Pol. J. Food Nutr. Sci. 2006, Vol. 15/56, No 4, pp. 427–431

ANTIBIOTIC RESISTANCE OF BACTERIA OF THE FAMILY *ENTEROBACTERIACEA* ISOLATED FROM VEGETABLES – SHORT REPORT

Łucja Łaniewska-Trokenheim, Marcin Sobota, Iwona Warmińska-Radyko

Department of Industrial and Food Microbiology, Faculty of Food Science, University of Warmia and Mazury in Olsztyn

Key words: vegetables, *Enterobacteriaceae,* antibiotics, resistance

Antibiotic resistance of 114 strains of the *Enterobacteriaceae* family bacteria isolated from vegetables, originating from retail, was investigated in the study. The highest number of the strains isolated were resistant to ampicilin (81.9%), whereas a lower number of the strains exhibited resistance to the following antibiotics: neomycin (29.3%), streptomycin (28.4%), rifampicin (21.5%), chloramphenicol (19.8%), colistin (12.9%), and nitrofurantoin (11.%). All the isolated strains appeared to be susceptible to vancomycin, kanamycin, doxycyclin, nalidixic acid and gentamycin.

INTRODUCTION

The Gram-negative bacteria occurring in vegetables constitute a large and diversified group of bacteria represented, most often, by the following genera: *Citrobacter, Enterobacter, Erwinia, Escherichia, Hafnia,* and *Proteus.* Inside healthy plant tissues there are no bacteria but only single cells. In contrast, on the surface of vegetables there occurs saprophytic and pathogenic microflora originating from soil, water, sewage and air [Hamilton-Miller & Shah, 2001]. The number of cells per one gram of vegetables ranges from a few hundreds to a few millions. The most contaminated with microorganisms are root vegetables.

A systematic increase of the population of drug-resistant strains is being observed, which poses difficulties in the selection of antibiotics. Bacteria are likely to gain genes that determine some forms of resistance to antibiotics from other bacteria (sometimes even from bacteria of different genera). That process proceeds owing to the so-called "horizontal transfer of genes" occurring through conjugation, transformation and transduction.

Resistance to antibiotics is a natural phenomenon occurring in nature. Yet, rapid appearance of resistance is determined by a variety of factors, including: excessive or improper application of antibiotic therapies [Falkiner, 1998]. This evokes a great selective pressure on microorganisms and allows only the most resistant strains to survive. Microorganisms contaminating food are likely to be resistant to chemiotherapeutic agents and, consequently, they are disseminated with food. Next, transpozones may transfer genes of resistance to various antibiotics onto microorganisms colonizing the gastrointestinal tract of humans. Another problem is the application of antibiotics in animal breeding not only for therapeutic purposes but also for feeding the animals to accelerate their body weight gain [Falkiner, 1998]. Reports have also been provided on the use of antibiotics in plant cultivation, *e.g.* in pomicultre in the form of aerosoles, which enables introduction of antibiotic-resistant bacteria into consumer's organism [McManus *et al*., 2002]. It has also been proved that some types of antibiotics are contaminated with DNA fragments of bacteria that produce them [Markiewicz & Kwiatkowski, 2001]. Taking all these into account, the appearance of bacteria resistant to antibiotics seems and an inevitable process. Resistant bacteria could have occurred on vegetables due to a variety of reasons, *e.g.* the use of antibiotics during cultivation of those plants or the application of fertilizers or water contaminated with antibiotic-resistant bacteria for irrigation [Czapski & Radziejewska, 2001].

The study was aimed at determining antibiotic resistance of strains of Gram-negative rods of the family *Enterobacteriaceae* isolated from vegetables.

MATERIAL AND METHODS

The experimental material were vegetables originating from retail. Root vegetables: radish, parsley root, carrot; and greens: head lettuce, chives, iceberg lettuce, wild celery, cabbage and dill.

Gram-negative rods of the family *Enterobacteriaceae* were isolated on VRBL culture medium (Merck). Colonies were identified with the use of biochemical tests API (bioMérieux). The next stage of the study involved determination of the susceptibility of the isolated strains to antibiotics. To this end, use was made of the diffusion method on Müller-Hinton culture medium (Merck) with disks by bioMérieux.

The susceptibility of the strains were determined towards

Author's address for correspondence: Łucja Łaniewska-Trokenheim, Department of Industrial and Food Microbiology, University of Warmia and Mazury, Pl. Cieszyński 1, 10-957 Olsztyn, Poland; e-mail: lucja.laniewska-trokenheim@uwm.edu.pl

the following antibiotics: ampicilin (AM), rifampicin (RA), nitrofurantoin (F/M), vancomycin (VA), kanamycin (K), gentamycin (GM), neomycin (N), streptomycin (S), nalidyxoic acid (NA), colistin (CL), doxycyclin (D), and chloramphenicol (C).

TABLE 1. Occurrence of Gram-negative bacteria in vegetables.

Origin	Identified Gram-negative bacteria	Number of isolated strains
Radish	*Citrobacter freundii*	6
	Enterobacter aerogenes	3
	Erwinia caratovora	2
	Hafnia sp.	9
	Proteus vulgaris	4
Parsley root	*Citrobacter freundii*	4
	Enterobacter aerogenes	6
	Escherichia coli	1
	Hafnia sp.	2
Lettuce	*Citrobacter freundii*	6
	Enterobacter aerogenes	13
	Escherichia coli	1
	Hafnia sp.	2
Chives	*Citrobacter freundii*	2
	Enterobacter aerogenes	5
	Erwinia caratovora	3
	Hafnia sp.	4
	Serratia marcescenes	1
Carrot	*Citrobacter freundii*	1
	Enterobacter aerogenes	2
	Hafnia sp.	1
	Klebsiella sp.	3
Iceberg lettuce	*Enterobacter aerogenes*	12
	Escherichia coli	2
	Hafnia sp.	2
Wild celery	*Escherichia coli*	1
	Hafnia sp.	1
	Proteus vulgaris	5
	Providencia sp.	2
Dill	*Enterobacter aerogenes*	1
	Hafnia sp.	1
Cabbage	*Hafnia* sp.	6

Diameter of growth inhibition zones of the strains examined was measured (mm). The division into resistant, medium-susceptible and susceptible strains was carried out based on the occurrence of size of the zones.

RESULTS AND DISCUSSION

The experiment resulted in the isolation of 114 strains of Gram-negative bacteria of the family *Enterobacteriaceae.* After the identification, the strains classified to the following genera: *Citrobacter freundii* – 21 strains, *Enterobacter aerogenes* – 41 strains, *Erwinia carotovora* – 5 strains, *Escherichia coli* – 5 strains, *Hafnia* sp. – 30 strains, *Klebsiella* sp. – 1 strain, *Proteus vulgaris* – 9 strains, *Providencia* sp. – 2 strains, and *Serratia marcescens* – 1 strain (Table 1).

The following genera of bacteria were isolated from vegetables with the highest frequency: *Enterobacter aerogenes* – 41 strains and *Hafnia* sp. – 30 strains as well as *Citrobacter freundii* – 21 strains. Due to sporadic occurrence on vegetables, lower numbers of genera were determined for: *Proteus vulgaris* – 9 strains, *Erwinia* sp. – 5 strains, *Escherichia coli* – 5 strains, *Providencia* sp. – 2 strains, as well as *Klebsiella* sp. and *Serratia marcescens* – 1 strain each (Table 1).

In the case of radish samples, 24 strains were isolated and classified to the following genera: *Hafnia* sp. – 9 strains, *Citrobacter freundii* – 6 strains, *Proteus vulgaris* – 4 strains, *Enterobacter aerogenes* – 3 strains, and *Erwinia carotovora* – 2 strains. From head lettuce samples, 22 strains were isolated and classified to the following genera: *Enterobacter aerogenes* – 3 strains, *Citrobacter freundii* – 6 strains, *Hafnia* sp. – 2 strains, and *Escherichia coli* – 1 strain. In the case of iceberg lettuce, 16 strains were isolated that were predominated by *Enterobacter aerogenes* – 12 strains. Next, 15 strains were isolated from chives, 13 strains form parsley root, 9 strains from wild celery, 8 strains from carrot, 6 strains from cabbage, and 2 strains from dill, including one strain of *Enterobacter aerogenes* and another one of *Hafnia* sp.

The frequency of occurrence of the antibiotic-resistant strains in vegetables was presented in Table 2. The susceptibility of the isolated strains to nitrofurantoin, rifampicin and chloramphenicol was presented in Table 3a. Most of the strains were resistant to 8 out of 12 antibiotics applied in the study. The highest number of strains demonstrated resistance to ampicilin: *Hafnia* sp. – 30 strains, *Citrobacter freundii* – 21

TABLE 2. Occurrence of strains resistant to antibiotics used in the study.

Species	Total number of strains	Antibiotics used in the study											
		FM	RA	C	VA	K	D	AM	NA	CL	N	GM	S
		Number of strains											
Citrobacter freundii	21	12	21	0	0	0	0	21	0	10	9	0	9
Enterobacter aerogenes	41	0	0	22	0	0	0	19	0	0	0	0	0
Erwinia carotovora	5	0	3	0	0	0	0	5	0	3	0	0	0
Escherichia coli	5	0	0	0	0	0	0	5	0	0	3	0	4
Hafnia sp.	30	0	0	0	0	0	0	30	0	0	12	0	12
Klebsiella sp.	1	0	0	0	0	0	0	1	0	0	1	0	1
Proteus vulgaris	9	0	0	0	0	0	0	9	0	0	6	0	6
Providencia sp.	2	0	0	0	0	0	0	2	0	0	2	0	1
Serratia marcescens	1	1	0	0	0	0	0	1	0	0	0	0	0

TABLE 3a. Antibiotic characteristics of the strains isolated.

Strains	Antibiotics used in the study								
	Nitrofurantoin			Rifampicin			Chloramphenicol		
	No. of resistant strains	No. of medium-susceptible strains	No. of susceptible strains	No. of resistant strains	No. of medium-susceptible strains	No. of susceptible strains	No. of resistant strains	No. of medium-susceptible strains	No. of susceptible strains
Citrobacter freundii	12	9	0	21	0	0	0	11	10
Enterobacter aerogenes	0	41	0	0	0	41	22	19	0
Erwinia carotovora	0	0	5	3	2	0	0	2	3
Escherichia coli	0	2	3	0	5	0	0	5	0
Hafnia sp.	0	15	15	0	30	0	0	15	15
Klebsiella sp.	0	0	1	0	1	0	0	1	0
Proteus vulgaris	0	3	6	0	9	0	0	5	4
Providencia sp.	0	1	1	0	2	0	0	1	1
Serratia marcescens	1	0	0	0	0	1	0	0	1

TABLE 3b. Antibiotic characteristics of the strains isolated.

Strains	Antibiotics used in the study								
	Vancomycin			Kanamycin			Doxycyclin		
	No. of resistant strains	No. of medium--susceptible strains	No. of susceptible strains	No. of resistant strains	No. of medium-susceptible strains	No. of susceptible strains	No. of resistant strains	No. of medium-susceptible strains	No. of susceptible strains
Citrobacter freundii	0	13	8	0	21	0	0	21	0
Enterobacter aerogenes	0	0	41	0	41	0	0	41	0
Erwinia carotovora	0	1	4	0	0	5	0	5	0
Escherichia coli	0	3	2	0	0	5	0	5	0
Hafnia sp.	0	15	15	0	17	13	0	30	0
Klebsiella sp.	0	1	0	0	0	1	0	1	0
Proteus vulgaris	0	5	4	0	3	6	0	9	0
Providencia sp.	0	1	1	0	2	0	0	2	0
Serratia marcescens	0	1	0	0	0	1	0	0	1

TABLE 3c. Antibiotic characteristics of the strains isolated.

Strains	Antibiotics used in the study								
	Ampicilin			Nalidyxoic acid			Colistin		
	No. of resistant strains	No. of medium-susceptible strains	No. of susceptible strains	No. of resistant strains	No. of medium-susceptible strains	No. of susceptible strains	No. of resistant strains	No. of medium-susceptible strains	No. of susceptible strains
Citrobacter freundii	21	0	0	0	21	0	10	11	0
Enterobacter aerogenes	19	22	0	0	0	41	0	41	0
Erwinia carotovora	5	0	0	0	2	3	3	2	0
Escherichia coli	5	0	0	0	0	5	0	2	3
Hafnia sp.	30	0	0	0	14	16	0	15	15
Klebsiella sp.	1	0	0	0	0	1	0	1	0
Proteus vulgaris	9	0	0	0	5	2	0	2	7
Providencia sp.	2	0	0	0	0	2	0	1	1
Serratia marcescens	1	0	0	0	1	0	0	1	0

TABLE 3d. Anibiotic characteristics of the strains isolated.

Strains	Antibiotics used in the study								
	Neomycyn			Gentamycin			Streptomycin		
	No. of resistant strains	No. of medium-susceptible strains	No. of susceptible strains	No. of resistant strains	No. of medium-susceptible strains	No. of susceptible strains	No. of resistant strains	No. of medium-susceptible strains	No. of susceptible strains
Citrobacter freundii	9	12	0	0	21	0	9	12	0
Enterobacter aerogenes	0	41	0	0	41	0	0	12	29
Erwinia carotovora	0	3	2	0	2	3	0	5	0
Escherichia coli	3	2	0	0	3	2	4	1	0
Hafnia sp.	12	18	0	0	13	17	12	18	0
Klebsiella sp.	1	0	0	0	1	0	1	0	0
Proteus vulgaris	6	3	0	0	5	4	6	3	0
Providencia sp.	2	0	0	0	2	0	1	1	0
Serratia marcescens	0	1	0	0	0	1	0	0	1

strains, *Enterobacter aerogenes* – 19 strains (Table 3c). A considerable number of strains appeared to be resistant to neomycin: *Hafnia* sp. – 12 strains, *Citrobacter freundii* – 9 strains, *Proteus vulgaris* – 6 strains (Table 3d), as well as to streptomycin: *Hafnia* sp. – 12 strains, *Citrobacter freundii* – 9 strains, *Proteus vulgaris* – 6 strains, *Escherichia coli* – 4 strains, *Klebsiella* sp. and *Providencia* sp. – 1 strain each (Table 3d). This has been confirmed by previous investigations into antibiotic resistance of Gram-negative bacteria isolated from food [Łaniewska-Moroz *et al.,* 1998].

The Gram-negative bacteria appeared not to be resistant to the following antibiotics: vancomycin, kanamycin, nalidyxoic acid and gentamycin (Table 3b,c,d) [Österblad *et al.,* 1999; Levy, 1984].

Since the last reports, the antibiotic resistance of bacteria of the family *Enterobacteriaceae* isolated from vegetables has been observed to change [Hamilton-Miller & Shah, 2001]. Amongst the isolated strains, there occurred strains resistant to doxycyclin, colistin, neomycyn and streptomycin (Table 3b,c,d). The highest numbers of bacteria were resistant to ampicilin, which points to an increasing occurrence of antibiotic-resistant bacteria among strains contaminating fresh vegetables (Table 3c) [Levy, 1992].

Bacteria of the family *Enterobacteriaceae* display a variety of mechanisms antibiotic resistance, *e.g.* enzymes inactivating antibiotics, mechanisms of antibiotics removal from bacterial cells or mechanisms of reduced permeability of cell walls for antibiotics. All those mechanisms determine resistance to individual chemiotherapeutic agents [Dzierżanowska *et al.*, 2004]. The percentage of Gram-negative bacteria resistant to particular chemiotherapeutic agents is observed to increase [Markiewicz & Kwiatkowski, 2001]. Another group of chemiotherapeutic agents that the Gram-negative bacteria are resistant to are glycylocicins – derivatives of tetracyclins [Kapperud *et al.*, 1995]. Vegetables available in retail and contaminated with antibiotic-resistant bacteria may readily penetrate into the gastrointestinal tract of a consumer [Österblad *et al.,* 1999, Taorimina, *et al.,* 1999]. Therefore, fresh, not cooked vegetables can be a potential source of bacteria resistant to antibiotics.

CONCLUSIONS

1. The experiment resulted in the isolation of strains of Gram-negative bacteria belonging to the family *Enterobacteriaceae* that were classified to the following genera: *Citrobacter freundii*, *Enterobacter aerogenes*, *Erwinia carotovora*, *Escherichia coli*, *Hafnia* sp., *Klebsiella* sp., *Proteus vulgaris*, *Providencia* sp., and *Serratia marcescens*.

2. The strains isolated were resistant to 8 out of 12 antibiotics used in the study. The highest number of the strains demonstrated resistance to ampicilin, including: *Hafnia* sp., *Citrobacter freundii*, *Enterobacter aerogenes*.

3. All the strains isolated appeared to be susceptible to: vancomycin, kanamycin, nalidyxoic acid and gentamycin.

REFERENCES

1. Czapski J., Radziejewska E., Methods of extending the shelf life of low-processed vegetables and fruit. Przem. Spoż., 2001, 1, 16–19 (in Polish).
2. Dzierżanowska D., Pawińska A., Kamińska W., Drug-resistant microorganisms in hospital infections. Post. Mikrobiol., 2004, 43, 81–105 (in Polish).
3. Falkiner F.R., The consequences of antibiotic use in horticulture. J. Antimicrob. Chemother., 1998, 41, 429–431.
4. Hamilton-Miller J.M., Shah S., Identity and antibiotic susceptibility of enterobacterial flora of salad vegetables. Int. J Antimicrob Agents., 2001, 18, 81–93.
5. Kapperud G., Rorvik L.M., Hasseltvedt V., Hoiby E.A., Iversen B.G., Staveland K., Johnsen G., Leitao J., Herikstad H., Andersson Y., Outbreak of *Shigella sonnei* infection traced to imported iceberg lettuce. J. Clin. Microbiol., 1995, 33, 609–614.
6. Levy S.B., Antibiotic-resistant bacteria in food of man and animals. 1984, *in*: In Antimicrobals and Agriculture (ed. M. Woodbine). Butterworth, London, pp. 525–531.
7. Levy S.B., The Antibiotic Paradox: How Miracle Drugs are Destroying the Miracle. 1992, Plenum Press, New York, pp. 53–67.
8. Łaniewska-Moroz Ł., Nalepa B., Kornacki K., Resistance of *Enterobacteriacea* family bacteria isolated from food

to chemiotherapeutic agents. Medycyna Wet., 1998, 54, 330–333 (in Polish).
9. Markiewicz Z., Kwiatkowski Z.A., Bakterie, antybiotyki, lekooporność. 2001, Wydawnictwo PWN, Warszawa, pp. 97–191 (in Polish).
10. McManus P.S., Stockwell V.O., Sudin G.W., Jones, A.L., Antibiotic use in plant agriculture. Annu. Rev. Phytopathol. E. Pub., 2002, 40, 443–465.
11. Österblad M., Pensala O., Peterzéns M., Heleniusc H., Houvinen P., Antimicrobal susceptibility of *Enterobacteriacea* isolated from vegetables. J. Antimicrob. Chemother., 1999, 43, 503–509.
12. Taorimina P.J., Beuchat L.R., Slutsker L., Infections associated with eating seed sprouts: an international concern. Emerg. Infect. Dis., 1999, 5, 626–634.

Received June 2006. Revision received September and accepted October 2006.

OPORNOŚĆ NA ANTYBIOTYKI PAŁECZEK Z RODZINY *ENTEROBACTERIACEA* WYIZOLOWANYCH Z WARZYW – KRÓTKI KOMUNIKAT

Łucja Łaniewska-Trokenheim, Marcin Sobota, Iwona Warmińska-Radyko

Katedra Mikrobiologii Przemysłowej i Żywności, Uniwersytet Warmińsko-Mazurski w Olsztynie, Olsztyn

Określono oporność na antybiotyki 114 szczepów pałeczek z rodziny *Enterobacteriaceae* wyizolowanych z warzyw, pochodzących z handlu detalicznego. Wśród wyizolowanych szczepów najwięcej było opornych na ampicylinę 81,9%, natomiast mniej szczepów wykazywało oporność na następujące antybiotyki: neomycynę 29,3%, streptomycynę 28,4%, rifampicynę 21,5%, chloramfenikol 19,8%, kolistynę 12,9%, nitrofurantoinę w 11,2%. Wszystkie wyizolowne szczepy były wrażliwe na wankomycynę, kanamycynę, doksycyklinę, kwas nalidyksowy oraz gentamycynę (tab. 3).