Comparative Immunology, Microbiology and Infectious Diseases 63 (2019) 131–135

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Phenotypic and genotypic presence of the *Yersinia* virulence plasmid do not affect the production of enterotoxin YstA by *Yersinia enterocolitica* strains

Agata Bancerz-Kisiel*, Magdalena Gral[1], Marta Odyniec, Wojciech Szweda

Department of Epizootiology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Oczapowskiego 2, 10-719, Olsztyn, Poland

ARTICLE INFO

Keywords:
Yersinia enterocolitica
Yersinia virulence plasmid
Enterotoxin YstA
Phenotypic and genotypic features

ABSTRACT

The aim of the study was to determine whether the presence of the *Yersinia* virulence plasmid could affect the production of enterotoxin YstA by *Y. enterocolitica* strains isolated from pigs which are the main source of infection for humans. The phenotypic features characteristic for the *Yersinia* virulence plasmid were detected on CRMOX agar in 8 out of 12 strains producing enterotoxin YstA, in 5 out of 12 doubtful strains, and in 11 out of 12 strains not producing YstA. Autoagglutination ability was detected in all 12 *Y. enterocolitica* strains that were positive in the suckling mice bioassay, in 11 doubtful strains and 10 negative strains. CRMOX+ colonies were generally *ystA*, *myfA, virF* and *yadA* positive, while CRMOX- colonies were only *ystA* and *myfA* positive. The amplicons of *yadA* were not detected in 2 (8.3%) out of 24 CRMOX+ and *virF* positive strains. The results of this study indicate that the presence of pYV does not affect the enterotoxin-producing ability of *Y. enterocolitica* strains.

1. Introduction

The genus *Yersinia* of the family *Enterobacteriaceae* includes three species that are pathogenic for humans and animals: *Yersinia (Y.) pestis, Y. pseudotuberculosis* and *Y. enterocolitica*. The latter is the main cause of yersiniosis around the world [1,2]. All of the above pathogenic species harbor a highly conserved pYV plasmid of *Yersinia* virulence which is responsible for the strains' pathogenicity [3]. *Y. enterocolitica* strains are generally heterogeneous in terms of both genetic and plasmid virulence markers, but bioserotypes pathogenic for humans are highly homogenous [4]. Out of the six known biotypes, *Y. enterocolitica* strains belonging to biotype 1B are considered highly pathogenic for humans and animals, whereas strains belonging to biotypes 2–5 as weakly pathogenic [5]. Biotype 1 A strains lack the classical virulence markers and are regarded as nonpathogenic. In Europe, pigs are the main reservoir of *Y. enterocolitica* strains dangerous for humans, but pathogenic strains have also been found in other animal species [6,7].

The pYV plasmid with a size of 64–75 kb encodes a variety of proteins whose production and secretion depends on calcium ion concentration [8]. These include: YadA (*Yersinia* adhesin), Yops (*Yersinia* outer membrane proteins), Sysc (specific Yop chaperons), Yscs (*Yersinia* secretion complex), and expression regulator virF (transcriptional activator of the Yersinia virulence regulon) [4,5,8]. pYV-mediated phenotypic characteristics are usually detected in autoagglutination, calcium dependency and Congo red absorption tests [9]. However, the pathogenicity of the examined strain should not be determined based on the results of the above tests alone because pYV is easily lost from bacterial cells during their growth in the laboratory [10]. Potentially pathogenic *Y. enterocolitica* strains which have lost the plasmid can be identified in pyrazinamidase and esculin tests where they produce negative results [9].

The detection of genetically-stable chromosomal virulence markers is a more accurate method of determining a strain's pathogenicity. The most important virulence markers are *ail, inv, myfA* and *yst* genes that encode protein Ail (attachment-invasion locus), invasin Inv, protein MyfA (mucoid *Yersinia* factor/fibrillae A) and enterotoxin Yst (*Yersinia*-stable toxin), respectively [4,5]. *Y. enterocolitica* strains isolated from clinical cases of yersiniosis produce enterotoxin YstA with a low molecular weight, which is active in a suckling mouse bioassay and considered as the main factor in diarrhea caused by pathogenic bioserotypes [11,12]. Interestingly, not all strains possessing the *ystA* gene produce YstA enterotoxin. According to some authors, YstA production can be restored in a silent state [13,14]. MyfA, an adhesin that is present only in pathogenic bioserotypes, is suspected to be the main factor that facilitates contact between the epithelium and enterotoxin and the induction of diarrhea [5].

The presence of a correlation between diarrhea induction by enterotoxin YstA encoded on the chromosome and pYV-encoded fimbrial

* Corresponding author.
E-mail address: a.bancerz-kisiel@uwm.edu.pl (A. Bancerz-Kisiel).
[1] Current address: International Institute of Molecular and Cell Biology in Warsaw, Ks. Trojdena 4, 02-109 Warsaw, Poland.

https://doi.org/10.1016/j.cimid.2019.01.020
Received 21 March 2018; Received in revised form 21 January 2019; Accepted 23 January 2019

Table 1
Bioserotypes of the *Y. enterocolitica* strains used in the study.

Enterotoxic properties	Number of strains	Bioserotypes		
		2/O:9	4/O:3	4/[a]
Positive	12	1	9	2
Doubtful	12	3	7	2
Negative	12	1	10	1

[a] NI – nonidentified.

outer-membrane adhesin YadA was first postulated by Delor and Cornelis [15]. They suggested that *Y. enterocolitica* requires a YadA colonization factor for the onset of diarrhea. A correlation between YadA and other chromosomally encoded proteins was also observed. Schmid et al. [16] demonstrated that YadA mediates internalization into host cells when invasin Inv is repressed. Białas et al. [17] observed that protein Ail cooperates with YadA to ensure a high level of serum resistance.

The aim of this study was to determine whether the presence of pYV can affect the production of enterotoxin YstA by *Y. enterocolitica* strains isolated from pigs.

2. Materials and methods

2.1. Bacterial strains

The materials for the study consisted of 36 *Y. enterocolitica* strains divided into three groups based on their enterotoxic properties. Each group consisted of 12 strains that were positive, doubtful or negative in the production of YstA. All strains were isolated from clinically healthy pigs, biotyped and serotyped (Table 1) [18]. Their enterotoxic properties were determined in the suckling mice bioassay described by Giannella with certain modifications [18]. All strains were characterized in terms of the most important virulence markers [18]. Interestingly, all strains were *ystA* positive, whereas only one group of strains displayed enterotoxic properties [18]. The strains were also analyzed for the presence of single nucleotide polymorphisms (SNP) in the *ystA* gene with the use of the High Resolution Melting (HRM) method to detect possible mutations in the *ystA* gene and to evaluate their influence on enterotoxic properties [19]. Due to the negative results of HRM analysis the search for factors responsible for the production of YstA in selected *ystA*-positive strains focused on the detection of pYV and the encoded YadA.

2.2. Phenotypic detection of pYV

The phenotypic features characteristic of plasmid-positive strains, including calcium dependence and Congo red binding, were determined on CRMOX agar (Congo Red – Magnesium Oxalate) according to the procedure described by Riley and Toma [9]. CRMOX+ *Y. enterocolitica* strains were detected based on the presence of small red colonies after 48 h at 37 °C, and CRMOX- *Y. enterocolitica* strains – based on the presence of larger colorless colonies. All CRMOX- colonies were analyzed for pyrazinamidase production and esculin fermentation in accordance with the PN-EN ISO 10273 standard. Autoagglutination was evaluated in Tryptone Soya Broth (TSB). The suspension was swirled after 18 h of culturing at 22 °C, and 0.1 ml of the suspension was sampled twice and transferred to two new 2 ml test tubes. The tubes were incubated for 18 h, one at 22 °C and the other at 37 °C. Strains that grew in irregular layers at the bottom of the test tube and left a clear medium at 37 °C, but produced a cloudy medium at 22 °C were regarded as autoagglutination-positive (AA+) strains. Autoagglutination-negative (AA-) strains were responsible for the cloudy appearance of both media cultured at different temperatures.

2.3. Genetic confirmation of pYV detection

To confirm phenotyping results, multiplex PCR was designed to amplify four genes, plasmid virulence markers *yadA* and *virF*, and chromosomal virulence markers *ystA* and *myfA*. *Y. enterocolitica* colonies from CRMOX were used for the isolation of genomic DNA. In two types of colonies (CRMOX+ and CRMOX- on one plate), DNA was isolated from both colonies and was analyzed separately. Genomic DNA was isolated with the Genomic Mini kit (A&A Biotechnology, Gdynia, Poland) according to the manufacturer's instructions. Multiplex PCR was carried out using HotStarTaq Plus DNA polymerase (Qiagen GmbH, Hilden, Germany) and the HotStarTaq Plus Master Mix Kit (Qiagen). The reaction mixture of 20 µl contained around 120 ng of isolated DNA (3 µl), 10 µl of the HotStarTaq Plus Master Mix 2x, 2 µl of CoralLoad Concentrate 10x, and 0.1 µl of each primer (with a final concentration of 0.5 µM), supplemented with up to 20 µl of RNase-free water. Primer sequences (synthesized by Genomed, Warsaw, Poland) were published previously (Table 2). Two controls were applied to each reaction: a positive control with DNA isolated from O:8 reference strains (ATCC 23715), and a negative control without DNA. The reaction was carried out in the following steps: preliminary denaturation at 95 °C for 5 min, followed by 30 cycles with: denaturation at 94 °C for 30 s, primer annealing at 60 °C for 30 s, and elongation at 72 °C for 1 min. After the last reaction, the final chain synthesis took place at 72 °C for 10 min. The size of the products was evaluated by comparison with the standard mass of GeneRuler 100-bp Ladder Plus (Fermentas UAB, Vilnius, Lithuania). The following reaction products were searched: fragments of the *ystA* gene (size of 134 bp), *myfA* gene (size of 272 bp), *virF* gene (size of 561 bp), and the *yadA* gene (size of 849 bp). Electrophoresis results were archived in the GelDoc imaging system (Bio-Rad Laboratories, Milan, Italy).

2.4. Sequencing

The obtained amplicons were purified and sequenced to confirm their specificity. The amplicons were purified with the Clean-up purification kit (A&A Biotechnology, Gdynia, Poland) according to the manufacturer's recommendations. Each purified amplicon was independently sequenced (Genomed S.A., Warszawa, Poland), and sequence data were compared with the nucleotide sequence of the previously identified *ystA*, *myfA*, *virF* and *yadA* genes in Y. enterocolitica using BLASTN ver. 2.2.18. [20].

3. Results

An analysis of calcium dependence and Congo red binding in the group of 12 *Y. enterocolitica* strains that were positive in the suckling

Table 2
Characteristics of the primers used in the study.

Gene	Primer sequences	Product size	Reference
ystA	5'GTCTTCATTTGGAGGATTCGGC3' 5'AATCACTACTGACTTCGGCTGG3'	134 bp	Platt-Samoraj et al. [28]
myfA	5'CAGATACACCTGCCTTCCATCT3' 5'CTCGACATATTCCTCAACACGC3'	272 bp	Gierczyński et al. [29]
virF	5'GGCAGAACAGCAGTCAGACATA3' 5'GGTGAGCATAGAGAATACGTCG3'	561 bp	Thoerner et al. [26]
yadA	5'CTTCAGATACTGGTGTCGCTGT3' 5'ATGCCTGACTAGAGCGATATCC3'	849 bp	Thoerner et al. [26]

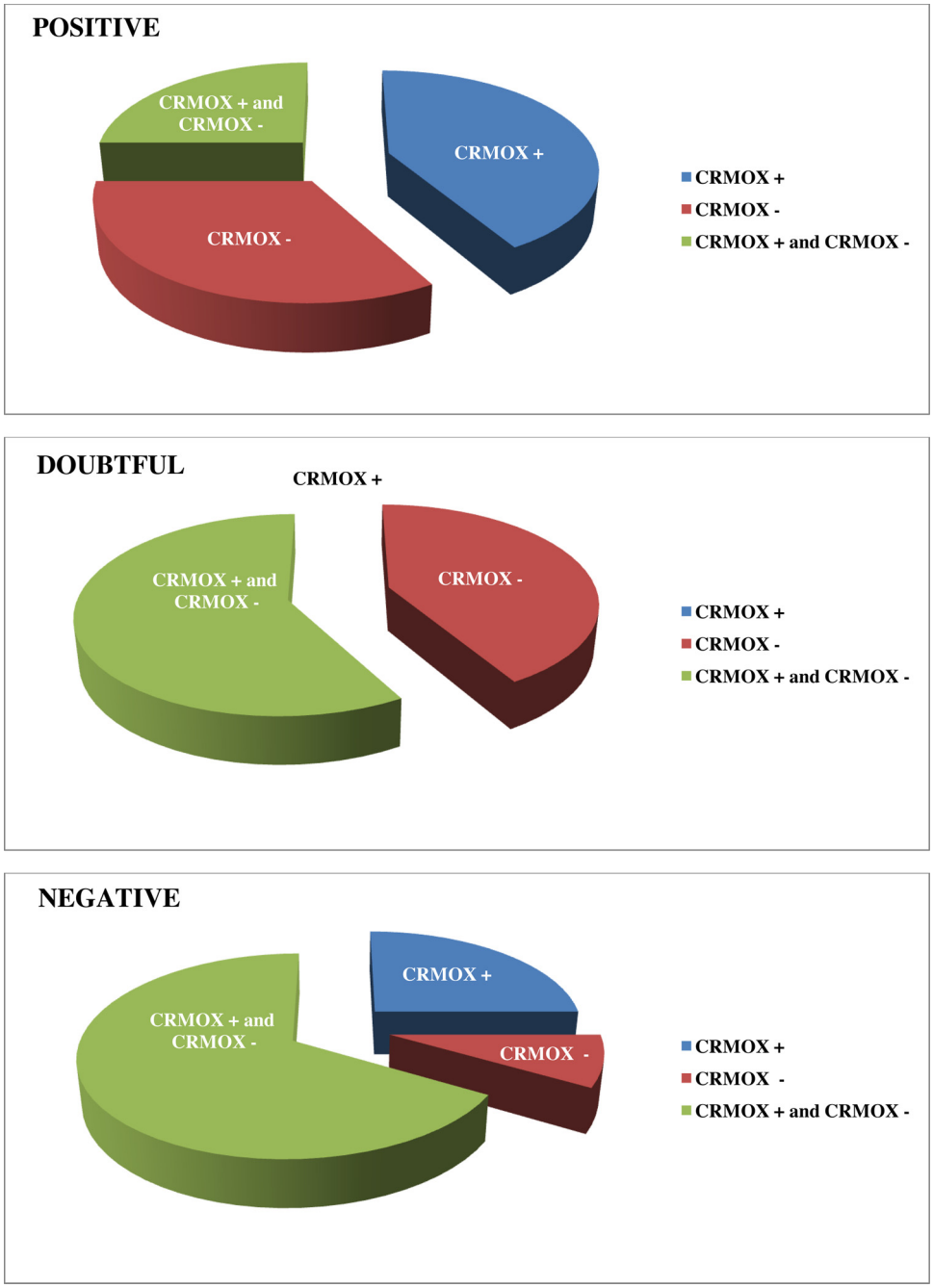

Fig. 1. The results of calcium dependence and Congo red binding detection tests on CRMOX agar in *Y. enterocolitica* strains belonging to three different groups. The CRMOX colonies detected in each group of *Y. enterocolitica* strains were grouped based on their enterotoxic properties.

mice bioassay revealed that 8 strains (66.7%) possessed phenotypic features characteristic of the virulence plasmid. Three of those strains were characterized by the presence of two types of colonies: CRMOX + and CRMOX-, whereas the 5 remaining strains harbored only CRMOX + colonies (Fig. 1). All CRMOX- colonies tested negative for pyrazinamidase production and esculin fermentation. The auto-agglutination test revealed that in all 12 strains, autoagglutination was observed only at 22 °C A molecular examination of *Y. enterocolitica* strains that were positive in the suckling mice bioassay confirmed the results obtained on CRMOX agar. All CRMOX + colonies were characterized by the presence of amplicons of four gene fragments: *ystA*, *myfA, virF* and *yadA,* whereas CRMOX- colonies were only *ystA* and *myfA* positive.

In the group of *Y. enterocolitica* strains classified as doubtful in the suckling mice bioassay, calcium dependence and Congo red binding were detected in only 5 out of 12 (41.7%) examined strains. All 5 strains were characterized by the presence of two types of colonies: CRMOX + and CRMOX- (Fig. 1). Similarly to the strains that were positive in the suckling mice bioassay, CRMOX- colonies were negative for pyrazinamidase production and esculin fermentation. Auto-agglutination ability was observed in 11 out of 12 *Y. enterocolitica* strains. The majority of CRMOX + colonies were characterized by the presence of four amplicons: *ystA*, *myfA, virF* and *yadA*. In one case, DNA isolated from a CRMOX + colony was *yadA* negative and only *ystA*, *myfA* and *virF* amplicons were detected. CRMOX- colonies were *virF* and *yadA* negative.

An analysis of *Y. enterocolitica* strains that were negative in the suckling mice bioassay revealed that as many as 11 (91.7%) strains

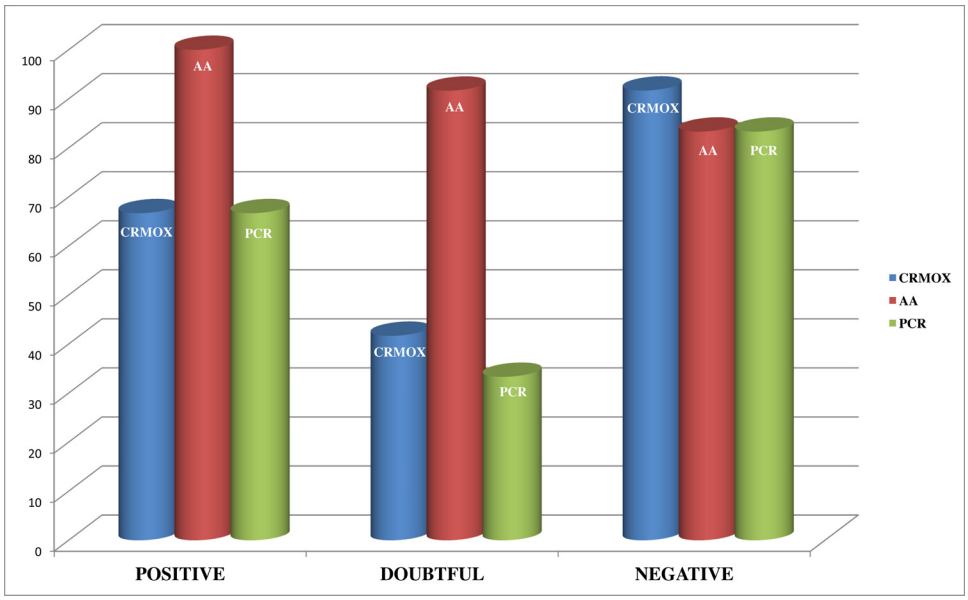

Fig. 2. The combined results of the analyses of phenotypic and genotypic features characteristic of plasmid-bearing *Y. enterocolitica* strains belonging to three different groups.
The percentage of positive plasmid detections in the CRMOX test, the autoagglutination test and molecular analyses was presented for each group of *Y. enterocolitica* strains based on their enterotoxic properties.

were calcium dependent and bound Congo red. Eight of those strains harbored both CRMOX + and CRMOX- colonies (Fig. 1). Similarly to the previous groups of strains, CRMOX- colonies were pyrazinamidase and esculin negative. Autoagglutination ability was observed in 10 out of 12 examined *Y. enterocolitica* strains. A molecular examination of *Y. enterocolitica* strains that were negative in the suckling mice bioassay generally confirmed the results obtained on CRMOX agar. Similarly to the group of doubtful strains, the DNA isolated from a CRMOX + colony was *yadA* negative in one case. The remaining CRMOX + colonies were characterized by the presence of amplicons of four gene fragments: *ystA*, *myfA, virF* and *yadA,* whereas CRMOX- colonies were only *ystA* and *myfA* positive.

The combined results of an analysis of the phenotypic and genotypic features of plasmid-bearing *Y. enterocolitica* strains with different enterotoxic properties are presented in Fig. 2.

4. Discussion

Y. enterocolitica is one of the most important pathogens that cause diarrhea in humans and animals [21]. However, not all mechanisms of yersiniosis pathogenesis have been elucidated. Bacteriological, biochemical and serological examinations support the detection and general characterization of *Y. enterocolitica* isolates, but molecular methods are required to investigate pathogenesis at the cellular level. Enterotoxin production is one of the key mechanisms of action in *Y. enterocolitica* strains [11,12,22]. This study aimed to determine whether the presence of pYV can affect the production of enterotoxin YstA by *Y. enterocolitica* strains.

Plasmid-containing pathogenic *Y. enterocolitica* strains are usually detected with the use of CRMOX agar [4,9,23]. This test has an advantage over other virulence tests in that each colony on CRMOX is tested for both calcium dependency and Congo red absorption. Additionally, strains which have lost the plasmid (CRMOX-) can be defined as potentially pathogenic if they are pyrazinamidase and esculin negative [9]. The method combining CRMOX, pyrazinamidase and esculin tests supports accurate differentiation between pathogenic and nonpathogenic *Y. enterocolitica* strains. Therefore, all strains used in this study should be defined as pathogenic because all CRMOX- colonies were pyrazinamidase and esculin negative. No correlations were found between CRMOX results and the bioserotype of the examined strains or between autoagglutination ability and bioserotype.

The presence of YadA protein can be confirmed indirectly in the autoagglutination test because only plasmid-containing *Y. enterocolitica* strains are capable of forming cell aggregates [24]. In a study by Zheng et al. [25], the results of the autoagglutination test were consistent in 92% with the molecular presence of *yadA* in the tested strains of *Y. enterocolitica.* A similar level of convergence was not observed in our study. The percentage of AA + strains was determined at 100%, 91.7% and 83.3% in positive, doubtful and negative strains, respectively. In our study, results of molecular analyses were rather correlated with calcium dependency and Congo red absorption tests on CRMOX agar.

Chromosomal virulence markers *ystA* and *myfA* were observed in all tested strains. This is a particularly important observation because it was suspected that MyfA protein facilitated contact between the epithelium and enterotoxin, and the induction of diarrhea [5]. Our study did not confirm this suggestion, and *myfA* was detected regardless of the enterotoxic properties of the examined strains. The presence of plasmid virulence markers *virF* and *yadA* was generally correlated with pYV-mediated phenotypic characteristics. CRMOX + colonies were mostly *ystA*, *myfA, virF* and *yadA* positive, whereas CRMOX- colonies were only *ystA* and *myfA* positive. The above correlation had been previously observed by Kot et al. [23] who demonstrated that 100% of CRMOX + colonies were characterized by the presence of plasmid virulence markers *virF* and *yadA.* In our study, *yadA* was not detected in 2 (8.3%) out of 24 CRMOX + and *virF*-positive strains. Similar results were obtained by Thoerner et al. [26] who found only *virF* strains in 4 (2.9%) out of 140 MOX + strains, but did not detect *yadA.* According to the above authors, phenotypic features are more subjective and less reliable than genotypic characteristics. This suggestion was confirmed by other authors who observed a reverse correlation – when plasmids are lost, beneficial genes are integrated into the bacterial chromosome [27].

5. Conclusions

The results of this study indicate that the presence of pYV does not affect the ability of *Y. enterocolitica* strains to produce enterotoxin YstA. The presence of phenotypic features characteristic of the *Yersinia* virulence plasmid, confirmed by the genotypic characteristics of plasmid virulence markers, was not correlated with the enterotoxic properties of the examined *Y. enterocolitica* strains.

Funding

This publication was supported by KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal - Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.

Conflict of interest

The authors declare no conflict of interest.

References

[1] R. Pirie, J. Williman, C. Nicol, K. Sexton, Review of Yersiniosis Notifications in New Zealand 2002–2006, (2008) http://www.foodsafety.govt.nz/elibrary/industry/review-notificationszealand-yersiniosis/index.htm.

[2] I. Stamm, M. Hailer, B. Depner, P.A. Kopp, J. Rau, *Yersinia enterocolitica* in diagnostic fecal samples from European dogs and cats: identification by fourier transform infrared spectroscopy and matrix-assisted laser desorption ionization-time of flight mass spectrometry, J. Clin. Microbiol. 51 (2013) 887–893 https://www.ncbi.nlm.nih.gov/pubmed/23284028.

[3] S. Bhaduri, C.H. Sommers, Detection of *Yersinia pestis* by comparison of virulence plasmid (pYV/PCD)-associated phenotypes in *Yersinia* species, J. Food Saf. 28 (2008) 453–466, https://doi.org/10.1111/j.1745-4565.2008.00123.x.

[4] R. Gierczyński, Phylogeny, pathogenicity and genetic diversity of *Yersinia enterocolitica*, Med. Dosw. Mikrobiol. 64 (2012) 159–181 https://www.researchgate.net/publication/232276507_Phylogeny_patogenicity_and_genetic_diversity_of_Yersinia_enterocolitica.

[5] E.J. Bottone, *Yersinia enterocolitica*: revisitation of an enduring human pathogen, Clin. Microbiol. Newsl. 37 (2015) 1–8, https://doi.org/10.1016/j.clinmicnews.2014.12.003.

[6] A. McNally, T. Cheasty, C. Fearnley, R.W. Dalziel, G.A. Paiba, G. Manning, D.G. Newell, Comparison of the biotypes of *Yersinia enterocolitica* isolated from pigs, cattle and sheep at slaughter and from humans with yersiniosis in Great Britain during 1999–2000, Lett. Appl. Microb. 39 (2004) 103–108 https://www.ncbi.nlm.nih.gov/pubmed/15189296.

[7] S. Joutsen, E. Sarno, M. Fredriksson-Ahomaa, N. Cernela, R. Stephan, Pathogenic *Yersinia enterocolitica* O:3 isolated from a hunted wild alpine ibex, Epidemiol. Infect. 141 (2013) 612–617 https://www.ncbi.nlm.nih.gov/pubmed/22697252.

[8] G.R. Cornelis, A. Boland, A.P. Boyd, C. Geuijen, M. Iriarte, C. Neyt, M.P. Sory, I. Stainier, The virulence plasmid of *Yersinia*, an antihost genome, Microb. Mol. Biol. Rev. 62 (1998) 1315–1352 https://www.ncbi.nlm.nih.gov/pubmed/9841674.

[9] G. Riley, S. Toma, Detection of pathogenic *Yersinia enterocolitica* by using congo red-magnesium oxalate agar medium, J. Clin. Microbiol. 27 (1989) 213–214 https://www.ncbi.nlm.nih.gov/pubmed/2913030.

[10] R. Gierczyński, Evaluation of the usefulness of selected virulence markers for identification of virulent *Yersinia enterocolitica* strains. II. Genotypic markers associated with the pYV plasmid, Med. Dosw. Mikrobiol. 52 (2000) 35–49 https://www.ncbi.nlm.nih.gov/pubmed/11107777.

[11] T. Ramamurthy, K.I. Yoshino, X. Huang, G. Balakrish Nair, E. Carniel, T. Maruyama, The novel heat-stable enterotoxin subtype gene (*ystB*) of *Yersinia enterocolitica:* nucleotide sequence and distribution of the *yst* genes, Microb. Pathog. 23 (1997) 189–200 https://www.ncbi.nlm.nih.gov/pubmed/9344780.

[12] I. Singh, S. Bhatnagar, J.S. Virdi, Isolation and characterization of *Yersinia enterocolitica* from diarrhoeic human subjects and other sources, Curr. Sci. 84 (2003) 1353–1355 https://www.researchgate.net/publication/285663676_Isolation_and_characterization_of_Yersinia_enterocolitica_from_diarrhoeic_human_subjects_and_other_sources.

[13] T. Grant, V. Bennett-Wood, R.M. Robins-Browne, Identification of virulence-associated characteristics in clinical isolates of *Yersinia enterocolitica* lacking classical virulence markers, Infect. Immun. 66 (1998) 1113–1120 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC108023/.

[14] G.R. Cornelis, C. Sluiters, I. Delor, D. Geib, K. Kaniga, C. Lambert de Rouvroit, M.P. Sory, J.C. Vanooteghem, T. Michiels, A. Ymo, A *Yersinia enterocolitica* chromosomal gene modulating the expression of virulence functions, Mol. Microb. 5 (1991) 1023–1034 https://www.ncbi.nlm.nih.gov/pubmed/1956283.

[15] I. Delor, G.R. Cornelis, Role of *Yersinia enterocolitica* Yst toxin in experimental infection of young rabbits, Infect. Immun. 60 (1992) 4269–4277 https://www.ncbi.nlm.nih.gov/pubmed/1398938.

[16] Y. Schmid, G.A. Grassl, O.T. Bühler, M. Skurnik, I.B. Autenrieth, E. Bohn, *Yersinia enterocolitica* Adhesin A induces production of interleukin-8 in epithelial cells, Infect. Immun. 72 (2004) 6780–6789 https://iai.asm.org/content/72/12/6780.

[17] N. Bialas, K. Kasperkiewicz, J. Radziejewska-Lebrecht, M. Skurnik, Bacterial cell surface structures in *Yersinia enterocolitica*, Arch. Immunol. Ther. Exp. (Warsz) 60 (2012) 199–209 https://www.ncbi.nlm.nih.gov/pubmed/22484801.

[18] A. Bancerz-Kisiel, A. Szczerba-Turek, A. Platt-Samoraj, W. Szweda, Distribution of the *ymoA* and *ystA* genes and enterotoxins Yst production by *Yersinia enterocolitica* strains isolated from humans and pigs, Pol. J. Vet. Sci. 15 (2012) 609–614 https://www.ncbi.nlm.nih.gov/pubmed/23390748.

[19] A. Bancerz-Kisiel, K. Lipczyńska, A. Szczerba-Turek, E. Gospodarek, A. Platt-Samoraj, W. Szweda, The use of the HRM method for identifying possible mutations in the *ymoA* gene region and evaluating their influence on the enterotoxic properties of *Y. enterocolitica* strains, BMC Vet. Res. 10 (2014) 207–211 https://www.ncbi.nlm.nih.gov/pubmed/25234736.

[20] S.F. Altschul, T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller, D.J. Lipman, Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, Nucl. Acids Res. 25 (1997) 3389–3402 https://www.ncbi.nlm.nih.gov/pubmed/9254694.

[21] EFSA (European Food Safety Authority), The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011, EFSA J. 11 (2013) 3129–3379 https://www.efsa.europa.eu/en/efsajournal/pub/3129.

[22] S.M. Tennant, N.A. Skinner, A. Joe, R.M. Robins-Browne, Homologues of insecticidal toxin complex genes in *Yersinia enterocolitica* biotype 1A and their contribution to virulence, Infect. Immun. 73 (2005) 6860–6867 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1230942/.

[23] B. Kot, A. Woźniak-Kosek, J. Kawiak, K. Bukowski, Zastosowanie metody PCR do wykrywania plazmidowych markerów chorobotwórczości szczepów *Yersinia enterocolitica* izolowanych od ludzi i świń, Med. Weter. 57 (2001) 727–730.

[24] I. Gackowska, A.J. Furowicz, D. Czernomysy-Furowicz, A. Jakubczak, Fenotypowa i genotypowa analiza właściwości chorobotwórczych szczepów *Yersinia pseudotuberculosis* oraz *Yersinia enterocolitica*, Med. Weter. 58 (2002) 303–305.

[25] H. Zheng, Y. Sun, S. Lin, Z. Mao, B. Jiang, Investigation of virulence genes in clinical isolates of *Yersiniayersinia enterocolitica*, FEMS Immunol. Med. Microbiol. 53 (2008) 368–374 https://www.ncbi.nlm.nih.gov/pubmed/18557936.

[26] P. Thoerner, C.I. Bin Kingombe, K. Bögli-Stuber, B. Bissig-Choisat, T.M. Wassenaar, J. Frey, T. Jemmi, PCR detection of virulence genes in *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* and investigation of virulence gene distribution, Appl. Environ. Microbiol. 69 (2003) 1810–1816 https://aem.asm.org/content/69/3/1810.

[27] E. Harrison, M.A. Brockhurst, Plasmid-mediated horizontal gene transfer is a coevolutionary process, Trends Microbiol. 20 (2012) 262–267 https://www.ncbi.nlm.nih.gov/pubmed/22564249.

[28] A. Platt-Samoraj, M. Ugorski, W. Szweda, A. Szczerba-Turek, K. Wojciech, Z. Procajło, Analysis of the presence of *ail, ystA* and *ystB* genes in *Yersinia enterocolitica* strains isolated from aborting sows and aborted fetuses, J. Vet. Med. B Infect. Dis. Vet. Public Health 53 (2006) 341–346 https://www.ncbi.nlm.nih.gov/pubmed/16930279.

[29] R. Gierczyński, M. Jagielski, W. Rastawicki, Evaluation of usefulness for selected virulence markers for identifying pathogenic *Yersinia enterocolitica* strains. IV. Genes *myfA* and *ureC*, Med. Dosw. Mikrobiol. 54 (2002) 347–355 https://www.researchgate.net/publication/10844752_Evaluation_of_usefulness_for_selected_virulence_markers_for_identifying_pathogenic_Yersinia_enterocolitica_strains_IV_Genes_myfA_and_ureC.