PROGRAM and Abstracts

EU FP7 PROMISE Regional MEETING

Foodborne Pathogens and critical genes:
inside/outside the EU food chain control

in frame of the Annual Meeting of the Hungarian Society for Microbiology

A Magyar Mikrobiológiai Társaság
2014. évi Nagygyűlése

www.promise-net.eu
www.mmt.org.hu
www.vmri.hu

Helikon Hotel, Keszthely, Hungary, 16th October, 2014.

EU FP7 PROMISE Regional Meeting

Foodborne Pathogens and critical genes: inside/outside the EU food chain control

October 16th , Thursday

Room No1.

15.00-17.40

Chair persons
Prof. Sonja Smole Mozina (co-coordinator-PROMISE), *Prof. Martin Wagner* (coordinator-PROMISE)
Welcome Address
by *Prof. Károly Márialigeti*, President of the Hungarian Society for Microbiology

15.00-15.20
PROMISE-1
MARTIN WAGNER[1], ANJA STRAUß[1], KATI SZAKMARY-BRÄNDLE[1], SABINE SCHLAGER[2], JANINE BEUTLICH[3], BEATRIX STESSL[1], ANCA IOANA NICOLAU[4]; LUMINITA CIOLACU[4], KATHRIN RYCHLI[1], DAGMAR SCHODER[1]

THE PROMISE PROJECT: PREVALENCE AND CHARACTERIZATION OF FOOD-BORNE PATHOGENS ISOLATED FROM NEGLECTED ROUTES OF TRANSMISSION TO CONSUMERS

[1]Institute for Milk Hygiene, Milk Technology and Food Science, University for Veterinary Medicine, Vienna, Austria; [2]Federal Institute for Risk Assessment, Berlin, Germany; [3]Austrian Agency for Health and Food Safety, Graz, Austria; [4]Lower Danube University of Galati, Galati, Romania

15.20-15.40
PROMISE-2
EVA KACLÍKOVÁ, JANKA KOREŇOVÁ, ADRIANA VÉGHOVÁ, JANA MINAROVIČOVÁ, PETER SIEKEL, THOMAS KUCHTA

PROBLEMS OF CONTAMINATION BY *LISTERIA MONOCYTOGENES* IN FOOD INDUSTRY IN SLOVAKIA

Food Research Institute, National Agricultural and Food Centre, Bratislava, Slovakia

15.40-16.00
PROMISE-3
ANCA IOANA NICOLAU[1], ANDREI.S. BOLOCAN[1], KATHRIN RYCHLI[2], ELENA ALEXANDRA ONICIUC[1], AVELINO ALVAREZ-ORDÓÑEZ[3], K. JORDAN[3], MARTIN WAGNER[2]

TO BE PERSISTENT OR NOT TO BE PERSISTENT? THAT IS THE QUESTION FOR *LISTERIA MONOCYTOGENES* STRAINS ISOLATED FROM A MEAT PROCESSING ENVIRONMENT

[1]Faculty of Food Science and Engineering, Lower Danube University of Galati, Galati, Romania; [2]Institute for Milk Hygiene, Milk Technology and Food Science, University of Veterinary Medicine, Vienna, Austria; [3]Teagasc Food Research Centre, Fermoy, Cork, Ireland

16.00-16.20 Kávészünet / **Coffee Break**

16.20-16.40
PROMISE-4

SONJA SMOLE MOŽINA[1], JASNA KOVAČ[1], NEŽA ČADEŽ[1], DILETTA DI MARCO[1], KATJA BEZEK[1, 2], BEATRIX STESSL[1], PETER RASPOR[1, 2], MARTIN WAGNER[3]

ANTIMICROBIAL RESISTANCE OF *CAMPYLOBACTER JEJUNI* IN THE CENTRAL EUROPEAN COUNTRIES

[1]Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; [2]Faculty of Health Sciences, University of Primorska, Izola, Slovenia; [3]Institute for Milk Hygiene, Milk Technology and Food Science, University of Veterinary Medicine, Vienna, Austria

16.40-17.00
PROMISE-5

IVAN RYCHLIK[1], ESTELLA PRUKNER-RADOVCIC[2], SONJA SMOLE-MOZINA[3], BÉLA NAGY[4]

CHARACTERISATION OF EGG LAYING HEN AND BROILER CECAL AND FECAL MICROBIOTA

[1]Veterinary Research Institute, Brno, Czech Republic; [2]Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia; [3]Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; [4]Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

17.00-17.20
PROMISE-6

ISTVÁN TÓTH, DOMONKOS SVÁB

BACTERIOPHAGES FROM CONFISCATED FOOD, LYTIC FOR FOOD-BORNE PATHOGENS

Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest

17.20-17.40
PROMISE-7

BÉLA NAGY[1], SONJA SMOLE-MOŽINA[2], JASNA KOVAČ[2], MARTIN WAGNER[3], DAGMAR SCHODER[3], ANJA STRAUSS[3], SABINE SCHLAGER[4], JANINE BEUTLICH[5], BERND APPEL[5], MARIJA LUŠICKY[6], MOJCA CIMERMAN[6], PAVEL APRIKIAN[7], ISTVÁN TÓTH[1], RENATA KUGLER[1], AMA SZMOLKA[1]

VIRULENCE AND ANTIMICROBIAL RESISTANCE DETERMINANTS OF VEROTOXIGENIC *ESCHERICHIA COLI* (VTEC) AND OF ESBL-PRODUCING MULTIDRUG RESISTANT *E. COLI* FROM FOODS OF ANIMAL ORIGIN ILLEGALLY IMPORTED TO EUROPE

[1]Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary; [2]Department of Food Science/Technology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; [3]Institute for Milk Hygiene, Milk Technology and Food Science, University of Veterinary Medicine, Vienna, Austria; [4]Institute for Medical Microbiology and Hygiene, AGES, Graz, Austria; [5]Federal Institute for Risk Assessment, Berlin, Germany; [6]Center for Microbiology, Institute of Public Health Maribor, Maribor, Slovenia; [7]IDGenomics, Seattle, WA, USA

SESSION ORGANIZERS: *Béla Nagy* and *István Tóth* (Promise Partner No9, VMRI, Budapest)

THE PROMISE PROJECT: PREVALENCE AND CHARACTERIZATION OF FOOD-BORNE PATHOGENS ISOLATED FROM NEGLECTED ROUTES OF TRANSMISSION TO CONSUMERS

M. WAGNER[1], ANJA STRAUß[1], KATI SZAKMARY-BRÄNDLE[1], SABINE SCHLAGER[2], JANINE BEUTLICH[3], BEATRIX STESSL[1], ANCA IOANA NICOLAU[4]; LUMINITA CIOLACU[4], KATHRIN RYCHLI[1], DAGMAR SCHODER[1]

[1]Institute for Milk Hygiene, Milk Technology and Food Science, University for Veterinary Medicine, Vienna, Austria; [2]Federal Institute for Risk Assessment, Berlin, Germany; [3]Austrian Agency for Health and Food Safety, Graz, Austria; [4]Lower Danube University of Galati, Galati, Romania

THE 7th FP EU funded project „Protection of Consumers through Mitigation of Segregation of Expertise (PROMISE)" studies food-borne pathogens transmitted into EU-28 through less researched gates. A number of EU-28 member states participated and took samples from different possible neglected routes of pathogen transmission: food confiscates from travellers arriving at airports (Austria, Germany, Spain), food confiscated from travellers passing ground border stations (Croatia, Slovenia), food sold at black markets (Romania), and food samples from a globally acting food importer (Greece). At Austria`s main airport Wien Schwechat, more than 11 tons of food are annually confiscated from travellers hand luggage. We accompanied the authority for 8 month and took 600 food samples of animal origin carried along by passengers that flew into Vienna from destinations assignable to 33 countries worldwide. The total amount of food confiscated by these 600 checks peaked to 1278 kg. By using ISO methods, we could confirm a prevalence of *Salmonella spp*, *Listeria monocytgenes*and VTEC of 1.1% (n=7), 2.5% (n=15), and 1.3% (n=8), respectively. *Campylobacter* could not be isolated from any food commodity what was surprising since several raw poultry meat samples were confiscated. *Salmonella* isolates belonged to serotypes Telaviv, Hadar, Ohio, Brandenburg, Mbandaka, and Anatum. Two *S.* Hadar isolates from food originating from Egypt and one *S.* Anatum isolate from food originating from Ethiopia tested multidrug resistant. *Listeria monocytogenes* mainly belonged to the MLST (ST) type 9 known to be prevalent in Asian countries. All seven VTEC strains confirmed were non-0157: O2:H27, O6:H10, O8:HNM, O39:H48, O178:H7 and Orough:H7. The study shows for the first time that food shuttled illegally into EU-28 by global travellers is contaminated at frequencies similar to those usually found when domestic samples are investigated. However, yet un-described genetic variants could be transmitted. To look into this issue, we performed a detailed analysis of virulence and occurrence of mutations in essential virulence genes in 15 *L. monocytogenes* strains isolated from a Romanian black market served by food vendors from Moldavia. Novel virulence profiles as studied in cell culture correlated with mutations in the *hly* and *inl*A genes and with the assignment to MLST types. Conclusively, our data show that food distributed through unregulated channels may be contaminated at rates known from food samples taken from domestic markets. However, a risk is given since strains with novel virulence traits might be transmitted through such gates. That Introduction of yet unknown genetic variants of pathogens may play an essential role in risk management was dramatically demonstrated by the recent EHEC outbreak in Germany and France where an obscure EHEC serovar O104 was the causal agent harbouring completely novel virulence traits. The pure amount of food shuttled illegally in hand luggage implies that the information of travellers should be enforced.

PROBLEMS OF CONTAMINATION BY *LISTERIA MONOCYTOGENES* IN FOOD INDUSTRY IN SLOVAKIA

EVA KACLÍKOVÁ, JANKA KOREŇOVÁ, ADRIANA VÉGHOVÁ, JANA MINAROVIČOVÁ, P. SIEKEL, T. KUCHTA
Food Research Institute, National Agricultural and Food Centre, Bratislava, Slovakia

Listeria monocytogenes is a pathogenic bacterium widely distributed in the environment that can cause listeriosis in humans. Cheese and meat products have been implicated in sporadic cases and in outbreaks of listeriosis worldwide, including Slovakia. The pathogen was previously detected in small and medium-sized food processing factories in Slovakia. The aim of this study was to obtain more detailed information on the character of contamination, by means of implementation of a tracing approach based upon advanced sampling and molecular methods for sub-species identification of *L. monocytogenes.* A total of 400 samples were collected and analysed in 2010 - 2014 from the internal environment of three cheese processing plants, and 242 swab samples were collected and analysed from the internal environment of one meat processing plant. Out of these, 60 samples were found to be positive for *L. monocytogenes*. Positive colonies were confirmed by real-time PCR and classified by PCR-serotyping as well as classical serotyping. Serotype 1/2a was found to dominate (80%) in all three cheese processing plants, while serotypes 1/2a and 4b dominated in the meat processing plant (51 % and 27 %, respectively). Isolates were then subjected to DNA restriction (AscI and ApaI) and pulsed field gel electrophoresis (PFGE). Three pulsotypes, 2 (30%), 9 (27%) and 1 (19%), were dominant in the meat plant. The repeated presence of isolates of certain pulsotypes during a three-year period indicated persistence of *L. monocytogenes* in the meat processing plant despite of regular cleaning and sanitation. The sources of contamination could originate from external environment (slaughter house) and, breaking the hygienic barriers, could cause contamination of the internal environment of the plant. Application of the tracing approach based upon advanced sampling and molecular methods for sub-species typing of *L. monocytogenes* facilitated detection of potentially persistent strains, identification of sources and routes of contamination and, subsequently, will allow to optimize the technical and sanitation measures to ensure hygiene of the food production.

Financial support of EU project PROMISE *is acknowledged.*

TO BE PERSISTENT OR NOT TO BE PERSISTENT? THAT IS THE QUESTION FOR *LISTERIA MONOCYTOGENES* STRAINS ISOLATED FROM A MEAT PROCESSING ENVIRONMENT

ANCA IOANA NICOLAU[1], A.S. BOLOCAN[1], KATHRIN RYCHLI[2], ELENA ALEXANDRA ONICIUC[1], A. ALVAREZ-ORDÓÑEZ[3], K. JORDAN[3], M. WAGNER[2]

[1]Faculty of Food Science and Engineering, Lower Danube University of Galati, Galati, Romania; [2]Institute for Milk Hygiene, Milk Technology and Food Science, University of Veterinary Medicine, Vienna, Austria; [3]Teagasc Food Research Centre, Fermoy, Cork, Ireland

Despite the progress made in recent years regarding understanding bacterial population structure and dynamics in a constant environment, identification of persistent strains of *L. monocytogenes* is still challenging. The present study analysed the *L. monocytogenes* strains isolated from a meat processing facility having in view to establishing whether or not persistence is involved in the contamination of the establishment. Forty two isolates from sixty one positive samples have been characterised by serotype multiplex PCR and PFGE in order to have an overall view of their diversity. In total 11 pulsotypes have been identified by PFGE, belonging to 2 serogroups: 1/2a/3a and 1/2c/3c. The PFGE dendrogram suggests the existence of two persistent pulsotypes (T4 and T8), if persistence is considered the isolation of the same pulsotype during several months to years. Susceptibility tests to disinfectants such as peracetic acid, benzalkonium chloride and hydrogen peroxide showed that pulsotypes T1 and T2, which have not been isolated frequently, were more resistant to benzalkonium chloride than to peracetic acid and hydrogen peroxide. No significant differences were detected between pulsotypes regarding their susceptibility to peracetic acid and hydrogen peroxide. The screening for identification of resistance genes (*qacH, brcABC*) and stress survival islet (SSI-1) revealed that both T1 and T2 pulsotypes harbour the benzalkonium resistance cassette (*brcABC*) and the SSI-1, which have been detected by other researchers in persistent strains. None of the pulsotypes have got qacH gene. The in-vitro adhesion ability of *L. monocytogenes* to abiotic surfaces at suboptimal temperatures (4°C, 10°C, 20°C) showed that T8 pulsotype had good adhesion ability, while T4 had the same ability as the other non persistent strains. Although not displaying typical characteristics for persistence, the T4 and T8 pulsotypes were identified in all sampling occasions, both in raw materials and on surfaces, suggesting a continuous contamination of the meat processing facility with these pulsotypes. Despite displaying characteristics for persistence, the T1 and T2 pulsotypes showed a non persistent phenotype. This can be attributed to the possibility of a spontaneous switch between persisters and non persisters, a phenomenon that can occur in both directions.In these circumstances, when referring to persistence of *L. monocytogenes* strains in an environment, it is better to use the terminology “presumed to be persistent” and “presumed to be non persistent” until clear prove is identified to support their classification.

The work has been funded by the Promise FP7 project (*ID 265877)*, the Sectorial Operational Programme Human Resources Development 2007-2013 of the Ministry of European Funds through the Financial Agreement POSDRU/159/1.5/S/132397, the bilateral project Romania-Austria 753/2014.

ANTIMICROBIAL RESISTANCE OF *CAMPYLOBACTER JEJUNI* IN THE CENTRAL EUROPEAN COUNTRIES

SONJA SMOLE MOŽINA[1], JASNA KOVAČ[1], NEŽA ČADEŽ[1], DILETTA DI MARCO[1], KATJA BEZEK[1, 2], BEATRIX STESSL[1], P. RASPOR[1, 2], M. WAGNER[3]

[1]Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; [2]Faculty of Health Sciences, University of Primorska, Izola, Slovenia; [3]Institute for Milk Hygiene, Milk Technology and Food Science, University of Veterinary Medicine, Vienna, Austria

Recently, several European countries have invested heavily in reducing pathogenic *Campylobacter* transmission, especially via specific food (mainly poultry meat) chains, but with very limited success – campylobacteriosis with the notification rate of 55.49/100,000 in year 2012 remains the leading food-borne illness and the most frequently reported zoonosis in humans in EU. *Campylobacter jejuni* is the most prevalent cause of bacterial gastroenteritis with still increasing trend in EU [1]. Moreover, the officially reported prevalence numbers are thought to be significantly underestimated, due to underdiagnosis and underreporting in different countries. Beside high prevalence in animals and retail (broiler) meat, the increasing antimicrobial resistance is a prompt problem, including multidrug resistant *Campylobacter* transmitted via food chain [2]. The resistance against ciprofloxacin, one of the first drugs of choice for treatment of campylobacteriosis has a more pronounced increasing trend in southern EU (e.g. Spain, with 84.1% of resistance rate among human isolates in 2012) and in central EU, including A, Sl and HU with 61.2, 70.7 and 79.4 % of resistance rate, respectively [3]. As an evident public health risk, *C. jejuni* was included also in the microbiological isolation scheme of the "Promise" project – analyzing products of animal origin (POAO), illegally imported by travelers to the EU. However, most illegally imported POAO were durable food products, e.g. fermented sausages and aged cheeses, and consequently, due to the low water activity and low pH, harbored no *C. jejuni*. Alternatively, a *C. jejuni* strain database was established covering also the isolates from other sources in the "Promise" partner countries (N=415). We determined antibiotic resistance profiles for seven antibiotics by broth microdilution method, with PCR detection of resistance genes and with mismatch amplification mutation assay and sequencing of specific regions like Quinolone Resistance-Determining Region (QRDR) in *gyrA gene*. Multilocus sequence typing in seven housekeeping genes (*aspA, glnA, gltA, glyA, uncA, tkt, pgm)* and PFGE was done for the isolates from human, animal, and meat sources as well as from environmental (surface water and other) samples. The isolates (n=103) were divided in groups based on the sequence of *gyrA* QRDR and compared with the MLST typing results. Correlation was confirmed between CC21 and *gyrA* cluster 2 (87,8%), as well as between *gyrA* cluster 7 and CC353 (32,3%) and CC354 (23,5%). We conclude that the high ciprofloxacin resistance prevalence observed indicates the clonal spread of quinolone resistance with CC21, not only locally as recently published [4], but also in geographically wider EU region.

Acknowledgement is due to "PROMISE" (FP7-265877).

[1] EFSA, (2014a) EFSA Journal 12: 3547-3859.
[2] Smole Možina, S. et al. (2011) TIFS 22: 91–98.
[3] EFSA, (2014b) EFSA Journal 12: 3590-3926.
[4] Kovač, J. et al. (2014) Epidemiol Infect 1-9. doi:10.1017/S0950268813003245

CHARACTERISATION OF EGG LAYING HEN AND BROILER CECAL AND FECAL MICROBIOTA

I.RYCHLIK[1], ESTELLA PRUKNER-RADOVCIC[2], SONJA SMOLE-MOZINA[3], B. NAGY[4]

[1]Veterinary Research Institute, Brno, Czech Republic; [2]Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia; [3]Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; [4]Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

In this study we characterised the development of caecal microbiota in egg laying hens over their lifespan, from the day of hatching until 60 weeks of age. Using pyrosequencing of V3/V4 variable regions of 16S rRNA genes for microbiota characterisation, we were able to define 4 different stages of caecal microbiota development. The first stage lasted for the first week of life and was characterised by a high prevalence of *Enterobacteriaceae* (phylum *Proteobacteria*). The second stage lasted from week 2 to week 4 and was characterised by nearly an absolute dominance of *Lachnospiraceae* and *Ruminococcaceae* (both phylum *Firmicutes*). The third stage lasted from month 2 to month 6 and was characterised by the succession of *Firmicutes* at the expense of *Bacteroidetes*. The fourth stage was typical for adult hens in full egg production aged 7 months or more and was characterised by a constant ratio of *Bacteroidetes* and *Firmicutes* formed by equal numbers of the representatives of both phyla. In the second part of this study we compared the fecal microbiota composition in egg laying hens and broilers originating from 4 different Central European countries. The core of chicken fecal microbiota was formed by 26 different families. Rather unexpectedly, representatives of *Desulfovibrionaceae* and *Campylobacteraceae*, both capable of hydrogen utilisation in complex microbial communities, belonged among this core of microbiota families. Understanding such metabolic bacterial mutualisms in complex microbiota systems may allow for interventions which might result in the targeted interventions reducing foodborne zoonoses such as replacement of *Campylobacteraceae* by *Desulfovibrionaceae* and a reduction of *Campylobacter* colonisation in broilers, carcasses, and consequently poultry meat products.

This study was supported by PROMISE project 7th Framework Programme.

BACTERIOPHAGES FROM CONFISCATED FOOD, LYTIC FOR FOOD-BORNE PATHOGENS

I. TÓTH, D. SVÁB

Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

Bacteriophages frequently carry non essential, first of all virulence genes including Shiga toxin production encoding genes and promote the evolution of both pathogenic and non pathogenic bacteria by integration to their genomes. However these prophages could be also induced and the lytic free phages could interfere with bacterial strains present in their environment. These lytic phages could modify the sensitivity of the isolation procedure. Altogether 207 confiscated food samples of animal origin were tested for the presence of Shiga/Vero toxin producing *E. coli* (STEC) and bacteriophages. By using ISO 16654:2001 for O157 and ELISA based Ridascreen Verotoxin we could not isolate *E. coli* O157 or STEC but we were able to isolate lytic phages. By using mitomycin C at 0.5 µl/ml final concentration as inducing agent and *E. coli* K-12 C600 strain as indicator strain lytic phages were isolated from 10 % (21/207) of the food samples. Electron microscopic studies revealed that the lytic phages represented different families including tailed *Myoviridae*, *Syphoviridae* and filamentous *Inoviridae*. The induced phage suspensions frequently contained more than one type of phages. The host specificity of lytic phages was tested by spot assay using *E. coli* strains representing enterohaemorrhagic (EHEC), enteropathogenic (EPEC), uropathogenic (UPEC) avian pathogenic (APEC) *E. coli* pathotypes, *Shigella sonnei*, *Cirobacter rodentium* and several *Salmonella* serovars including Enteritidis, Typhymurium, Hadar and Infantis. Different lytic patterns were observed and all the tested *E. coli* and *S. sonnei* strains were lysed by at least one phage. Interestingly the isolated lytic phages lysed the EHEC and atypical O157 strains tested and the *S.* Typhimurium study strain was also lysed by four phages. Results indicate that further studies are justified to elucidate the inhibitory effect of lytic phages that might influence the isolation of food-borne pathogens from food.

Financial support of EU FP project Promise (265877) and OTKA (K81252) is acknowledged.

VIRULENCE AND ANTIMICROBIAL RESISTANCE DETERMINANTS OF VEROTOXIGENIC *ESCHERICHIA COLI* (VTEC) AND OF ESBL-PRODUCING MULTIDRUG RESISTANT *E. COLI* FROM FOODS OF ANIMAL ORIGIN ILLEGALLY IMPORTED TO EUROPE

B. NAGY[1], SONJA SMOLE-MOŽINA[2], JASNA KOVAČ[2], M.WAGNER[3], DAGMAR SCHODER[3], ANJA STRAUSS[3], SABINE SCHLAGER[4], JANINE BEUTLICH[5], B. APPEL[5], MARIJA LUŠICKY[6], MOJCA CIMERMAN[6], P. APRIKIAN[7], I. TÓTH[1], RENATA KUGLER[1], A. SZMOLKA[1]

[1]Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary; [2]Department of Food Science/Technology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; [3]Institute for Milk Hygiene, Milk Technology and Food Science, University of Veterinary Medicine, Vienna, Austria; [4]Institute for Medical Microbiology and Hygiene, AGES, Graz, Austria; [5]Federal Institute for Risk Assessment, Berlin, Germany; [6]Center for Microbiology, Institute of Public Health Maribor, Maribor, Slovenia; [7]IDGenomics, Seattle, WA, USA

Microbial risk due to illegal food import has not been investigated so far. Here we aimed to reveal frequency, phenotype and genotype of verotoxigenic *E. coli* (VTEC) and ESBL-producing multidrug resistant (MDR) *E. coli* isolated from foods of animal origin confiscated at the EU airport borders. Of the 1500 food samples confiscated at the airports of Austria, Germany and Slovenia, the most frequent were cheese and meat products primarily from Turkey and from Balkan countries. The VTEC bacteria were isolated using ISO 16654:2001 for O157 and Ridascreen® ELISA based PCR testing of *stx* genes or ISO/ TS13136 for non-O157 VTEC, resulting in 15 isolates of VTEC (1%). In addition 600 samples from the Vienna airport were also tested for ESBL-producing MDR *E. coli*, using cefotaxime-McConkey agar. We identified 14 *E.coli* strains as ESBL/MDR *E. coli*. (0,9%) for phenotyping for antimicrobial resistance and for genotyping by microarray (Identibac®,AMR05). The 15 VTEC isolates were phenotyped as Stx toxin producing non-O157 strain. Only one isolate, from Turkish cheese, proved to be EHEC (O26:H46). The remaining 14 strains represent uncommon VTEC serotypes with *stx1* and/or *stx2* genes. Microarray analysis (Identibac®, Ec03) revealed a wide range of other non-LEE encoding virulence genes. Pulsed field electrophoresis (PFGE) showed high genetic diversity of the strains. Multilocus sequence typing (MLST) established three new ST types (ST4505, 4506 and 4507) in the MLST database, and indicated the existence of 5 small clusters with no relation to origin or serotype/genotype of the strains, but representing several human-related ST types. All VTEC isolates were sensitive to 18 antimicrobials relevant to human and/or animal health, and did not contain resistance genes._ESB/MDR *E. coli* were resistant to at least 3 classes of antimicrobials. Microarray analysis detected TEM-1 in all but one strain and a variety of genes encoding resistances to other ESBLs (CTXM-1, OXA-1), trimethromprim, tetracycline, aminoglycosides and class1/class2 integrons (8/14 isolates). *E.coli* virulence microarray detected 2-6 virulence genes in all but one MDR *E.coli*, and one of the strains qualified as an atypical EPEC. Even though the frequency and attributes of isolated VTEC and ESBL/MDR *E.coli* did not represent an immediate major risk through illegal food import for the countries involved, it is suggested that the unusual serovars of VTEC as well as the virulence and antimicrobial resistance determinants of ESBL/MDR *E.coli* detected here, may indicate a future emerging threat by strains in illegally imported foods.

EU FP7 PROMISE (Grant No: 265877), to Dr. Mária Herpay, National Institute for Epidemiology, Budapest.