
BZB2110	ColE7 producer
BZB2114	ColIa producer
BZB2115	ColIb producer
BZB2116	ColK producer
BZB2123	ColN producer
BZB2283	MccB17 producer
PAP1	ColM producer
PAP2	ColS4 producer
PAP54	MccC7 producer
PAP222	ColV producer
PAP247	ColE8-J producer

Overlay test

The overlay test was based on the method described by Pugsley and Oudega (1987). The UPEC strains were inoculated on LB plates (20 colonies grid) using toothpicks. Following overnight incubation the cells were lysed with chloroform, to release the colicins. After aeration, the plates were overlaid with 4 ml of soft agar with 0,2 ml of an overnight culture of either AB1133 or one of the strains from Pugsley's collection. The plates were then incubated overnight and next day examined for zones of colicin activity (clear zones around the colonies).

Plasmid isolation

Plasmid DNA was prepared by the alkaline lysis method described in Sambrook et al. (1989).

Mating assay

Conjugation experiments were performed overnight on LB plates. The mating mixture was transferred to LB plates supplemented with appropriate antibiotics to select for transconjugants. The conjugative plasmids encoding antibiotic resistances were initially, depending on the antibiotic resistance profile, transferred to either DH5α, HB101, RU4404, RU4406 or TR51 and further to a another laboratory strain to confirm the self conjugative transfer ability. All conjugative plasmids were finally transferred to strain DH5α.

Results

Colicinogenic UPEC strains and their colicins

In order to identify the colicinogenic UPEC strains, strain AB1133, which is known to be sensitive to all colicins, was used in an overlay test as described above. 42 UPEC strains (38%) were able to lyse strain AB1133, hence exhibited colicinogenic activity. To attempt to characterise colicin encoded by

the individual UPEC strains, the 20 strains of Pugsley's collection of colicin producing strains were used in overlay tests. The tests showed, that most of the colicinogenic UPEC strains (67%) produced either more than one colicin, or a colicin, not harboured by strains of the Pugsley collection. However, 14 UPEC strains were shown to produce only one colicin, namely 8 strains (19% of the colicinogenic strains) produced only ColV, 3 strains (7%) ColM and 3 strains (7%) produced only ColE1 (Tab. 2).

Table 2: Colicinogenic DL strains.
Tabela 2: Kolicinogeni sevi DL.

Strain	Number of lysed Pugsley's strains	Identified colicin	Plasmid detected	Conjugative plasmid
DL2	20		+	+
DL3	20		+	–
DL6	20		+	+
DL10	20		+	–
DL14	20		+	+
DL22	20		+	+
DL24	20		+	–
DL27	19	ColV	+	–
DL35	20		+	–
DL37	20		+	+
DL40	19	ColM	–	–
DL46	20		+	+
DL48	20		+	+
DL49	20		+	–
DL51	20		+	–
DL52	19	ColV	+	–
DL53	20		+	–
DL56	20		+	+
DL57	19	ColV	+	–
DL58	20		+	–
DL59	20		–	–
DL60	20		+	–
DL62	19	ColV	–	–
DL63	19	ColE1	+	–
DL64	19	ColV	+	–
DL66	19	ColV	+	–
DL67	19	ColV	+	–
DL71	19	ColE1	+	–
DL72	19	ColE1	+	–
DL75	20		+	–
DL76	20		+	+
DL83	19	ColV	+	–
DL89	19	ColM	+	–
DL91	19	ColM	+	–
DL92	20		–	–
DL93	20		+	–
DL95	20		–	–
DL99	20		+	–
DL104	20		+	–
DL107	20		+	–
DL108	20		+	+
DL110	20		+	+

Sensitivity of Pugsley's colicinogenic strains for the colicinogenic UPEC strains

The sensitivity of Pugsley's colicinogenic strains for the colicinogenic UPEC strains was also demonstrated in the overlay tests. The ColA, ColB, ColD, ColE2, ColE3, ColE4, ColE5, ColE6, ColE7, ColIa, ColIb, ColK, ColN, MccB17, ColS4, MccC7 and ColE6-J colicin producer strain from Pugsley's collection were lysed by all colicinogenic UPEC strains, the ColM and ColE1 producer strains by 93% of the UPEC colicinogenic strains and the ColV producer strain by only 81% of the UPEC colicinogenic strains (Graph 1).The high sensitivity of the Pugsley colicinogenic strains for the colicinogenic UPEC strains is also evident from Tab. 2, which shows that 67% of the colicinogenic UPEC strains were able to lyse all 20 of the used colicin producer strains and 33% of the colicinogenic UPEC strains were able to lyse 19 of the Pugsley strains.

The ability to produce colicins and plasmids

Preparation of plasmid DNA with alkaline lysis from colicinogenic strains revealed that 37 (88%) of the colicinogenic strains harboured plasmids. In only 5 strains (DL40, DL59, DL62, DL92 and DL95) no plasmid DNA was detected (Tab. 2).

Colicinogenic proprieties encoded on conjugative plasmids

In the mating assays, 19 DL strains (DL2, DL6, DL7, DL8, DL14, DL17, DL22, DL37, DL41, DL43, DL46, DL48, DL56, DL76, DL81, DL84, DL108, DL109 and DL110) were found to harbour conjugative plasmids encoding antibiotic resistances. 11 of these strains (DL2, DL6, DL14, DL22, DL37, DL46, DL48, DL56, DL76, DL108 and DL110) were also found to be colicinogenic (Tab. 2). To determine whether the colicinogenicity is encoded on a conjugative plasmid (pDL), the DH5α laboratory strains harbouring the conjugative plasmids from the DL strains were subjected to overlay tests. When the DH5α strains harbouring pDL conjugative plasmids were overlaid with the Pugsley strains, all 20 of the Pugsley strains were lysed. Further, when the original DL strains were overlaid by DH5α strains harbouring pDL conjugative plasmids, the DH5α pDL strains were not lysed by the original DL strain. The obtained results clearly demonstrated that colicinogenicity in all of these strains was encoded by a conjugative plasmid and hence, was transferable to other strains.

The ability to produce colicins and some other virulence factors

The obtained data concerning colicinogenicity of the tested strains presented in this paper were compared with our unpublished data on the prevalence of virulence factors; namely haemolysin (*hly*), cytotoxic necrotising factor (*cnf*) and invasin (*ibeA*). Our results showed that 19% of the haemolytic UPEC strains and 44% of the non-haemolytic strains were also colicinogenic, 28% of the *cnf* encoding strains and 41% of the strains not encoding *cnf* were colicinogenic, while 40% of the *ibeA* encoding strains and 38% of the strains not encoding *ibeA* were colicinogenic (Fig. 1).

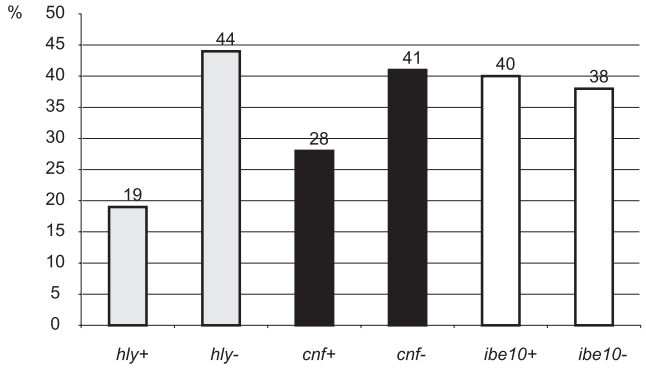

Fig. 1: Incidence of colicinogenic strains in correlation with some virulence factors. The incidence of colicinogenic strains is expressed in percentage of strains encoding or not encoding haemolysin (*hly*), cytotoxic necrotising factor (*cnf*) and invasin (*ibeA*).

Slika 1: Pogostnost kolicinogenih sevov v povezavi z nekaterimi virulenčnimi dejavniki. Pogostnost kolicinogenosti je izražena v odstotkih sevov z oziroma brez zapis za hemolizin (*hly*), citotoksičen nekrotizirajoč dejavnik (*cnf*) in invazin (*ibeA*).

Discussion

The DL strain collection of uropathogenic *E. coli* strains was screened for colicin production. 38% of the examined strains exhibited in the performed overlay tests a phenotype consistent with colicin production. Since the overlay tests were performed without mitomycin C treatment, which is known to induce temperate bacteriophage, it can be assumed that the obtained results have not been misinterpreted due to bacteriophage. However, to completely rule out the possibility of misinterpretation of bacteriophage activity as colicin production further tests would be needed.

Typically, 25–50% of *E. coli* isolates are colicinogenic (Riley & Gordon 1996). Usually, the percentages are higher in pathogenic than commensal strains, they are also higher in human compared to animal strains (Riley & Gordon 1996). In this study the obtained percentage (38%) of colicin producing strains in the DL collection is comparable to that of other studies, McGeachie (1965) found 36% of colicinogenic strains among uropathogenic *E. coli* strains and O'Brien et al. (1996) found 41% of colicin producers among uropathogenic *E. coli* strains. The obtained percentage is also comparable to the incidence of colicinogenicity (41%) in strains from colons of healthy persons (Šmarda & Obdržalek 2001).

It is established that the frequency of different colicin types varies substantially between populations (Riley & Gordon 1996), hence the colicin pattern in the DL collection should differ from patterns in other *E. coli* collections. The applied tests allowed the assignment of colicins of those strains producing only one colicin, namely ColV by 19% of the colicinogenic strains, ColM and ColE1 by 7% of the colicinogenic strains. Colicins of all other colicinogenic strains (67%) could not be assigned as these strains produce more than one colicin or they produce a colicin type not presented in the Pugsley collection of colicinogenic strains. However, the combination of ColV, ColM and ColE1 colicins in one collection is, to our knowledge, unique.

High levels of colicin resistance are known to occur in natural *E. coli* populations (Feldgarden & Riley 1998), hence the high sensitivity of Pugsley's collection for the colicinogenic strains (the majority, 67%, of colicinogenic strain were able to lyse all 20 strains of Pugsley's collection), is a bit surprising. But it is explainable with the assumption, that the UPEC strain encode more than one colicin or a colicin not produced by the Pugsley strains collection.

It is known, that the genetic determinants of most of the colicins are located on the plasmids, apart from few, which are chromosomally encoded (Daw & Falkiner 1996). Hence, it is not surprising that in 88% of colicinogenic strains we were able to detect plasmid DNA. A very similar percentage, 86%, of plasmid harbouring colicin producing strains was found by Riley and Gordon (1992) in the ECOR collection, a collection of strains representing clinical and non-clinical isolates from man, domestic and zoo animals.

To determine whether the colicins are really encoded on the detected plasmids, the plasmids should be transferred to laboratory strains and subsequently tested for colicin production. Unfortunately, the capabilities of transferring the plasmids to laboratory strains are limited due to large plasmid size and lack of selection possibilities. However, several conjugative plasmids encoding antibiotic resistances were found in the DL collection and were successfully transferred. The overlay tests performed on these strains showed that all such plasmids (11) also encoded colicin production.

The role of colicins in microbial communities is still not clear (Riley & Wertz 2002). Bacteriocins may serve as anti-competitors enabling the invasion of a strain into an established microbial community or they may play a defensive role and act to prohibit the invasion of other strains or species into an occupied niche or limit the advance of neighbouring cells (Riley & Wertz 2002). Further it was also suggested that colicins, at least ColV, more exactly the virulence factors also encoded by ColV plasmids might play a role in pathogenesis (Waters & Crosa 1991). In a study of UPEC strains Šmarda & Obdržalek (2001) observed that the incidence of colicinogenicity significantly differs between haemolytic and non-haemolytic strains (42% of non-haemolytic and only 22% of haemolytic strains were colicinogenic). A comparable difference was also observed in the presented study, as 44% of the non-haemolytic and 19% of the haemolytic strains were colicinogenic. Further, a similar even though less distinctive difference, was observed with the *cnf* encoding (28%) and *cnf* non encoding strains (41%). These results suggest, that the production of colicins is associated with some virulence factors, since otherwise the incidence of colicinogenic strains should be equal in both groups, as it is in the presented study for the *ibeA* encoding (40%) and non *ibeA* encoding strains (38%). Even though the obtained results could indicate that colicinogenic strains are less virulent, further studies are needed to establish the role colicins play in natural populations.

Conclusions

To summarise and conclude:

1. 110 uropathogenic *Escherichia coli* (UPEC) strains were screened for colicin production;
2. 38% of the tested UPEC strains have been found to be colicinogenic;
3. 67% of the colicinogenic UPEC strains were able to lyse all 20 used colicin producer strains, hence these UPEC strains encode and produce either more than one colicin, or a colicin not tested;
4. 19% of the colicinogenic strains produced only ColV, 7% only ColM and 7% only ColE1;
5. plasmids were found in 88% of the colicinogenic strains;
6. 26% of colicinogenic strains harboured conjugative plasmids encoding antibiotic resistance(s) and colicinogenic proprieties and
7. 19% of haemolytic UPEC strains and 44% of non-haemolytic strains were also colicinogenic, 28% of *cnf* encoding strains and 41% of strains not encoding *cnf* were colicinogenic, while 40% of *ibeA* encoding strains and 38% of strains not encoding *ibeA* were colicinogenic.

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Povzetek

V predstavljeni raziskavi smo 110 uropatogenih sevov bakterije *Escherichia coli* (UPEC), ki so jih na Inštitutu za mikrobiologijo in imunologijo Medicinske fakultete v Ljubljani izolirali iz urina bolnikov z urinarno infekcijo, s pomočjo 20 kolicinogenih sevov iz Pugsleyeve zbirke testirali za produkcijo kolicinov. Testiranje je pokazalo, da je bilo 42 sevov UPEC (38%) kolicinogenih. Ker smo testiranje izvedli brez indukcije z mitomicinom C, za katero je znano, da sproži lizogene bakteriofage, lahko predpostavljamo, da dobljeni rezultati temeljijo samo na aktivnosti kolicinov in ne bakteriofagov. Dobljen odstotek kolicinogenih sevov (38%) se ujema tudi z rezultati objavljenimi v podobnih študijah. Vsi kolicinogeni sevi UPEC so povzročili propad producentskih sevov kolicinov ColA, ColB, ColD, ColE2, ColE3, ColE4, ColE5, ColE6, ColE7, ColIa, ColIb, ColK, ColN, MccB17, ColS4, MccC7 in ColE6-J iz Pugsleyeve zbirke. 93% kolicinogenih sevov UPEC je povzročilo propad ColM in ColE1 producentskega seva in 81% kolicinogenih sevov UPEC je povzročilo propad producentskega seva kolicina ColV. 67% kolicinogenih sevov UPEC je povzročilo propad vseh 20 kolicinogenih sevov Pugsleyeve zbirke in 33% kolicinogenih sevov UPEC je povzročilo propad 19 sevov iz Pugsleyeve zbirke. Večina kolicinogenih sevov (67%) sintetizira vsaj dva kolicina, ali pa kolicin, ki ni bil zastopan v Pugsleyevi zbirki kolicinogenih sevov. Kolicine sevov UPEC, ki sintetizirajo samo 1 kolicin, smo prepoznali; 8 sevov UPEC (19%) je sintetiziralo samo ColV, 3 sevi (7%) so sintetizirali samo ColM in 3 sevi (7%) so sintetizirali samo ColE1. Sicer je znano, da različne populacije *E. coli* sintetizirajo različne kombinacije kolicinov, a kombinacija ColV, ColM in ColE1, do sedaj še ni bila ugotovljena. Iz 88% kolicinogenih sevov smo uspeli izolirati plazmidno DNA po metodi alkalne lize. S poskusi konjugacije smo v 11 sevih DL našli konjugativne plazmide z zapisi za odpornost proti antibiotikom in za sintezo kolicinov. Vloga kolicinov v naravnih populacijah še ni popolnoma razjasnjena. Za plazmid ColV je znano, da ima poleg zapisa za kolicin V tudi zapise za virulenčne dejavnike. V naši raziskavi smo ugotovili, da je bilo 19% hemolitičnih sevov UPEC in 44% nehemolitičnih sevov UPEC tudi kolicinogenih; 28% sevov z zapisom *cnf* in 41% sevov brez zapisa *cnf* je bilo kolicinogenih, medtem ko je 40% z zapisom *ibeA* in 38% brez tega zapisa bilo kolicinogenih. Čeprav dobljeni rezultati nakazujejo, da so kolicinogeni sevi manj virulentni kot nekolicinogeni sevi, so za potrditev te domneve potrebne še nadaljnje raziskave.

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