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Molecular characterization of *Escherichia coli* from dishwasher rubber seals

Molekularna opredelitev bakterij *Escherichia coli* z gumijastih tesnil pomivalnih strojev

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Abstract: In this study 35 *Escherichia coli* isolates obtained from sampled dishwashers rubber seals were analysed with polymerase chain reactions (PCR) using specific primers for phylogenetic group, multilocus sequence type (MLST) determination the presence of 43 virulence-associated genes (VAGs) linked with intestinal and extraintestinal *E. coli* infections and the presence of some typical *E. coli* virulence plasmids' sequences in order to assess their virulence potential and/or specific genes, associated with the adaption to a specific environment. It was found that all of the 35 *E. coli* isolates belonged to the commensal non-pathogenic phylogenetic group A and that the diversity of these *E. coli* isolates, according to MLST analysis, was relatively low. Further, the prevalence of virulence-associated genes among the dishwasher rubber seal *E. coli* isolates was also low. Only the following VAGs were detected: *fimH*, *crl*, *fluA*, *picU*, *irp*, *fyuA*, *sitA*, *aslA*. Of the five plasmid replication regions tested only RepFIA and RepFIIA were detected. The two sequences associated with conjugative plasmids namely *traJ* and *traT*, were detected in only one isolate. Based on the obtained results the studied isolates can be designated as commensal *E. coli* with low pathogenic potential. Due to the low diversity of sequence types, even among isolates obtained from dishwashers from different locations, there is a possibility that strains from certain clonal groups are more adapted to specific habitats outside warm-blooded hosts than strains of other sequence types.

Keywords: dishwasher, *E. coli*, genes, One Health, plasmids, virulence

Izvleček: V tej raziskavi smo analizirali 35 izolatov bakterije *Escherichia coli*, pridobljenih iz vzorčenih gumijastih tesnil kuhinjskih pomivalnih strojev, da bi ocenili njihovo potencialno patogenost in/ali prisotnost genov povezanih z adaptacijo na specifično okolje. Z verižno reakcijo s polimerazo (PCR), ob uporabi ustreznih oligonukleotidov, smo vse izolate uvrstili v filogenetske skupine, določili njihov sekvenčni tip na podlagi analize multilokusnega zaporedja (MLST), ugotavljali prisotnost 43 genov, povezanih z virulenco (VAG), ki so pomembni v črevesnih in zunajčrevesnih okužbah z *E. coli* ter ugotavljali prisotnost nekaterih značilnih zaporedij plazmidov, povezanih z virulenco *E. coli*. Ugotovili smo, da je vseh 35 izolatov *E. coli* pripadalo komenzalni nepatogeni filogenetski skupini A in da je bila raznolikost teh izolatov, glede na analizo MLST, relativno majhna. Poleg tega je bila razširjenost genov, povezanih

z virulenco, med preučevanimi izolati *E. coli* nizka. Ugotovili smo le naslednje VAG: *fimH*, *crl*, *fluA*, *picU*, *irp*, *fyuA*, *sitA*, *aslA*. Od petih testiranih plazmidnih replikacijskih regij sta bili potrjeni samo RepFIA in RepFIIA. Zaporedji *traJ* in *traT*, povezani s konjugativnimi plazmidi, smo zasledili samo v enem izolatu. Na podlagi dobljenih rezultatov lahko preučevane izolate označimo kot komenzalne *E. coli* z nizkim patogenim potencialom. Glede na majhno diverziteto sekvenčnih tipov, tudi med izolati pridobljenimi iz pomivalnih strojev iz različnih krajev, pa obstaja možnost, da so sevi iz določenih klonalnih skupin bolj prilagojeni na specifične habitate izven toplokrvnih gostiteljev, kot sevi drugih sekvenčnih tipov.

Ključne besede: *E. coli*, Eno zdravje, geni, plazmidi, pomivalni stroj, virulenca

Introduction

Recently, the importance of the approach, known as "One Health" has been increasingly recognized in the world. This approach is based on the awareness that microbes, animals and humans share ecosystems and that research on the diversity and complexity of interactions is needed. Further, this approach recognizes that the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and interdependent (Miao et al. 2022).

Escherichia coli (*E. coli*), which is one of the best studied microorganisms in the world and a well-known genetic model organism, is a typical example of a bacterium that can be found in the environment, in water, soil, as well as in animal and human hosts (Jang et al. 2017). It usually lives in a mutualistic relationship with the host, but can also be pathogenic and cause serious, even fatal, intestinal and extra-intestinal infections (Allocati at al. 2013). *E. coli* is excreted from the host by faeces into the environment, where it can spread and then be reintroduced into different animal and human hosts by different routes (Jang et al. 2017). It is known for its pronounced genetic diversity, reflected in many different strains (Chaudhuri and Henderson 2012). The *E. coli* pathogenic strains carry many different virulence factors genes including colonization and fitness factors, toxins, and other effectors intertwining with the host's physiology (Kaper et al. 2004). Furthermore, it is known that certain phylogenetic groups are associated with pathotypes, for example the B2 phylogenetic group is associated with *E. coli* strains causing extraintestinal infections (Čurová et al. 2020). On the other hand, commensal strains isolated from France and Sweden in the 2000s and from North America (USA), Japan, and Australia also mainly belong to the B2 group (43%), while commensal strains isolated from France and Croatia in the 1980s and from Africa (Mali and Benin), Asia (Pakistan), and South America (French Guiana, Colombia, and Bolivia) belong mainly to the A group (55%) (Tenaillon et al. 2010). The genetic diversity of *E. coli* is also reflected in the presence of different mobile genetic elements, among which plasmids, especially the conjugative ones, are notorious for their effect on the genetic diversity and for carriage of virulence-associated genes (Kaper et al. 2004).

Although *E. coli* can exhibit host-associated and free-living/environmental state, the data on environmental *E. coli* strains are particularly scarce as there is a huge bias towards data on pathogenic strains (Starčič Erjavec and Žgur-Bertok 2015). In order to understand the heterogeneity of *E. coli* strains, their ability to adapt and spread within and outside hosts and the interactions between these strains, including gene exchange with horizontal transfers, it is necessary to obtain more data on characteristics of strains from specific human-associated environments such as household dishwashers.

Zupančič et al. (2019) found that *E. coli* are relatively frequent members of dishwasher rubber seal bacterial communities. In their study 35 *E. coli* isolates were obtained from eight out of 30 sampled dishwashers rubber seals. The aim of the present study was to conduct molecular characterisation of these 35 isolates, including the determination of phylogenetic group and multilocus sequence type (MLST) and screening of virulence-associated genes and typical replications regions associated with *E. coli* virulence plasmids.

Materials and methods

Bacterial isolates and growth conditions

A total of 35 *E. coli* isolated in six out of 30 dishwashers randomly selected in kitchens inside private dwellings located in seven Slovenian cities (i.e., Ljubljana, Velenje, Žalec, Celje, Mislinja, Sežana, and Portorož) were obtained from the Mycosmo Microbial Culture Collection Ex at the Biotechnical Faculty in Ljubljana (more informations about the dishwashers are available in Zupančič et al. 2019, additional file S1). All isolates were grown in liquid LB or on LB plates at 37 °C. Liquid cultures were vigorously aerated by shaking at 180 rpm.

Determination of phylogenetic groups of E. coli *isolates*

The phylogenetic groups for all of the *E. coli* isolates were determined by PCR, as described by Clermont et al. (2000) and improved by Clermont et al. (2013). The phylogenetic groups obtained by the method of Clermont et al. (2000) were assigned to their phylogenetic (sub)groups, according to the interpretation of Escobar-Páramo et al. (2006).

Multilocus sequence typing of E. coli *isolates*

Multilocus sequence typing was carried out according to Wirth et al. (2006) using PCR primers and protocols specified on the *E. coli* MLST website (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli) to amplify housekeeping genes *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*. The purified PCR products were sent to Microsynth AG (Switzerland) for DNA sequencing. The sequences were analysed for allelic profiles and sequence types through the *E. coli* MLST website.

Detection of virulence-associated genes (VAGs) and plasmid replication regions of the E. coli *isolates*

The presence of 43 VAGs, including adhesins (*fimH*, *crl*, *eae*, *bmaE*, *gaf*, *aaf*, *papG*I, *papG*II, *papG*III, *matA*, *sfa*/*foc*, *iha*, *afa*/*dra*, and *hra*), autotransporters (*sat*, *vat*, *hbp* (*tsh*), *picU*, and *fluA* (*Ag43*)), iron acquisition systems (*fyuA*, *irp*, *sitA*, *iutA*, *iucD*, and *iroN*), genes involved in increased serum survival and protectins (*iss*, $ompT_{APEC}$, *kpsMT*II, *neuCS*, *cvi)*, toxins (*stx1*, *stx2*, *astA*, *eltA*, *cnf2*, *hlyA*), invasins (*aslA*, *ibeA*, *gimB*, *tia*), and uropathogenic specific protein *usp,* was tested in all isolates. This was achieved using amplification procedures as described previously (Dozois et al. 1992, Yamamoto et al. 1996, Yamamoto and Echeverria 1996, Maurer et al. 1998, Paton and Paton 1998, Schubert et al. 1998, Hoffman et al. 2000, Johnson and Stell 2000, Vila et al. 2000, Janβen et al. 2001, Ruiz et al. 2002, Ewers et al. 2007, Runyen-Janecky et al. 2003, Starčič et al. 2003, Tóth et al. 2003, Watt et al. 2003, Parham et al. 2005, Vidal et al. 2005) and the instructions for the 'DEC Primer Mix'[Statens Serum Institut, Denmark]. *FluA* was amplified using the primer pair FluAF 5'-GCGGTGTACTGCTGGCCG-3' and FluAR 5'-CGTTGTGGCTGCCCAGAC-3', under the following cycling conditions: initial denaturation for 5 min at 95 °C, 30 cycles of 30 s at 94 °C and 30 s at 60 °C, followed by extension for 90 s at 72 °C. Likewise for *usp*, using UspDe-F 5'-ATGCTACTGTTTCCGGGTAGTGTGT-3' / UspDe-R 5'-CRTGTAGTCKGGGSGTAACAAT-3': initial denaturation for 5 min at 95 °C, 30 cycles of 30 s at 94 °C and 30 s at 55 °C, followed by extension for 2 min at 72 °C. Both final extensions were performed at 72 °C for 10 min. The plasmid replication regions were determined by PCR as described previously for RepFIA, RepFIIA (Starčič Erjavec et al. 2003), RepFIC (Carattoli et al. 2005) and IncP (Sobecky et al. 1997). For RepFIB, the following protocol was used: initial denaturation at 94 °C for 4.5 min, followed by 30 cycles, each consisting of 30 s of denaturation at 94 °C, 30 s of annealing at 63 °C, and 1.5 min of elongation at 72 °C. Additionally, the *E. coli* isolates were screened for F-like plasmid conjugative transfer genes *traJ* (the main positive regulator of conjugal transfer) and *traT* (surface exclusion and serum resistance), as described previously (Starčič Erjavec et al. 2003, Johnson and Stell 2000).

Results

All tested dishwasher E. coli *isolates belong to the non-pathogenic phylogenetic group A*

Phylotyping revealed, that all of the 35 *E. coli* isolates from the dishwasher rubber seals belonged to the commensal non-pathogenic phylogenetic subgroup A_0 according to Clermont et al. (2000) and Escobar-Páramo et al. (2006), and to phylogenetic group A according to Clermont et al. (2013).

Multilocus sequence typing analysis of E. coli *dishwasher isolates reveals low strain diversity*

The diversity of the *E. coli* isolates according to MLST analysis was relatively low. Twenty-seven (77%) of the 35 *E. coli* isolates were assigned to four known sequence types: ST189 (12 isolates, 44%), ST216 (7 isolates, 26%), ST399 (7 isolates, 26%), and ST1316 (1 isolate, 4%). The remaining eight isolates belonged to a new sequence type (Tab. 1).

Prevalence of virulence-associated genes among the E. coli *isolates from dishwasher rubber seals is low*

The prevalence of virulence-associated genes among the dishwasher rubber seal *E. coli* isolates was low (Tab. 1, Fig. 1). All isolates carried the curli fimbriae regulator gene *crl* and, except for strain L-436, the type 1 fimbrial adhesion gene *fimH*. Isolates L-687 and L-748 were positive for the autotransporter gene *picU*. PicU has mucinolytic activity through which colonisation of the host intestine is facilitated, by helping the pathogenic enteric bacteria to penetrate the mucus layer that coats the intestinal epithelium (Bhullar et al. 2015). Among the genes associated with iron acquisition, *sitA* was detected in isolate L-436, and *irp* and *fyuA* in isolates L-595 and L-1071. Further, 14 isolates (L-439, L-437, L-591, L-1101, L-778, L-781, L-785, L-786, L-790, L-808, L-814, L-815, L-821, L-836) were positive for *aslA*, a gene that is associated with invasion of the blood–brain barrier (Hoffman et al. 2000).

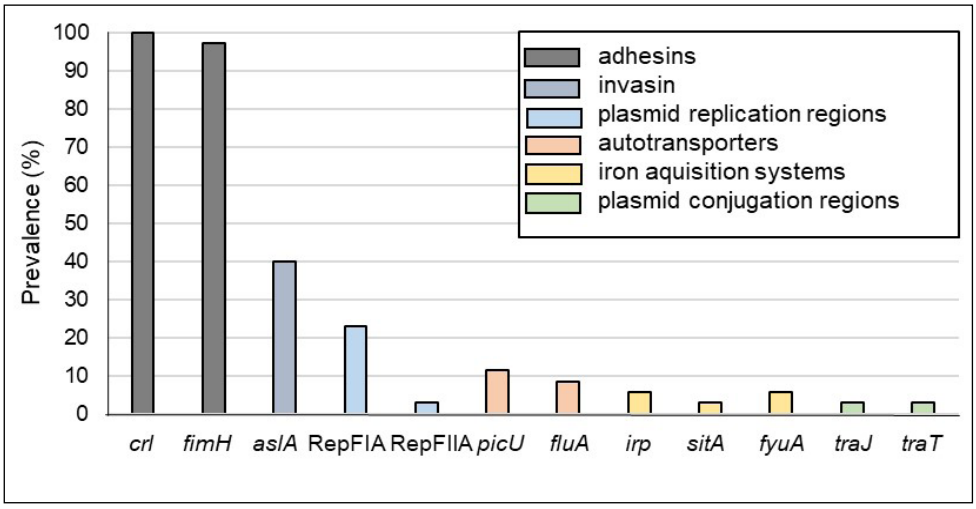

Figure 1: Prevalence of virulence-associated genes and plasmid replication regions in all *E. coli,* isolated from dishwasher rubber seals.

Slika 1: Prevalenca genov, povezanih z virulenco in plazmidnimi regijami za podvojevanje, v vseh izolatih *E. coli* z gumijastih tesnil pomivalnih strojev.

Prevalence of virulence plasmid associated sequences among the E. coli *isolates from dishwasher rubber seals is low*

In *E. coli*, plasmids can encode virulence factors and antibiotic resistance genes. Therefore, all of the isolates were analysed for plasmid-related characteristics; namely, the replication region and the *tra* region associated with conjugation. Of the five plasmid replication regions tested, only two were detected: RepFIA in eight isolates (L-396, L-434, L-431, L-433, L-594, L-736, L-983, L-982), and RepFIIA in one isolate (L-436) (Tab. 1). One isolate (L-429) was shown to have F-like plasmid *tra* region genes, *traJ* and *traT* (Tab. 1).

Table 1: MLST sequence type, virulence-associated genes and virulence plasmid associated sequences among the *E. coli* isolates from the dishwasher rubber seals. The following genes were not detected and are not included in the table: Adhesins: *eae*, *bmaE*, *gaf*, *aaf*, *papG*I, *papG*II, *papG*III, *matA*, *sfa/foc*, *iha*, *afa/dra* and *hra*, Protectins: *ompT, kpsMT*II, *neuCS, cvi* and *iss,* Autotransporters: *sat, vat* and *hpb/tsh,* Iron acquisition genes: *iutA, iucD* and *iroN*, Toxins: *stx1, stx2, astA, , cnf2, hlyA* and *elt,* Invasins: *ibeA, gimB* and *tia,* Uropathogenic specific protein: *usp*. The RepFIC, RepFIB and IncP plasmid replication regions were not present, and are not included in the table.

Tabela 1: Sekvenčni tip MLST, z virulenco povezani geni in zaporedja, značilna za plazmide, povezanih z virulenco pri izolatih *E. coli* z gumijastih tesnil kuhinjskih pomivalnih strojev. Naslednjih genov nismo zasledili pri preučevanih izolatih in zato niso vključeni v tabelo: Adhezini: *eae*, *bmaE*, *gaf*, *aaf*, *papG*I, *papG*II, *papG*III, *matA*, *sfa/foc*, *iha*, *afa/dra* in *hra*, Protektini: *ompT, kpsMT*II, *neuCS, cvi* in *iss,* Avtotransporterji: *sat, vat* in *hpb/tsh,* Sistemi za privzem železa: *iutA, iucD* in *iroN*, Toksini: *stx1, stx2, astA, , cnf2, hlyA* in *elt,* Invazini: *ibeA, gimB* in *tia,* Uropatogeni specifični protein: *usp*. Plazmidnih replikacijskih regij RepFIC, RepFIB in IncP prav tako nismo zasledili in niso vključene v tabelo.

Dish-washer	*E. coli* isolate	Sequence type	Adhesins		Autotrans-porters		Iron acquisition			Invasin	Plasmid replication regions		Plasmid conjugation regions	
			fimH	*crl*	*fluA*	*picU*	*irp*	*fyuA*	*sitA*	*aslA*	RepFIA	RepFIIA	*traT*	*traJ*
1	L-429	ST 399	+	+	+	–	–	–	–	–	–	–	+	+
	L-595	ST 399	+	+	+	–	+	+	–	–	–	–	–	–
	L-1071	ST 399	+	+	+	–	+	+	–	–	–	–	–	–
6	L-1101	ST 189	+	+	–	+	–	–	–	+	–	–	–	–
	L-790	ST 189	+	+	–	–	–	–	–	+	–	–	–	–
	L-396	ST 399	+	+	–	–	–	–	–	–	+	–	–	–
10	L-431	UN	+	+	–	–	–	–	–	–	+	–	–	–
	L-433	UN	+	+	–	–	–	–	–	–	+	–	–	–
	L-594	UN	+	+	–	–	–	–	–	–	+	–	–	–
	L-736	UN	+	+	–	–	–	–	–	–	+	–	–	–
	L-983	UN	+	+	–	–	–	–	–	–	+	–	–	–
	L-982	UN	+	+	–	–	–	–	–	–	+	–	–	–
13	L-434	ST 216	+	+	–	–	–	–	–	–	+	–	–	–

	L-589	ST 216	+	+	–	–	–	–	–	–	–	–	–	–
	L-590	ST 216	+	+	–	–	–	–	–	–	–	–	–	–
	L-592	ST 216	+	+	–	–	–	–	–	–	–	–	–	–
	L-745	ST 216	+	+	–	–	–	–	–	–	–	–	–	–
	L-747	ST 216	+	+	–	–	–	–	–	–	–	–	–	–
	L-748	ST 216	+	+	–	+	–	–	–	–	–	–	–	–
22	L-436	ST 1316	–	+	–	–	–	–	+	–	–	+	–	–
25	L-591	ST 189	+	+	–	–	–	–	–	+	–	–	–	–
	L-778	ST 189	+	+	–	–	–	–	–	+	–	–	–	–
	L-781	ST 189	+	+	–	–	–	–	–	+	–	–	–	–
	L-785	ST 189	+	+	–	–	–	–	–	+	–	–	–	–
	L-786	ST 189	+	+	–	–	–	–	–	+	–	–	–	–
	L-808	ST 189	+	+	–	–	–	–	–	+	–	–	–	–
	L-814	ST 189	+	+	–	–	–	–	–	+	–	–	–	–
	L-815	ST 189	+	+	–	–	–	–	–	+	–	–	–	–
	L-821	ST 189	+	+	–	–	–	–	–	+	–	–	–	–
	L-593	ST 399	+	+	–	–	–	–	–	–	–	–	–	–
	L-940	ST 399	+	+	–	–	–	–	–	–	–	–	–	–
	L-687	ST 399	+	+	–	+	–	–	–	–	–	–	–	–
	L-439	ST189	+	+	–	+	–	–	–	+	–	–	–	–
	L-437	UN	+	+	–	–	–	–	–	+	–	–	–	–
	L-836	UN	+	+	–	–	–	–	–	+	–	–	–	–

UN, unknown.

Discussion

The primary source of the studied dishwasher associated *E. coli* isolates might be both the household water supply system connected to the dishwasher and the contaminated vessels (Zupančič et al. 2019). In either case, it can be expected that studied *E. coli* strains are mainly commensal. Indeed, all of the *E. coli* isolates were assigned to phylogenetic group A_0, which is associated primarily with non-pathogenic commensal strains (Stoppe et al. 2017). This phylogenetic placement agrees with their low virulence potential and environmental adaptability. All of these isolates carried genes involved in adhesion; *crl*, and except one, *fimH*, the key factors in adhesion and biofilm formation on abiotic surfaces (Pratt and Kolter 1999). Furthermore, 14 isolates carried genes for AslA, a member of the arylsulphatase family of enzymes. In bacteria, the *asl* genes are expressed under conditions of sulphur starvation, in order to scavenge sulphate from exogenous substrates. In human meningitis

strains of *E. coli* K1, AslA contributes to invasion of the blood–brain barrier (Hoffman et al. 2000). The prevalence of other tested virulence-associated genes was very low. Only three isolates carried one or two genes involved in iron acquisition. Three isolates carried *fluA* for autotransporter Ag43a, which mediates diffuse adherence and bacterial intercellular aggregation, thus promoting biofilm formation and persistence (Zude et al. 2013). *flu* genes are predominantly found in clinical isolates, although Restieri et al. (2007) reported that 56% of commensal isolates screened in their study were positive for Ag43 coding genes. Furthermore, four isolates carried the autotransporter gene *picU*, which has been associated with phylogenetic group B2 isolates. Pic has mucinolytic activity, promotes species-specific haemagglutination, host colonisation, and immune evasion, by direct cleavage of complement proteins. The potential role of Pic in commensal strains is unknown (Abreu et al. 2015, Bhullar et al. 2015).

Contrary to our initial expectations related to tap water as the environmental inoculum, MLST analysis indicated low diversity of *E. coli* isolates on dishwasher rubber seals. This might be partly due to isolation of the same strain from individual dishwashers, although isolates with the same sequence type isolated from the same dishwasher often carried different tested genes, e.g.: dishwasher 1 isolate L-429 (*traJ*, *traT*) and isolates L-595 and L-1071 (*irp*, *fyuA*) all belong to ST399. From dishwasher 25, isolates of three different sequence types were detected, namely ST189, ST399 and an as yet unknown sequence type. Again, isolates from the same ST also differed; e.g., ST189 strain L-439 was positive for *picU* and *aslA*, and isolate L-778 was positive only for *aslA*. The same applied to dishwasher 6, with isolates L-1101 (*picU*, *aslA*) and L-790 (*aslA*); both ST189. The same sequence type of isolated bacterial strain was found in different dishwashers (different time in use) with different frequency of use, which were even geographically located in different cities (e.g. Ljubljana and Celje), thus indicating the strong selective pressure for certain sequence type genetic background in such specific extreme environments.

Analysis of the traits characteristic for horizontal gene transfer (*traT*, *traJ* and IncP-replication region) showed that vast majority of the studied isolates does not possess conjugative F-like and IncP plasmids. In the light of the knowledge that virulence factor genes can be spread in bacterial populations via conjugative plasmids, the studied isolates are potential recipients which could have high acceptance for conjugative IncF or IncP group plasmids and hence might obtain additional virulence-associated genes via conjugation. Therefore, further studies revealing the ability of acquiring conjugative plasmids are needed.

Conclusion

To the best of our knowledge, this is the first molecular characterization of *E. coli* isolates from Slovenian dishwasher rubber seals. Based on the obtained results the studied isolates can be designated as commensal *E. coli* with low pathogenic potential. However, further studies should shed light on the genome traits enabling specific adaptation of some clonal linages to harsh environments such as the studied dishwasher rubber seals and the potential of horizontal gene exchange in such bacterial communities.

Povzetek

Koncept Enega zdravja vse bolj pridobiva na pomenu, saj smo spoznali, da je zdravje ljudi, domačih in divjih živali, rastlin in širšega okolja (vključno z ekosistemi) tesno povezano in soodvisno. *Escherichia coli* (*E. coli*), ki je eden najbolje raziskanih mikroorganizmov na svetu in dobro poznan genetski modelni organizem, je tipičen primer bakterije, ki jo najdemo v okolju, v vodi, zemlji, pa tudi v živalskih in človeških gostiteljih. *E. coli* sicer velja za komenzalno, nepatogeno bakterijo, ki s svojim gostiteljem živi v mutualističnem odnosu, a obstajajo sevi, ki zaradi nabora virulentnih dejavnikov lahko povzročajo okužbe. Za patogene seve *E. coli* so značilni številni različni dejavniki virulence, vključno z dejavniki, ki omogočajo kolonizacijo, sintezo toksinov in efektorskih molekul, ki se vpletajo v fiziologijo gostitelja. Nadalje je znano, da so določene filogenetske skupine povezane s patogenostjo sevov. Podatkov o okoljskih sevih *E. coli* in njihovih lastnostih je v literaturi malo. Namen te raziskave je

tako bil opredeliti molekularne značilnosti izolatov *E. coli* z gumijastih tesnil pomivalnih strojev in oceniti njihov patogeni potencial. V tej raziskavi smo 35 izolatov bakterije *E. coli*, osamljenih iz osmih vzorčenih gumijastih tesnil kuhinjskih pomivalnih strojev, analizirali z verižno reakcijo s polimerazo (PCR) z uporabo specifičnih začetnih oligonukleotidov za njihovo filogenetsko skupino in sekvenčni tip na osnovi multilokusnega zaporedja (MLST), za prisotnost 43 genov, povezanih z virulenco (VAG) *E. coli* pri črevesnih in zunajčrevesnih okužbah (geni adhezinov *fimH*, *crl*, *eae*, *bmaE*, *gaf*, *aaf*, *papG*I, *papG*II, *papG*III, *matA*, *sfa*/*foc*, *iha*, *afa*/*dra*, in *hra*; avtotransporterjev *sat*, *vat*, *hbp* (*tsh*), *picU*, in *fluA* (*Ag43*); sistemov za privzem železa *fyuA*, *irp*, *sitA*, *iutA*, *iucD*, in *iroN*; protektinov *iss*, *ompTAPEC*, *kpsMT*II, *neuCS*, *cvi*; toksinov *stx1*, *stx2*, *astA*, *eltA*, *cnf2*, *hlyA*; invazinov *aslA*, *ibeA*, *gimB*, *tia,* in uropatogenega specifičnega proteina *usp*) ter za prisotnost nekaterih značilnih zaporedij plazmidov, tudi povezanih z virulenco *E. coli* (zaporedja plazmidnih replikacijih regij RepFIA, RepFIB, RepFIIA, RepFIC, IncP ter zaporedji, povezani s konjugacijo, *traJ* in *traT)*. Ugotovili smo, da je vseh 35 izolatov *E. coli* pripadalo komenzalni, nepatogeni filogenetski skupini A po Clermontu in sod. (2013) in da je bila raznolikost teh izolatov glede na analizo MLST relativno majhna. Sedemindvajset izolatov smo uvrstili v enega izmed štirih sekvenčnih tipov: ST189 (12 izolatov), ST216 (7 izolatov), ST399 (7 izolatov), in ST1316 (1 izolat). Tudi razširjenost VAG med preučevanimi izolati *E. coli* je bila nizka. Potrdili smo le naslednje VAG: *fimH*, *crl*, *fluA*, *picU*, *irp*, *fyuA*, *sitA*, *aslA*. Vsi izolati so imeli genski zapis *crl*, *fimH* pa smo našli pri vseh, razen pri enem izolatu. Pri 14 izolatih smo potrdili prisotnost *aslA*, pri štirih izolatih *picU*, pri treh izolatih *fluA* ter pri dveh izolatih *irp* in *fyuA* (Tab. 1, Sl. 1). Od petih testiranih plazmidnih replikacijskih regij sta bili odkriti samo RepFIA in RepFIIA. Zaporedji *traJ* in *traT*, povezani s konjugativnimi plazmidi, sta bili odkriti samo pri enem izolatu (Tab. 1, Sl. 1). Majhna raznolikost sekvenčnih skupin (ST) ter podatka, da so bili iz istega pomivalnega stroja izolirani sevi iste ST z nekoliko drugačim naborom genov za dejavnike virulence, ter prisotnost sevov iz enake ST iz strojev na različnih lokacijah, bi lahko bila posledica na specifično okolje prilagojenih klonalnih skupin. Kolikor vemo, je to prva študija molekularne opredelitve izolatov *E. coli* z gumijastih tesnil pomivalnih strojev v Sloveniji. Na podlagi dobljenih rezultatov lahko preučevane izolate označimo kot komenzalne *E. coli* z nizkim patogenim potencialom.

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