ESPOO 2008 VTT SYMPOSIUM 252

DETECTION AND IDENTIFICATION OF HARMFUL MICROBES

1st Workshop arranged by SAFOODNET – Food Safety and Hygiene Networking within New Member States and Associated Candidate Countries

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Keywords: detection, identification, sampling, molecular biology methods, PCR based detection, phage typing, antibiotic resistance, antimicrobial activities, harmful microbes, pathogens, *Salmonella* spp., *Mycobacterium* spp., *Listeria* spp., *Staphylococcus* spp., microbial contamination routes, food processing

DETECTION AND IDENTIFICATION OF HARMFUL MICROBES

1ST WORKSHOP ARRANGED BY SAFOODNET – FOOD SAFETY AND HYGIENE NETWORKING WITHIN NEW MEMBER STATES AND ASSOCIATED CANDIDATE COUNTRIES; FP6-022808-2006

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PREFACE

Food Safety and Hygiene Networking within New EU Member States and Associated Candidate Countries (SAFOODNET FP6-022808) is a specific support action EU-project building-up a sustainable network in food safety. It aims at knowledge sharing to prevent risks related to microbial hazards, to find future RTD needs and apply for RTD funding in food processing and packaging safety. The action focuses towards Czech Republic, Denmark, Estonia, Finland, Hungary, Latvia, Slovenia and Turkey in the pilot actions, seminars, and workshops on process hygiene and end product safety. Interested researchers and SME representatives from other new EU countries and ACCs are encouraged to participate in the activities. The objectives of SAFOODNET are to: 1) disseminate knowledge from national and international food safety projects in open seminars, workshops, practical exercises, RTD activities and pilot actions resulting in new research projects for food industry especially SMEs; 2) establish an expert group (EG) in which authorities, scientists, industrial representatives build-up or strengthen existing networks and identify specific needs for future RTD activities in food safety and 3) bridge networks within the new EU, fostering scientific co-operation and knowledge transfer in food safety.

The 1st SAFOODNET workshop was arranged to a targeted audience of young scientists and junior employees in food processing industry, research on food hygiene and food safety as well as authority. The title of the 3-day long workshop was *Detection and identification of harmful microbes* and it was entirely dedicated to microbiological methods and methodology available for detection, identification and tracking of harmful contaminants and pathogens with concurrent practical exercises focusing on detection of: 1) *Salmonella* spp., 2) *Mycobacterium* spp. and 3) *Listeria* spp. and *Staphylococcus* spp. This workshop focused on both conventional and molecular biology based methods that are usable for industry e.g. in hygiene management. Relevant information on the contaminating microbiota and its surroundings enables control of the process hygiene in the processing plant. Under suitable conditions microbes can namely grow very quickly and consequently cause a drastic change in the microbial population at a certain site e.g. due to a change in the temperature. Rapid, relevant microbial monitoring methods ensure that any developing

problems are detected as early as possible. Occasional proliferation of microbes from stagnant areas e.g. from biofilm formations in dead ends can contaminate the entire system and must be detected rapidly to avoid human suffering due to illnesses and financial losses due to product withdrawals. The main requirements for a good microbiological method are reliability, sensitivity, economy and sometimes also selectivity.

The forthcoming activities in SAFOODNET-project are an open seminar on risk management and an workshops incl. practical exercises to disseminate methodologies and methods in microbial risk management; networking at events and through project web-site (http://safoodnet.vtt.fi); establishment of an expert group to suggest and evaluate pilot actions for future RTD needs in food safety; performance of food safety pilot actions in food industry especially in SMEs and building-up a new channel for co-operation within the new EU.

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LECTURES

MICROBIAL SAMPLING OF SURFACES IN DIRECT AND INDIRECT CONTACT WITH PRODUCTS PROCESSED

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High hygiene with low occurrence of spoilage microbes and no occurrence of pathogens is a must in processing high quality food products with long shelf-life. Especially when there are problems in process hygiene the collection of process contaminants and their further characterisation is needed to be able to define and establish the contamination routes. Thus knowledge of the domestic flora is an important background fact in solving process hygiene problems. The hygiene testing on food processing equipment surfaces is normally carried out using conventional culturing or contact agar methods. However, it is difficult to measure the real extent of biofilm formation and the biotransfer potential of biofilms, because the conventional microbiological methods used in assessing equipment hygiene were not developed for assessment of biofilm formation. The biofilm assessment can be incorrect, because the microbes in the biofilms are strongly adhered to the surfaces they grow on, if the swabbing is not performed carefully. The work must be performed using Good Laboratory Practices (GLP) and the culturing under reproducible conditions to obtain reliable results. Some details in GLP during sampling, detection and identification are:

- planning the working procedure in advance,
- preparation of clear and concise written instructions e.g. on how to sterilise tools, diluents and nutrients, on aseptic practises, on how to control aseptic working methods, on how to clean aseptic working areas, on how to control the air quality in the work area, on how to dress, on how and when to wash the hands as well as on how to control the microbial cleanliness of working surfaces,
- preparation of concise working instructions of official standard methods,
- taking part in intercalibrations or use reference samples,

- keeping track on the work through proper documentation e.g. who performed the work, when it was carried out and who read the results,
- calibrating the pipettes at least four times a year; the dimensional accuracy must be checked always when starting analyses,
- carrying out the work sitting with rapid, stable movements without flailing,
- keeping sterile and non-sterile tools on their own places,
- calibrating scales and thermometers regularly,
- controlling the safety of the autoclave regularly,
- checking the gas pipelines for any leakage regularly,
- earthen electric laboratory equipment,
- keeping the worktop clean; the worktop should not function as a stock and
- checking the hygiene in the working area at least once a month.

The microbial yield from sampling is also dependent on other factors e.g. surface material and topography, consistency of microbial soil, amount and age of microbes. VTT arranged a collaborative study focused on comparing the yield of microbes detached from the test surface using swabbing, RODAC contact agar plates and Hygicult® dipslides to known microbe amounts and species spread on the test surface. In our experiments it was clearly revealed that even vigorous swabbing only detached a small part of the surface-adhered cells. This means that techniques based on swabbing only provide limited information on the true surface hygiene. Contact methods are easy to use and labour-saving, because it is not necessary to transfer the microbes from the swab to the cultivation medium. However, these methods are also based on the detachment of surface-attached microbes, which also is the limiting factor in the swab method. According to the results of the RODAC contact agar plate, Hygicult® dipslide and swabbing methods, the detection level of contaminants on artificially soiled stainless-steel surfaces was 15–20% of the theoretical yield depending on the microbes detected.

Sampling methods followed by culturing of biofilm microbes could be improved, e.g. by using non-toxic detergents in wetting the surfaces. Furthermore, the detachment of biofilm microbes with ultrasonication was improved when

compared to swabbing. The microbial cells maintained their viability and cultivability in the ultrasonic treatments at all the power levels tested. Although detachment of microbial cells by ultrasonication was efficient, remnants of extracellular materials and materials used for pre-soiling of the test surfaces were detected by epifluorescence microscopy. Rapid detection used in routine hygiene monitoring can be based on bioluminescence i.e. measurement of the adenosine triphosphate (ATP). The ATP method is suitable for hygiene monitoring, since it detects both living microbes and organic residues. However, a severe disadvantage of the method is its rather high detection limit for bacteria. The method is thus unreliable in situations in which even very low numbers of residual microbe e.g. hygiene indicator organisms and pathogens are to be monitored after cleaning. Another rapid and easy method for checking surface hygiene is to detect protein residues from surfaces. However, the sensitivity of some commercial protein detection kits tested was quite low at least to pure microbial residues. Some methods can be used to measure the hygiene level directly from the surface without detaching neither the microbes nor the organic soil e.g. microscopying, which is a accurate method but only applicable in hygiene research. Various stains, different types of microscopying e.g. epifluorescence microscopying, confocal laser scanning microscopying, scanning electron microscopying, and multifunctional image analysis systems offer many opportunities in developing the hygiene monitoring systems.

In conclusion, the conventional hygiene control often underestimates the number of microbes or amount of soil on the surfaces both in direct and indirect contact with products processed, because surface-attached microbes and biofilms are difficult to detach from surfaces. Furthermore, the detection and identification is slow. Thus, we need improved methods both in sampling and in detection and identification. Improved sampling can be achieved by developing surface-active agents for wetting surface during sampling. The sampling can also be improved by using ultrasonication. Improved detection and identification can e.g. be achieved through developing practical kits based on molecular biology techniques. Furthermore, the identification of critical areas in the process to be sampled must be based on unprejudiced selection of sampling places.

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SAMPLING FOR *SALMONELLA* DETECTION ON POULTRY AND PIG FARMS AND IN SLAUGHTERHOUSES

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Insufficiently heat treated foodstuffs containing eggs and poultry meat contaminated with *Salmonella* spp. is in many countries including the Czech Republic the most frequent source of alimentary infections in humans. The Directive 2003/99/EC and Regulation 2160/2003/EC have been issued for an effective control of zoonoses in the whole food-supply chain ('farm to fork approach') and for limitation of public health risks (Table 1). The objective of the new EU legal regulations is to improve the programmes for monitoring and control of zoonoses in food animals. The programme is supplemented with new types of foodborne pathogens, animal categories, harmonization of sampling methods for monitoring, diagnostic methods and reporting systems so as to be comparable in all EU member states. The recently issued report of the European Food Safety Authority (EFSA) analyzed the results of a base line on *Salmonella* prevalence in flocks of laying hens in 27 EU member states and in Norway. Significant differences of prevalence which ranged from 0 to 79.5% were reported in individual countries. In the Czech Republic, 62.5% prevalence of the serotype *S*. Enteritidis (SE) and *S*. Typhimurium (STM) has been reported within the above study, which is the highest prevalence in Europe. This high incidence detected from feces and dust in flocks of lying hens presents a significant public health thread for *Salmonella* transmission in production chain of table eggs (Tables 2 & 3). Our investigations demonstrated that in spite of high number of positive samples of feces, dust and smears from the environment of infected flocks of lying hens, with a predominant occurrence of SE, *Salmonella* prevalence on egg shells and in egg contents were very low. Major risk factors on farms of laying hens infected with Salmonella were low level of hygiene, insufficient sanitation of stable environment and poor technology in the laying houses, which altogether creates suitable conditions for Salmonella spreading and persistence.

Table 1. Common elements in design of national control programmes for Salmonella *(adapted from Annex IIB of Regulation 2160/2003/EC).*

Population of animals	Production phase – location of sampling for culture
Fowl – *Gallus gallus*	
Breeding flocks	
– Breeding flocks (rearing)	– 1-day-old chicks – 4-week-old chicks – 2 weeks prior to entering the egg unit
– Flocks of adult breeding birds	– every second week during the laying period
Laying hen flocks	
– Flocks of pullets	– 1-day-old chicks – 2 weeks prior to entering the egg unit
– Flocks of adult hens	– every 15 weeks prior to entering the laying period
Broiler flocks	animals living for slaughter
Turkey Flocks	animals living for slaughter
Pigs	
– Breeding pig herds	animals living for slaughter or carcasses in slaughterhouse
– Slaughter pig herds	animals living for slaughter or carcasses in slaughterhouse

Salmonellae enter the food chain via infected poultry, which results in intravital – primary contamination. Another significant way of spreading is postvital – secondary contamination which occurs during the processing of broiler carcasses when not only the carcasses but also the organs and technological equipments are contaminated through feathers, feces, gut contents and other organic materials. New technologies have been implemented in the food industry besides new legal measures to protect human health and reduce the incidence of pathogenic microorganisms in poultry production. These include hygiene and decontamination procedures and HACCP systems oriented to minimization of the pathogenic microorganisms in slaughtered poultry. Limiting of these pathogens on farm level is the necessary precondition for reduced transmission of the pathogens to the processing industry. Our investigations showed high occurrence of *Salmonella* spp. in cecum of the slaughtered broiler chickens, however, on carcass surface and in skin samples was the number of findings lower. Incidence of germs depended on epidemiological situation in flocks of broiler chickens, on transportation stress prior to slaughtering, technological and hygiene level in slaughterhouse as well as on the way of sampling, sample

examinations and other factors. Although novel slaughter technologies can markedly reduce microbial contamination during processing, especially air cooling of slaughtered poultry, they are not effective enough to devitalize *Salmonella* reliably. Part of chilled poultry, which remains contaminated by *Salmonella*, is after packing distributed to the markets where it may become a vehicle of contamination under the conditions of poor hygiene and/or undercooking.

Table 2. The national programme for Salmonella *control in reproduction poultry flocks of egg and meat type – the scheme of sampling for monitoring the serotypes* S. *Enteritidis,* S. *Typhimurium,* S. *Infantis,* S. *Virchov and* S. *Hadar.*

Sample collection ensured by the breeder (farm owner):
A. One-day-old chicks – 10 smears from the inside of chick transport boxes (sample of the whole batch of chicks) – chickens that died during transport (sample of the whole batch, max. 60 heads) B. Two weeks prior to onset of laying period – 16 weeks of age – collection of pooled sample of feces (2 samples of min. 150 g) or socks – 1 pair from each flock C. Every two weeks during the laying period – collection of pooled sample of feces (2 samples of min. 150 g) or socks from each flock
Sample collection by the State Veterinary Administration (official samples):
A. at the age of 4 weeks – collection of pooled sample of feces (2 samples of min. 150 g) or socks – 1 pair from each flock B. 4 weeks after the onset of laying period at 24 weeks of age – collection of pooled sample of feces (2 samples of min. 150 g) or socks – 1 pair from each flock C. at week 20 of the laying period (at the age of 40 weeks) – collection of pooled sample of feces (2 samples of min. 150 g) or socks – 1 pair from each flock D. 4 weeks before termination of the laying period (at the age of 62 weeks) – collection of pooled sample of feces (2 samples of min. 150 g) or socks – 1 pair from each flock

Pig herds are in most European countries important reservoirs of *Salmonella*; the prevalent serotype being *S.* Typhimurium and *S.* Derby. Through weaned piglets from the infected litters or newly purchase animals, Salmonellae are transmitted

into the fattening. Fattening pigs from infected herds, transportation stress and other factors (lairage etc.) pose major risk of carcass and the slaughter line contamination by *Salmonella* via feces, gut content and cut lymph nodes.

Table 3. The national programme for Salmonella *control in flocks of laying hens producing table eggs – the scheme of sampling for monitoring the serotypes* S. *Enteritidis and* S. *Typhimurium.*

Sample collection ensured by the breeder (flock owner):
A. One-day-old chicks – 10 smears from the inside of chick transport boxes (sample of the whole batch of chicks) – chickens that died during transport (sample of the whole batch of chicks, max. 60 heads) B. At the age of 24 weeks – collection of pooled sample of feces (2 samples of min. 150 g) or socks – 1 pair from each flock C. Every 15 weeks during the laying period (age of laying hens 39 and 54 weeks) – collection of pooled sample of feces (2 samples of min. 150 g) or socks – 1 pair from each flock
Sample collection by the State Veterinary Administration (official samples):
A. Once a year at the age of 24 or 39 weeks – collection of pooled sample of feces (2 samples of min. 150 g) or socks, or dust samples (min. 100 g) from each flock B. 6–8 weeks prior to of egg-laying termination at the age of 60 weeks or after moulting at the age of 100 weeks – collection of pooled sample of feces (2 samples of min. 150 g) or socks, or dust samples (min. 100 g) from each flock

We found out that Salmonellae were most frequently isolated from cecum and mesenteric lymph nodes in the slaughtered pigs. Although *Salmonella* prevalence in fattening pigs was very high (>50%) in some of the herds, their findings in carcass swabs and slaughter line smears were sporadic (<1%). These findings on *Salmonella* distribution only confirm the importance of high level of hygiene and technology during the slaughter process. Preventive measures in slaughterhouses based on HACCP system can limit fecal contamination with *Salmonella* of pork carcasses and meat products, thus minimizing health risks for consumers.

IMMUNOMAGNETIC SEPARATION OF SELECTED BACTERIAL SPECIES

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Traditional methods of bacterial detection are cultivation methods on agar plates, which have limitations of time-consuming and lacking precision. Many innovative bacterial detection methods have been developed, but just few of them have the potential for becoming a standardized method.

Immunomagnetic Separation (IMS) is a technology based upon uniform super paramagnetic spheres, which are used to separate specific biological targets from samples. IMS is a very popular approach, it has been widely and successfully used in many biological fields including molecular biology, immunology and microbiology.

This method involves immobilizing antibodies to micro-sized paramagnetic beads and the antibody coated beads to trap directly targeted bacteria from liquid. The beads are easily manipulated under the magnetic field, which facilitates efficient bacteria retrieval and concentration. Furthermore, the small size and shape of the beads allow them to be uniformly dispersed in the sample for accelerating the rate of interaction between bead and target. The attachment of target-specific antibodies to the surface of the beads allows capture and isolation of intact bacteria directly from a complex matrix (Figure 1).

When added to a heterogeneous suspension, the beads will bind to the desired target (cells, nucleic acids, proteins or other bio-molecules). This interaction is based on the specific affinity of the ligand on the surface of the beads. The resulting target-bead complex can be removed from the suspension using a permanent magnet that is equipped with strong rare earth magnets (neodymium-iron-boron permanent magnet) to ensure the optimal isolation of bead-bound target and easy removal of supernatant. Bead-bound complexes are drawn to the side of the tube facing the magnet, and the supernatant can be easily removed with a pipette. The inherent benefits of magnetic handling allow for easy washing, separation and concentration of the target without any need for

centrifugation or columns. The concentrated bacterial complexes are either directly inoculated onto the selective agars or they are used for other immuno or molecular based detection methods.

These favourable characteristics reduces largely the assay time and help the analytical procedure to be smooth, so that it is more applicable to higher sample throughput and automation.

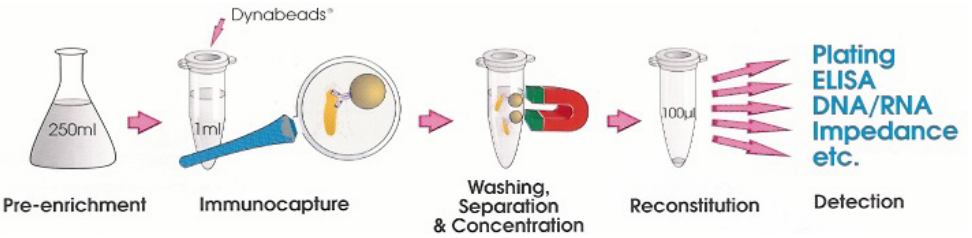

Figure 1. Protocol for detection of bacterial pathogens from food matrices (Dynal).

Immunomagnetic separation methods have been specifically designed and developed for separation of bacteria, viruses and protozoa from different sample matrices including both the clinical samples and food. Currently many commercially prepared beads are on market e.g. for foodborne or waterborne pathogens:

- *Salmonella* spp.
- *Listeria* spp.
- *Listeria monocytogenes*
- VTEC *E. coli*
- *Legionella* spp. and
- cryptosporidia.

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PCR Detection of Pathogenic Microorganisms

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PCR Characteristic and Design

Polymerase chain reaction (PCR), a process of *in vitro* nucleic acid amplification, was predicted by the Cetus scientist Kary Mullis (http://www.karymullis.com/) in 1983. On the base of several technologies that matured in the 1980s, including automated oligonucleotide synthesis, PCR became a reality in a relatively short time. Their first practical application was applied on amplification of β-globin genome sequences and published by Saiki *et al*. in 1985.

PCR permits the selective *in vitro* amplification of a specific DNA region by process which mimics the phenomena of *in vivo* DNA replication. A single-stranded DNA template, short oligonucleotide sequence complementary to the ends of a defined sequence of the DNA template (primers), deoxynucleotide triphosphates (dNTPs) and a DNA polymerase enzyme are required for performing amplification. These basic components enable to synthesize a new DNA strand complementary to the desired template under appropriate conditions. Single-stranded DNA template is generated directly in the process of PCR by heat-denaturing double stranded DNA. Synthetic oligonucleotide primers can be synthesized in one's own laboratory or purchased. Heat stable enzyme, Taq DNA polymerase, was introduced in 1988. This enzyme can withstand temperatures up to 95–97°C and enables automation of PCR. The PCR requires three steps:

1. denaturation of double stranded DNA at 92–96°C,
2. annealing of the oligonucleotide primers to a complementary site of the template at 45–72°C, and
3. extension of the primers at the 3´OH end by successive additions of dNTPs at 72°C.

The scheme of these three steps (denaturation, annealing, extension) is called a cycle. The repetition of such cycles leads to amplification of template DNA. Each of two oligonucleotide primers selectively binds to one of the complementary strands. If these primers are extended past each other in a cycle of amplification, than each newly created DNA strand will contain a binding site for the other primer. In this fashion each new DNA strand becomes a template for any further cycle of amplification, thus, enlarging the template pool from cycle to cycle. Repeated cycles of amplification theoretically lead to exponential synthesis of a DNA fragment with a length defined by the 5´termini of the primer pair employed.

CONCEPT OF "REAL-TIME" QUANTITATIVE PCR

The conventional PCR has some limitations from which quantification of specific targets in samples is the most important one. Although concept of competitive PCR was designed for semi-quantitative analysis only the method of "real-time fluorescence-based PCR" enables generating of real quantitative data. This method is based on the contention that there is a quantitative relationship between the amounts of target nucleic acid present at the start of a PCR assay and the amount of product amplified during its exponential phase. The principle of real-time PCR is simple: The presence of amplification products results in fluorescence, which is detected and monitored as amplification occurs. Since the fluorescence output is linear to sample concentration over a very broad range, this linear correlation between PCR product and fluorescence intensity is used to calculate the amount of template present at the beginning of the reaction. Rather than having to look at the amount of PCR product, real-time assays determine the number of cycles after which amplification of a PCR product is first detected. The crucial conceptual innovation is the threshold cycle (C_t), the cycle at which fluorescence exceeds fluorescence threshold.

The real-time PCR is a technique whose time has time. Over the last eight years this techniques has been transformed from an experimental tool to a mainstream scientific technology. It may or may not be more sensitive than conventional nested assay, have a greater detection range, with significantly less inter-assay variation and is highly reproducible and reliable. In theory, real-time PCR combines the objectivity of fluorescence detection with the simplicity of the original PCR reaction, and results obtained using fluorescence-based PCR

chemistries are now accepted as the "Gold Standard" for quantification of viral load in clinical samples, quantification of bacterial pathogens such as *Listeria monocytogenes*, *Helicobacter pylori*, and many others, and also for a lot of applications in human genetics.

SPECIFICITY AND SENSITIVITY OF PCR

Both, conventional and real-time PCR assays are techniques used universally in microbiology today. The most important parameters used in pathogen micro-organisms are sensitivity, specificity and reproducibility. A maximized sensitivity, specificity and reproducibility are required to avoid false-negative results. In many cases, sensitivity increases with increasing specificity. This is due to a decrease in non-specific competing reactions with substrates and polymerase. With increasing sensitivity, reproducibility also increases, because samples with a very low target DNA copy number are more often positive.

Specificity is an ability of PCR to amplify only target product and ability of primers to recognise only certain target loci in genome of micro-organism. Sensitivity is a minimal copy number of target sequence which can be amplified to detectable amount of product.

In bacteriology so name primary detection is ability to amplify a pathogen in real tissue samples (blood, soft tissues, liquid body fluids, food, etc.). In such samples a strict specificity and high sensitivity are limited factors which determine if PCR is applicable. A secondary detection is amplification of specific sequences in cell culture samples. In this case the sensitivity is not decision-making factor, but requirements for specificity are essential.

PCR AND DETECTION OF FOODBORNE MICROBIAL PATHOGENS

More than 250 foodborne diseases are now recognized, and most require specific laboratory diagnosis. Traditionally, a strategy for identifying most microbial pathogens in food involves enrichment culture, cultivation on selective agar media, and ultimately a series of biochemical tests on presumptive colonies to identify the micro-organisms. Such standard microbiological techniques are slow and laborious and often require several days to weeks to perform. For some important pathogens no satisfactory culturing techniques exists. Also, selective

conditions of conventional culturing may lead to loss of plasmids with toxin genes. The PCR method offers several advantages for rapid and sensitive detection of pathogens:

1. Short time requirement improves public health security and minimizes personnel costs.
2. The method is able to identify microorganisms that are difficult to culture.
3. The culture and enrichment of pathogens are not necessary for quality control.
4. PCR reagents are more readily available and easier to store than to those required for serological procedures.
5. Animal models are not needed.
6. The choice of primers determines specificity, which contrasts with the frequent cross-reactivities of antisera utilized in immunoassays.
7. Elaborate diagnostic equipment and media are not required, thereby increasing the flexibility.
8. Automation of PCR results in excellent cost efficiency.

MANIPULATION WITH BIOLOGICAL MATERIAL

Successful detection and proper identification of pathogens depends not only on properly designed parameters of conventional or real-time PCR. The following factors determine the success of amplification:

1. Way of the sample collection; sufficient amount of biological material must be collected.
2. Sample manipulation; especially temperature in which the samples are stored before their transportation to diagnostic laboratory is critical.
3. Sample storage; temperature, period from sample collection to DNA isolation and period from DNA isolation to amplification are the most important.

Due to the fact that PCR is an enzymatic process with extreme sensitivity, false-positive results may easily occur, commonly caused by carry-over or cross-contamination. Precautions have to be taken to circumvent the problems and negative control reaction must be added to all analysis. It is important to detect

also false-negative results depending on inhibition of the amplification caused by the presence of inhibitors, inefficient extraction of pathogen DNA, low number of pathogen present in the sample or pipetting errors. To check the efficiency and the sensitivity of the amplification, it is advisable to apply standard molecules as indicators of the procedures. An internal control molecule co-amplified in the same tube with the same primers as the target DNA can be used to the reliability of the PCR results. Absence of the internal control amplicons indicates inhibition of the amplification.

CHARACTERIZATION OF BACTERIAL SPECIES BY BIOCHEMICAL PROPERTIES

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TAXONOMIC CHARACTERIZATION OF BACTERIA

The identification is based on taxonomic principles. In the diagnostic laboratory, many samples must be characterized each day and results obtained as quickly as possible. Tests must be easy to learn, low in cost and rapidly performed. These classical methods for speciation of bacteria are based on morphological and metabolic characteristics. The diagnostic tests have been selected on the basis that empirically they provide discriminating information. There are numerous different tests for each of the many target pathogens.

The basis of bacterial identification is rooted in taxonomy, which is concerned with cataloging bacterial species and nowadays generally uses molecular biology (genetic) approaches. New information has resulted in renaming of certain bacterial species and in some instances has required totally re-organizing relationships within and between many bacterial families. Genetic methods provide much more precise identification of bacteria but are more difficult to perform than physiology-based methods.

Classification involves the recognition of similarities and relationships as a basis for the arrangement of the bacteria into taxonomic groups or taxa. The basic taxon is the species. Identification involves the recognition of a bacterium as a member of one of the established taxa, appropriately named, by the comparison of a number of characters with those in the description. A bacterial species is a conceptual entity that is hard to define, despite its role as the basic taxonomic grouping. Bacteriologists accept the imprecision and recognize that a species represents a cluster of clones exhibiting some variations in minor properties.

DIAGNOSTIC ISOLATION AND IDENTIFICATION STEPS OF BACTERIA

Step 1. Samples are streaked on culture plates and isolated colonies of bacteria (which are visible to the naked eye) appear after incubation for one to several days. Observation of these colonies for size, texture, color, and (if grown on blood agar) hemolysis reactions, is highly important as a first step in bacterial identification. Whether the organism requires oxygen for growth is another important differentiating characteristic.

Step 2. Colonies are Gram stained and individual bacterial cells observed under the microscope. Under the microscope, the appearance of bacteria is observed. Questions to be asked include: Are they Gram positive or negative? What is the morphology (rod, coccus, spiral, pleomorphic etc.)? Do cells occur singly or in chains, pairs etc.? How large are the cells? Besides the Gram stain, there are other less commonly employed stains available (e.g. for spores and capsules).

Step 3. The bacteria are determined using these isolated colonies. This often requires an additional 24 hours of growth. Another similar colony from the primary isolation plate is then examined for biochemical properties. In some instances, the bacteria are identified with commercially available antibodies recognizing defined surface antigens. Other commercial molecular tests are now widely used.

TESTS FOR METABOLISM OF CARBOHYDRATES AND OTHER COMPOUNDS

Bacteria differ widely in their ability to metabolize carbohydrates and related compounds. This can be demonstrated by various tests to identify the bacteria.

TESTING TO DISTINGUISH BETWEEN AEROBIC AND ANAEROBIC BREAKDOWN OF A CARBOHYDRATE (OF TEST)

This method depends upon the use of a semi-solid tubed medium containing the carbohydrate (glucose) together with a pH indicator. Duplicate tubes of medium are inoculated by stabbing and one tube is covered with a layer of sterile mineral oil. This medium differentiate fermenting (e.g. *Enterobacteriaceae, Aeromonas*

spp. and *Vibrio* spp.), oxidizing (e.g. *Pseudomonas* spp.) and glucose not-utilizing organisms (e.g. *Alcaligenes* spp.). It should be noted that this medium may also be used for recording gas production and motility.

TESTS TO SHOW THE RANGE OF CARBOHYDRATES AND RELATED COMPOUNDS USED

A large variety are used and they are often referred to loosely as "sugars" e.g.

- Monosaccharides
  Pentoses – arabinose, xylose, rhamnose
  Hexoses – glucose, fructose, mannose, sorbose, galactose
- Disaccharides
  Sucrose, maltose, lactose, trehalose, cellobiose
- Trisaccharides
  Starch, inulin, dextrin, glycogen
- Polyhydric alcohols
  Glycerol, erythritol, adonitol, mannitol, dulcitol, sorbitol, inositol
- Glycosides
  Salicin, aesculin
- Organic acids
  Dextro-, laevo-, meso-tartrate, citrate, mucate, gluconate, malonate.

For practical purposes these tests are of two kinds. The majority are tests simply for the production of acid and gas or acid alone. A minority of the tests are more complicated. This is a test for the ability of bacterial culture to utilize compound as the sole carbon and energy source for growth.

TESTS FOR SPECIFIC BREAKDOWN PRODUCTS

Many bacteria ferment carbohydrates with the production of acetyl methyl carbinol or its reduction product butylene glycol. The substances can be tested for by a colorimetric reaction: Voges-Proskauer (acetoin production) test. Another test is demonstration of the ability of an organism to oxidize gluconates to the 2 keto-gluconate (gluconate test).

TESTS TO SHOW ABILITY TO UTILIZE A PARTICULAR SUBSTRATE E.G. PROTEINS AND AMINO ACIDS

Proteolytic organisms digest proteins and consequently may liquefy gelatin or coaguled serum:

- *Liquefaction of gelatin* is the commonest of proteolytic property. The method employing sterile discs of formaldehyde-denatured gelatin containing powdered charcoal is a very rapid and convenient test for proteolysis. Liquefaction of the gelatin is shown by the settling of free carbon particles to the test medium.
- *Indole test* demonstrates the ability of certain bacteria to decompose the tryptophane to indole which accumulates in the medium. Indole is than tested for by a colorimetric reaction with *p*-dimethyl-aminobenzaldehyde (Kovac's reagent).
- *Phenylalanine deaminase test* indicates the ability of bacteria to deaminate phenylalanine with the production of phenylpyruvic acid which will react with ferric salts to give a green colour.

TESTS FOR ENZYMES

Tests for studying enzymatic activities:

- *Catalase test.* This demonstrates the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide.
- *Oxidase test*. This test depends on the presence in bacteria of certain oxidases that catalyse the transport of electrons between electron donors in the bacteria and redox dye-tetramethyl-*p*-phenylene-diamine. The dye is reduced to deep purple colour. The test is used for screening species of *Aeromonas, Pseudomonas, Vibrio, Neisseria* and *Campylobacter*, which give positive reactions and for excluding the *Enterobacteriaceae*, all species of which give negative reactions.
- *Urease test.* Bacteria may decompose urea by means of the enzyme urease. The occurrence of this enzyme can be tested for by growing the organism in the presence of urea and testing for alkali (NH_3) production by means of a suitable pH indicator.

– *ONPG (β-galactosidase) test*. Lactose fermentation depends on the production of two enzymes – an induced intracellular enzyme, β-galactosidase, which hydrolyses lactose, and a permease which regulates the uptake of lactose into the cell. Certain bacteria possess the β-galactosidase enzyme but not the permease. These potential lactose fermenters may not produce acid at all in traditional peptone water media or may take several days to do so. ONPG test, which determines the presence of the enzyme β-galactosidase by utilizing o-nitrophenyl-β-galactopyranoside, is used to differentiate late lactose fermenting organisms and of particular use in the identification of enterobacteria.

– *Nitrate reduction test.* This is a test for the presence of the enzyme nitrate reductase which causes the reduction of nitrate, in the presence of suitable electron donor, to nitrite which can be tested for by an appropriate colorimetric reagent. Almost all *Enterobacteriaceae* reduce nitrate.

COMBINED TESTS

Combined tests for differentiation of bacteria:

– *Triple sugar iron (TSI) agar*: This is a non-inhibitory indicator medium used primarily to confirm that colonies isolated on BG or XLD media are those of salmonellae. TSI agar contains 0.1% glucose, 1% lactose and 1% sucrose and chemicals to indicate H_2S production.

– *Commercial identification systems*: Identification methods are readily available from commercial sources. Some (e.g. API, Biolog) are essentially a series of miniaturized classical biochemical tests. Dehydrated substances in plastic cups are reconstituted when a suspension of the bacteria under investigation is injected. A biochemical profile of the organisms is obtained after 24–48 h incubation at 37 °C. The profile is translated into a numerical code which can be read from a key (Code Book). Computer-based identification or on-line services for identification of bacteria are available.

CHROMOGENIC AND FLUOROGENIC MEDIA

All the major food pathogens can be screened using chromogenic/fluorogenic media in a wide variety of food samples: *Salmonella, Campylobacter, Listeria, Listeria monocytogenes, Staphylococcus/S. aureus,* coliforms/*E. coli* as well as

specific target organisms such as, *E. coli* O157. Chromogenic and fluorogenic media are selective agars that contain enzyme substrates linked to a chromogen (colour reaction), fluorogen (light reaction) or a combination of both. The enzyme substrates, e.g. ONPG, CPRG, and MUG are organism specific or where they are not, the target organism is selected for by suppression of competing microflora. The target pathogen is characterized by enzyme systems that metabolize the substrate to release the chromogen/fluorogen. This results in a colour change in the medium and/or fluorescence under long wave UV light. Modifications to the incubation regime can add to the selectivity of the media and in some cases the target organism is confirmed without further testing.

ANTIBIOTIC RESISTANCE IN PATHOGENIC MICROORGANISMS

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Antibiotics are drugs of biological, semi-synthetic or synthetic origin that are used in antimicrobial therapy. The main property of antimicrobial agents is their selective toxicity to bacteria; therefore, they are design to attack only bacterial cellular structures. Two main effects to microorganisms can be attributed to antibiotics, bactericidal and bacteriostatic. Bactericidal antibiotics directly kill the micro-organism, the latter affect reversibly and inhibit bacterial growth in the host.

According to the mechanism of their action, antibiotics can be divided into five groups. For example, β-lactam antibiotics inhibit synthesis of the cell wall by the interaction with penicillin binding proteins (PBP). Another kind of action is the inhibition of proteosynthesis (typical for aminoglycosides, tetracycline or chloramphenicol). Ansamycin acts as the inhibitor of RNA synthesis by the inhibition of RNA-polymerase and quinolones influence the DNA synthesis by the inhibition of DNA-gyrase. Finally, sulfonamides and trimethoprim act as competitive inhibitors in the synthesis of the folic acid.

Like all drugs used in medical treatment, antibiotics may exhibit some negative impacts, either biological or immunological. They can be toxic in higher doses for the host, they can negatively affect normal/natural/common intestinal microflora or caused inadequate allergic reactions. Considerable problems arise from the antibiotic resistance that is nowadays massively spread throughout bacterial populations.

Resistance to antimicrobial agents is a common response of microorganisms to the changing environmental conditions and a number of mechanisms for decreasing concentration and impact of antibiotics have been described. These include destruction or modification of antibiotic by variety of enzymes

(hydrolases, transferases), exclusion of the antibiotic by changes in the permeability of the cell wall or by active transport mechanism (efflux pump). Another mechanism is based on the expression of an altered molecule that functionally substitutes the target one.

Bacteria can acquire resistance by several ways, either by accumulation of point mutations in the target gene (the typical example is gene coding for the variant of the gyrase enzyme leading to the nalidixic acid-resistant strains) or much more frequently by integrating of new resistance genes due to the horizontal transfer. Horizontal gene transfer is very common in Gram-negative bacteria and dissemination of antibiotic resistances due to the conjugation now appears to be the best tool for large-scale genetic exchange between many different bacterial species and genera.

The dissemination of antibiotic resistances is enabled by mobile genetic elements on which resistance genes are usually located. These include IS elements (Insertion Sequences), transposons, gene cassettes incorporated into integrons, and R-plasmids.

IS elements and transposons can move within the genome due to the function of the transposase protein encoded by the *tnp* gene of the elements. Transposons represent composite elements that are bounded on both ends by IS elements and include one or more antibiotic resistance genes. In Gram-negative bacteria, most of the transposons belong to the Tn*3* family of transposable elements and the typical representative is Tn*21*. The Tn*21* subgroup of the Tn*3* family contains closely related elements that are responsible for multiple resistance to antibiotics. The members of Tn*21* subgroup are frequently found on high molecular weight conjugative plasmids enabling their further dissemination. Tn*21* subgroup comprises many related transposons differing by the resistance gene content. For example Tn*21* transposon carries *aadA1* gene coding for the streptomycin resistance, and Tn*1721* harbours genes for tetracycline resistance.

Antibiotic resistance genes can also be found as gene cassettes incorporated into the integrons. Gene cassettes are small mobile elements that carry only one antibiotic resistance gene lacking the promoter, and so-called 59-bp element. Gene cassettes can exist either as free covalently closed circles or incorporated into the integron structure. Integrons are genetic elements encoding a site

specific recombinase (integrase) which is responsible for insertion and excision of gene cassettes. The process of incorporation is based on the homologous recombination between 59-bp element of the gene cassette and the *attI* site specific for the integron.

Up to now, four classes of integrons have been described based on the similarity of the genes coding for integrase proteins. In Gram-negative bacteria, the class 1 integrons are widely distributed. They consist of two conserved sequences, the 5´CS and 3´CS, carrying *intI* gene for the integrase protein and *sul1* gene for resistance to sulfonamides, respectively. In between these two conserved sequences, one or more gene cassettes can be integrated.

In *Salmonella* genus, class 1 integrons are most frequently associated with the emergence of pentadrug resistant *S.* Typhimurium DT104 clone. In this clone, two class one integrons are integrated into the chromosome forming the resistance region of the *Salmonella* Genomic Island 1 (SGI1). In this resistance region, five antibiotic resistance genes are located. These are $bla_{\text{PSE-1}}$, *floR*, *aadA2*, *sul1* and *tetG* encoding resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline, respectively. Pentadrug resistant *S.* Typhimurium DT104 isolate was detected for the first time in the United Kingdom in mid 1980's and since that time it has reached a worldwide distribution. In the Czech Republic, this clone first appeared in 1990 and now almost 90% of antibiotic resistant *S.* Typhimurium strains belong to pentaresistant DT104 isolates. Reasons for such successful spread are still unknown. It has been supposed that this clone might be of increased virulence or could be better adapted for survival in the environment due to the efficient biofilm formation.

Since integrons are responsible for the acquisition of new resistance genes they are therefore considered to be the assembly units involved in the formation of large resistance islands. Further, integrons are frequently found to form parts of transposons that can thus function as vectors for their subsequent dissemination. The typical vehicles of such composite elements are R-plasmids.

R-plasmids are characterized as high-molecular weight plasmids harbouring genes for resistance to multiple antimicrobials. Two functionally distinct regions can be recognized in the structure of R-plasmids, resistance transfer factor (RTF)

responsible for autonomous replication (*rep*), the production of pilli and for self-transmissibility (*tra*), and R-determinants consisting of individual transposons. Many multiresistance plasmids of gram-negative bacteria appear to have evolved from a common ancestor and later on have successfully spread into the current population of bacteria. In *Salmonella*, similar plasmids were found among current plasmids coding for antibiotic resistances and among a collection of plasmids from the pre-antibiotic era isolated between 1917 and 1950. The typical example and the archetype of many current R-plasmids is represented by plasmid NR1. This plasmid of 94.5 kb was originally isolated from *Shigella flexneri* in Japan in the late 1950's, and is composed of three transposons, Tn*9* with *catA1* gene, Tn*21* with *aadA1* gene cassette and *sul1* gene and Tn*10* with *tetA* gene, coding for the chloramphenicol, streptomycin, sulfonamide, and tetracycline resistance, respectively.

Due to the considerable mobility of transposons and due to the ability of integrons to incorporate new gene cassettes, the structure and resistance gene content can vary in R-plasmids. R-plasmids might therefore serve not only as vectors in horizontal transfer but can serve as the place where the new combinations of antibiotic resistance determinants can develop.

PHAGE TYPING OF BACTERIAL SPECIES

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Bacteria may need to be characterized for numerous reasons either to determine their taxonomic position or to provide an indication of their pathogenic potential. Such studies generally involve characterization to the genus and species level. Highly discriminatory methods for intra-species characterization are required for epidemiologic purposes in order to establish sources and routes of infection. Methods used for bacteria typing are resolved into phenotype and genotype based ones. Phage typing is one of the most frequently used standardized method determining the phenotype (the behaviour of the bacteria) and it became a well-established procedure in the routine epidemiological investigation of foodborne infections of bacterial origin.

Nearly all bacteria may be typed by their susceptibility to a range of bacteriophages (viruses whose host is a bacterial cell). Bacteriophage (phage) are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery. Bacteriophages are specific in action. A particular phage may be very specific and will infect only a few strains of a certain bacterial species. On the other extreme, another phage may infect strains of two or more species of a particular genus. Susceptibility to lysis by a particular phage may be the only apparent phenotypic difference between two bacterial strains and may be the only means by which a strain causing an outbreak of disease can be recognized. This observation is the basis for phage-typing, a procedure for characterizing and detecting bacterial strains by their reaction (susceptibility or resistance) to various known phages. As typing phages lysogenic phages (carried or temperate), phages produced by adaptation and virulent phages have been used.

THE STANDARD PROCEDURE

Tested bacterial cultures are grown with shaking in nutrient broth to the exponential growth phase (1–2 h), flooded onto the well dried nutrient agar plates and spotted with testing phages in routine test dilution (RTD). After incubation usually at 37 °C for 18–24 h aerobically the reactions with individual phages are evaluated as sensitive (confluent or semi-confluent lysis) or negative (no or very low number of plaques). Individual patterns are evaluated according the scheme and strains are ranked in phage types (PT).

This method is standardized on the international level, it is rapid, easy to perform and cheap, on the other hand only reference laboratories are supplied by the sets of international phages and control strains.

Phage typing procedure has been applied to several bacteria. In foodborne pathogens it is currently used for typing of certain *Salmonella* serotypes (*S.* Typhi, *S.* Paratyphi A, *S.* Paratyphi B, *S.* Java, *S.* Enteritidis, *S.* Typhimurium, *S.* Hadar, *S.* Virchow, *S.* Thompson, *S.* Agona), *Listeria monocytogenes*, *Campylobacter jejuni,* VTEC and *Staphylococcus aureus*.

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MOLECULAR EPIDEMIOLOGY OF MYCOBACTERIAL INFECTIONS

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The members of *Mycobacterium tuberculosis* complex (*MTC*) and *Mycobacterium avium* complex (*MAC*) are the most prevalent mycobacteria, which infect both animals and humans. Recent molecular studies have shown the presence of very close genetic relationships between different members of the above mentioned respected complexes. The members are known to harbour different numbers of tandem repetition in specific loci and multiple copies of a number of different genomic insertion elements (IS*6110*, IS*901* and IS*1245* etc.) that are usually specific for each species or subspecies. These features permit insertion elements to be used for species or subspecies identification and also for the typing of these strains. A crucial aspect of any control program is the ability to determine where transmission is occurring in other to prevent further spread of infection and prevent active disease by identifying newly infected animals or people.

The most common typing method used in molecular epidemiology of *MTC* members was IS*6110* RFLP analysis and spoligotyping (Pavlik *et al*., 2002; Erler *et al*., 2004; Prodinger *et al.*, 2005). Recently, a new method based on a variable number of tandem repeat (VNTR-MIRU typing methods; Supply *et al.,* 2006) was applied.

For the study of molecular epidemiology of *MAC* typing analyses were used, in particular restriction fragment length polymorphism (RFLP; van Soolingen *et al*., 1998; Pavlik *et al*., 1999; Dvorska *et al*., 2003; Dvorska *et al*., 2004) and pulsed gel field electrophoresis (PFGE; de Juan *et al*., 2005; Sevilla *et al*., 2007). Both methods are time consuming, requiring large mass of culture and do not discriminate between some not related strains. For these reasons new methods based on PCR are under investigation.

The insidious nature of tuberculosis coupled with the long environmental persistence of the mycobacteria strongly indicates a need to prevent the establishment of the disease in domestic animals and those in captivity. The molecular epidemiology studies can help to explain the presence of all members of *MTC* and *MAC* in both infected tissues and the environment (i.e. drinking water, soils, manure and dust). In some cases it is difficult, if not impossible to protect humans, animals and birds from exposure and infection. Moreover, control and/or eradication programs could be complicated with the existence and strong involvement of wild life and environmental reservoirs. Thus, early detection of infected birds and animals is significantly helpful to hamper the distribution of the disease.

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OVERVIEW OF DNA TYPING METHODS

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An increased demand for differentiation of pathogenic microorganisms circulating in the environment naturally results into an increased demand on new typing methods. Although the traditional methods like biochemical characterisation, serotyping or phage typing still remain valuable tools in differentiation of bacterial pathogens, these are frequently supplemented by the methods based on the analysis of genetic material.

Characterisation of bacterial isolates usually starts by serology. Serological typing is quite widespread and is in routine use in *E. coli* or *Salmonella*. Such typing is based on the variability of sugar residues forming O antigen, on variability in amino acid sequence forming bacterial flagella, or on the presence of additional polysaccharides forming capsule.

When this typing is not sensitive enough, it can be extended by phage typing or resistance to antibiotics. Phage typing is based on different sensitivity of field isolates to a standardized set of bacteriophages. The sensitivity or resistance to a particular phage can be dependent on presence or absence of different receptors on the surface of a bacterial cell, it can be also dependent on presence or absence of different restriction modification system, or can be dependent on presence of prophage present in the genome of analysed strains. Resistance to antibiotics, although not usually used for typing, may also serve as characteristic marker. It has to be considered that some of the resistance may be plasmid encoded and therefore need not be a stable marker.

Out of the DNA based typing techniques, plasmid profile analysis is the oldest one. It is based on the fact that different field strains may or may not contain plasmids of different sizes. When these are release from bacterial cell by a suitable protocol and separated by gel electrophoresis, spectrum of DNA fragments can be observed and compared. To increase differentiation power of the plasmid profile

analysis, prior the electrophoresis, the plasmid DNA can be digested by restriction endonucleases. Limiting factor for plasmid profile analysis might be the fact that some bacterial species do not contain plasmid regularly. Second point to be considered when using plasmid profile analysis is relative instability of plasmids in the host cell and therefore relative instability of this marker.

When chromosomal DNA is used as a target for bacterial strain differentiation, although there are multiple typing methods, 3 of them worth real consideration. Current gold standard of bacterial DNA typing is the macro-restriction analysis. During this procedure, the chromosomal DNA is carefully purified from bacteria embedded in agarose plugs and digested by restriction endonucleases which cut the DNA at limited number of positions. This is usually achieved with restriction endonucleases recognizing 8 bp long sequence like NotI or SpeI. Sometimes even some 6 bp long sequences appear very rarely in bacterial genomes and restriction endonucleases recognizing such sequences can be used for macro-restriction analysis as well. Typical example for this is *Salmonella* chromosomal DNA and XbaI restriction endonuclease. Long DNA fragments generated by the "rare cutters" are then separated by pulsed field gel electrophoresis, which enables separation of DNA fragments hundreds of thousand bp in size.

Another alternative is based on PCR detection of mobile DNA sequences. These can be different plasmids, insertion sequences or integrated prophages. These PCRs, frequently assembled in a multiplex format have been successfully used in *E. coli* clustering of field strain and are being introduced in *S.* Typhimurium typing too. Indeed, when we performed 4 triplex PCRs specific mostly for prophage sequences, we observed nearly the same discriminatory power as for the macrorestriction analysis while the results were much simple to obtained.

The last technique which can be used for strain characterisation is the microarray genomotyping. Great advantage of this method is that the whole genome of a bacterial strain under investigation is analysed in one time. During this procedure, labeled DNA from a strain under investigation is let to hybridise with a DNA chip containing a whole genome of the bacterial species of interest. Disadvantage at the moment is a rather high cost of the analysis and also the fact, that only the genes present on the DNA chip but absent in the genome of investigated strain can be detected. If the strain under investigation posses additional genes which are not present on the chip, these will remain unrecognised.

In a summary, the most widespread methods used bacterial differentiation are the serological methods and phage typing. When more detailed characterisation is needed, and some of the DNA typing of pathogenic bacteria is required, macrorestriction analysis is used the most frequently and currently is considered as a gold standard in bacterial DNA typing. It appears that at least in some of the case, the macrorestriction analysis could be replaced by multiplex PCRs targeted to mobile DNA fragments. Less frequently plasmid profile analysis as rather obsolete, or microarray analysis, as on the other hand not too standardised newly introduced protocol are also be in use.

PARTICIPANT ABSTRACTS

DETECTION OF FOODBORNE PATHOGENS AND BACTERIA CAUSING ZOONOSIS

DETECTION OF *RICKETTSIA CONORII* USING PCR AND IMMUNOFLUORESCENCE METHODS

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The aim of the study was to compare the results of PCR and immunofluorescence methods for the detection of *Rickettsia conorii* in sheep serum samples collected from sheep farms located in different areas of Cyprus. The PCR assay conditions were as specified in the Blood and Cell culture DNA Kit (GENOMED GmbH). The immunofluorescence assay conditions were as specified in the *R. conorii* – Spot IF Kid (bioMerieux). *R. conorii* is widely spread in Cyprus among animal farms mainly because the climate favours the breading of brown ticks, which are the main carriers of the disease. *R. conorii* is a zoonosis transmitted to human mainly after contact with infected animal. The principal route is by aerosols. Common symptoms of disease in humans include febrile illness, nausea, vomiting, abdominal pain, encephalitis, hypotension, acute renal failure, and respiratory distress. Case fatality is very low. During the study 20 samples were examined for *R. conorii* using the methods of PCR and immunofluorescence.

The results showed a suggestive past infection in 9 out of 20 samples tested with immunofluorescence, greater than 1:256 presence of IgG antibody to *R. conorii* detected. The PCR results for those 9 samples were negative. A suggestive recent infection in 5 out of 20 samples tested was found with immunofluorescence, greater than 1:64 presence of IgM antibody to *R. conorii* detected. The PCR results for those 5 samples were positive. The immunofluorescence showed no presence of IgG or IgM antibodies in 3 out of 20 samples. The PCR results for those 3 samples were positive suggesting that the infection took place very recently, maximum 6 days ago. The immunofluorescence showed no presence of IgG or IgM antibodies in 3 out of 20 samples and the PCR results for those 3 samples were negative suggesting no infection (Table 1).

PCR showed to be an ideal method in detecting the infection of *R. conorii* at the very early stages of the disease. The immunofluorenscence based method is efficient only after one week of infection. However, PCR cannot be used to

detect *R. conorii* in cases of past infection. The main conclusion is that in cases where the clinical symptoms are early detected PCR is more suitable for rapid detection of the infection. In cases of massive sampling for the detection of the disease in a flock, past and present immunofluorenscence is more suitable. Examination of samples using both methods is the best practice for detection of *R. conorii* leading the researcher to solid results.

Table 1. Detection of Rickettsia conorii *infection using PCR and immunofluorescence methods.*

Samples	Immunofluorescence	PCR	Diagnosis
9	>1:256 presence of IgG antibody	Negative	Past infection
5	>1:64 presence of IgM antibody	Positive	Recent infection
3	No presence of IgG or IgM antibodies	Positive	Very recent infection
3	No presence of IgG or IgM antibodies	Negative	No infection

A CAPILLARY POLYMERASE CHAIN REACTION FOR *SALMONELLA* DETECTION FROM POULTRY MEAT

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In this study, a capillary polymerase chain reaction (cPCR) was applied for *Salmonella* detection from poultry meat. The *Salmonella* detection limits of the optimized cPCR were determined with DNA templates from the samples of tetrathionate broth (TTB), Rappaport Vassiliadis broth (RVB) and selenite cystine broth (SCB) artificially contaminated with 10-fold dilutions of 6×10^8 CFU ml^{-1} of pure *Salmonella enterica* spp. *enterica* serovar Enteritidis 64K stock culture. Detection limits of cPCR from TTB, RVB and SCB were found to be 6, 6×10^1 and 6×10^4 CFU ml^{-1}, respectively. In addition, detection limits of bacteriology were also determined as 6 CFU ml^{-1} with TTB and SCB, and 6×10^1 CFU ml^{-1} with RVB. A total of 200 samples, consisting of 100 chicken and 100 turkey meat samples, were tested with optimized cPCR and bacteriology. Eight and six per cent of the chicken meat samples were found to harbour *Salmonella* by cPCR and standard bacteriology, respectively. Of six *Salmonella* isolates, four belonged to serogroup D, two to serogroup B. The TTB cultures of both artificially and naturally contaminated samples were found to be superior to those of RVB and SCB cultures in their cPCR results. This cPCR, utilizing template from 18-h TTB primary enrichment broth culture, takes approximately 40 min in the successful detection of *Salmonella* from poultry meat.

DISCRIMINATION OF *SALMONELLA* TYPHIMURIUM STRAINS BY MLVA AND PCR-PROPHAGE TYPING METHODS

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The aim of this study was to investigate the genetic variability of *Salmonella* strains caused by lysogenic bacteriophages and to compare the results with multilocus VNTR analysis of the same strains. 152 S. Typhimurium strains isolated from human clinical samples and from food and veterinary sources in Slovak Republic were included in this study. The diversity of strains was measured using PCR detection of selected prophage regions derived from phages P22, Gifsy-1, Gifsy-2, Fels-1, ST104 and SopEΦ. MLVA typing was based on five VNTR loci. The presence of one to eight different genes for a given phage was screened in *Salmonella* strains and we observed a high degree of variability in incidence for specific genes. The *gtg*B (Gifsy-2), g45 (ST104) and g8 (P22) genes exhibited the highest prevalence in our testing set, ranging from 83% to 97%. In contrast, the lowest frequency was observed for g13 (1.5%, P22) and *sop*E (2.6%, SopEΦ). Three genes (*eae*1, *sie*A of P22 and *nan*H of Fels1) were not detected in any of the *Salmonella* strains tested. Based on the presence of particular prophage genes, the strains were divided into 37 different PCR-prophage profiles out of which 20 profiles were represented by only a single strain. In MLVA typing, 152 Salmonella strains were separated into 82 MLVA strings. The degree of variability differed between the tested loci, both STTR3 and STTR9 loci being less polymorphic than the STTR5, STTR6 and STTR10 loci. In a comparison with other *S.* Typhimurium, the DT104 strains contained mostly alleles with larger numbers of repeats. It was observed that similar grouping of *Salmonella* strains using PCR-prophage detection and MLVA and the results corresponded well with the phage type of strains. However, several *Salmonella* strains, which were closely related according to MLVA, were detected, but yet they differed in PCR prophage profiles. These observations supported a view that integration/excision of bacteriophages in Salmonella strains was frequent shaping the bacterial genome. The PCR-prophage typing can successfully be used for molecular typing of S. Typhimurium strains.

THE PREVALENCE OF *CAMPYLOBACTER* SPP. AT THE FARM AND RETAIL LEVEL

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The aim of the study was to investigate the *Campylobacter* spp. contamination at the farm and retail level. A total of 102 faecal samples of poultry origin were collected in 2004. A total of 610 raw poultry meat samples were purchased from retail outlets in Estonia. *Campylobacter* spp. was detected using a modification of the NMKL Method no 119, 2nd ed. 1990, which includes the enrichment in Preston or in Bolton broth. Enrichment media was plated out on selective modified Charcoal Cefoperazone Deoxycholate agar (mCCDA; Oxoid). Typical colonies were streaked on Brucella agar, and verified by the Gram-reaction, motility-test, oxidase and catalase test, and hippurate hydrolysis. Fifty-two samples were collected from the farm A and of those 29 (55.8%) were positive. All samples of farm B origin were negative. Farm A samples were collected at the beginning of October and samples from farm B were collected at the beginning of November in order to investigate the presence of seasonal variation. There was a seasonal variation in flock colonization with *Campylobacter* spp. The overall prevalence of *Campylobacter* spp. in raw poultry products was 12%. Of the isolates, 77% were identified as *C. jejuni*. In conclusion, raw poultry products of Estonian origin are contaminated by *Campylobacter* species. An Estonian *Campylobacter* control programme is currently under development, and additional studies of different foods, at farms and processing plants are needed.

FLUORESCENT *IN SITU* HYBRIDIZATION – A RAPID METHOD FOR DETECTION OF FOODBORNE PATHOGENS

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Fluorescent *in situ* hybridisation (FISH) is a sensitive and rapid molecular technique, which is useful for many phylogenetic, ecologic, diagnostic, and environmental studies in all fields of microbiology. It is based on the hybridisation of a genomic sequence (oligonucleotide probes) characteristic of a micro-organism with a specific probe labelled with a fluorochrome and the simultaneous identification of the pathogens by fluorescent microscopy. FISH procedure includes four major steps: fixation and permeabilisation of the bacterial cells, hybridisation of the probe and the target sequence, stringency washes to remove excess probes and immediate visual detection and documentation of the labelled cells by fluorescent microscopy. The conventional cultural methods for the detection of pathogen bacteria in foods only allow presumptive results after 5 days. Over the past years, many rapid methods for the detection of pathogenic microorganisms have been developed, generally less time consuming and labour intensive than conventional microbiological methods. These rapid methods include automated cultural methods, commercial tests, immunomagnetic techniques, hybridisation and PCR. Some advantages of FISH over conventional cultural methods includes decreased proneness to inhibitory substances; identification of viable but non-cultivable cells; rapid availability of quantitative results; the possibility of simultaneous identification of different species in the same sample; relatively low cost per experiment; and easy to carry out. Although its application is fast and cheap, development and evaluation of probes for diagnostic purposes should be restricted to expert reference laboratories with facilities for culturing as well as molecular techniques. FISH allows the detection of both cultivable and nonculturable micro-organisms, and can be used in order to determine complex microbial communities. The in situ hybridization technique is less sensitive than PCR, although its sensitivity of detection could be increased considerably after an

enrichment step. The major advantage of in situ hybridization is the association of a reaction (nucleic acid hybridization) with cytological information such as shape and size of bacterial cells, grouping of these cells, relationships with other cells (including eukaryotic or host cells) or structures. FISH with rRNA oligonucleotide probes has been used for detection and identification of different microbial species, the application of this technique in food samples has already been described for detection of foodborne pathogens such as *Staphylococcus* spp., *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes* and *Legionella* spp. allowing the screening of a higher number of samples in less than 24 h.

RAPID DETECTION OF *B. CEREUS* THROUGH ENTEROTOXINS IN SOME SPICES SOLD FOR CONSUMPTION IN IZMIR

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Bacillus cereus is the most important cause of food poisoning from this group due to its ability to produce enterotoxins and emetic toxin. There are two principal types of food poisoning caused by *B. cereus*, namely, the emetic and the diarrhoeal types. *B. cereus* is also known to spoil food products. Therefore, its presence in food processing plants should be minimal. Good diagnostic tools are thus required to ensure the hygienic quality of susceptible food items. Several selective plating methods have been described for detecting *B. cereus*. The selection is based, for instance, on the ability of *B. cereus* to grow in the presence of polymyxin B and its lecithinase reaction. These methods require, with confirmatory testing, up to 4 days to perform. The presence of *B. cereus* strains that cause food poisoning can also be indicated by their toxins. The aim of the present study was to detect *B. cereus* via their major enterotoxins non-hemolytic enterotoxin (NHE) and hemolysin BL (HBL) by the commercial lateral flow test kit rapidly. Samples of 50 different spices purchased from local markets were examined to determine the incidence of *B. cereus*. The spices examined were composed of a variety of spices, such as black pepper, cumin, ground hot red pepper, flaked hot red pepper, cinnamon, offered for sale in the markets and district bazaars in Izmir. 10 g of the sample was added to 90 ml case-amino acids glucose yeast extract (CGY) Broth (with 1% glucose) and homogenized. Then 200 µL of the homogenized sample was mixed in 20 ml CGY Broth (with 1% glucose) in 200 ml flask and incubated at 37°C for 18–24 h. After incubation commercial enterotoxin test kit were applied and results obtained in 30 min. In case of positive results cultural confirmation with additional biochemical tests were carried out. Enterotoxin producing *B. cereus* was present in 15% of spices. The average count of *B. cereus* varied from 10^2 to 10^5/g.

DETECTION OF PATHOGENIC MICROORGANISMS USING AN ELECTRONIC NOSE

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Electronic noses, which are used for characterizing complex vapors and aromas, may be useful for detection of bacterial contamination. Classical bacterial contamination detection methods for food products especially for perishable foods is of limited value in early stages because these foods could be eaten before the results of microbiological tests are available. Since many of the detection methods are time consuming, this long delay involved in determining the status of food products have generated an interest in developing rapid methods of detection especially using non-destructive techniques. Metabolic activities of microorganisms in food products produce metabolites in the form of gases. Sensing these metabolites in the headspace of food products could be used to determine the quality of that product. The use of electronic noses for determining the quality of food products as a means of non-destructive method is becoming widespread, fast, and reliable. Recently, electronic noses have been used to detect the volatile compounds produced by growing bacteria. In one of the studies milk spoilage caused by single bacterial species *Pseudomonas fluorescens* or *Bacillus coagulans* was during storage discriminated using an electronic nose. Another study showed that microorganisms e.g. *Bacillus subtilis* CRA 14160, *Penicillium verrucosum* Vmmope 20-07 and *Pichia anomala* NCPF were detected using an electronic nose before the spoilage was visible on the bread. Other researchers also concluded that the spoilage of beef strip loins, inoculated with *Salmonella typhimurium* and stored at 20 °C, was determined using an electronic nose and the *Salmonella* counts correlated well with the electronic nose data. The instrument was also used for detection of microorganisms in potable water. In the view of these research studies, electronic nose data correlated well with the microbial counts and can thus be used as a potential instrument for a rapid and non-destructive microbial detection method.

DETECTION OF PATHOGENS IN FOOD, FEED, POULTRY FAECES, CLINICAL MATERIALS AND SMEARS

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In our laboratory we do different kinds of examination for pathogenic microorganisms (*Salmonella*, *Campylobacter*, *Listeria monocytogenes*, *Staphylococcus aureus*) in different samples. Every month we receive about 2000 samples, that we examine for *Salmonella*. In the *Salmonella* isolation we use the ISO method with modifications (Modified semi solid Rappaport Vassiliadis medium). When we need rapid result, we use Mini Vidas and Tecra Unique rapid methods.

DETECTION AND IDENTIFICATION OF FOODBORNE PATHOGENS

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I am an employee of the Institute of Public Health of the Republic of Slovenia, Communicable Diseases Centre, Department for Medical Microbiology, where I am working with enteric pathogens, which cause also foodborne diseases and which are isolated from both clinical and food samples. The most frequently isolated bacteria are *Salmonella* spp. and *Campylobacter* spp., and less frequently are diarrheagenic *E. coli*, *Yersinia enterocolitica* and *Shigella* spp. ISO and classical methods are used for *Salmonella* isolation and detection and the serotyping is performed according to the Kauffman-White scheme. Antibiotic resistance is provided with disk diffusion method according to NCCLS.

IMPLEMENTATION OF PULSED-FIELD GEL ELECTROPHORESIS IN DETECTION OF *SALMONELLA* TYPHIMURIUM FROM HUMAN ISOLATES

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Pulsed-Field Gel Electrophoresis (PFGE) is of great value in epidemiological analysis for differentiation of pathogenic strains and in the monitoring of their spread among communities. This technique has been successfully employed in tracking diseases caused by a number of different bacterial pathogens. Daily we use PFGE for determinating borrelial species. We employed this technique to develop rapid PFGE for detecting *Salmonella* Typhimurium phage types. We focus our study on phage type DT104 (definitive type 104). Serotypes of *S*. Typhimurium with this phage are usually resistant to many antibiotics. 95% of isolates of *S*. Typhimurium DT 104 are genetically identical, they are monoclonal and can be identified by their PFGE patterns. We tested 145 isolates of *S*. Typhimurium from human isolates for their antimicrobial susceptibility for 12 antibiotics with disc diffusion test. We found 35 isolates with fenotype of antimicrobial susceptibility caracteristic for *S*. Typhimurium DT 104. We extracted DNA from bacterial colonies of multi resistant isolates of *S.* Typhimurium after growing overnight on plates. Cell suspension with 20% of transmittance, was used for extraction. Extracted DNA was mixed with agarose and digested with *Xba*I enzyme. On the basis of resistant phenotypes and PFGE paterns of our tested isolates we confirmed that 28 out of these 35 isolates belonged to *S.* Typhimurium DT 104 (19% of all isolates). *S.* Typhimurium DT 104 has evidently reached our territory.

PARTICIPANT ABSTRACTS

RISK ASSESSMENT AND RISK MANAGEMENT IN FOOD PROCESSING

FOOD SAFETY AND SOME PREVENTIVE MEASURES IN BAKERIES

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Food safety is a growing global concern not only because of its continuing importance for public health but also because of its impact on international trade (Barendsz *et al.*, 1998). Bakery products are an important part of a balanced diet and, today, a wide variety of such products can be found on supermarket shelves. This includes unsweetened goods (bread, rolls, buns, crumpets, muffins and bagels), sweet goods (pancakes, doughnuts, waffles and cookies) and filled goods (fruit and meat pies, sausage rolls, pastries, sandwiches, cream cakes, pizza and quiche). However, bakery products, like many processed foods, are subject to physical, chemical and microbiological spoilage (Smith *et al*., 2005). *Penicillum* spp. and *Aspergillus* spp. are most common fungal contaminants which have been isolated from bakery products. Mycotoxigenic moulds can produce mycotoxins such as aflatoxins and ochratoxin A in inoculated breads. Pathogens, such as *Salmonella* spp, *Listeria monocytogenes* and *Bacillus cereus* have also been isolates from bakery products. Spores of *B. subtilis, B. licheniformis* or *B. mesentericus* can survive baking process, cause bread rope disease and therefore affect the quality of bread. Food process hygiene is very important because many people could be affected by foodborne illnesses. Most microbiological spoilage problems with bread occur after baking, during cooling process, slicing and packing. Therefore, cleaning and disinfection of slicers, conveyor belts, cooling lines and packing machines is very important to decrease the risk of further spoilage. Also the temperature of production air and its microbiological condition affect final product quality. Physical spoilage can occur when production process has not monitored enough. It can be pieces of packaging material, human hair, nails, jewelleries, pieces of equipment etc. Adequate and timely maintenance for all process machines is also necessary to avoid serious problems. Chemical spoilage can be residues of cleaning or disinfection chemicals, which are not washed out from utensils and process machines. Personnel hygiene and also training have a great role in food safety.

Effective HACCP or own-checking plan (OCP) have to be implemented in all food productions to guarantee the food safety and control over the whole process, starting from raw material. There are also several other methods for decreasing the risk of microbiological spoilage such as using preservatives, packaging under modified atmosphere using CO_2 and N_2 as well as radiation after packaging e.g. UV, infrared and microwaves. Some of these methods are very expensive. An increasing number of companies are striving for a certificate, to realize both 'external benefits' as part of their market strategy and 'internal benefits' to open up a way to enormous improvements and efficiency (Barendsz *et al*., 1998). ISO 9001, ISO 22000 and British Retail Consortium (BRC) Food standards are some options, which can help to improve the quality management system and product quality as well as food safety.

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RISK ASSESSMENT IN PRODUCTION OF SMOKED TURKEY HAM

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The scope of this study was to estimate the risk of serious illness due to *L. monocytogenes* after consuming ready-to-eat smoked turkey ham produced, marketed and stored under usual conditions in Cyprus. This study focused on the 'fork' risk assessment since most of the exposure data for *L. monocytogenes* in smoked turkey ham currently available is relate to the frequency and extent of contamination at retail level. The current risk assessment is evaluating the steps of smoked turkey that might pose risks to the safety of the final product. It focuses mainly on the probability of contamination of such products in the production step after cooking and before packaging i.e. during the handling taking place in the slicing room. Several post-retail factors that can influence the risk on a consumer acquiring foodborne listeriosis, e.g. temperature and time frame of refrigerated storage are also evaluated. In the Community summary report for zoonoses from 2005 it was mentioned that only 24% of reported *Listeria* outbreaks had information on contributing factors including inadequate heating of contaminated raw material, improper storage temperature, deficiency in food handling and preparation, contamination by infected persons, poor hygiene and breakdown of HACCP systems. Factors contributing to a foodborne outbreak are often a combination of these factors. An efficient use of techniques in molecular biology would make it a bit easier to find the source of *Listeria* contamination in the processing. This type of data can then be further used in a food safety scenario based on the HYGRAM® model for the processing of smoked turkey ham.

FOOD PROCESSING HYGIENE IN PRIMARY SCHOOL CANTEENS IN ANKARA

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The aim of the study was to determine food processing hygiene quality in primary school canteens in Ankara, Turkey. This study on detection of *Escherichia coli* in raw Sucuk (Turkish sausage flavoured with garlic), which were collected from randomly selected 25 primary school canteens, was carried out in the Çankaya district of Ankara, Turkey. All specimens were examined for *E. coli* and coliforms with EC Compact Dry method, which is ready to use method for microbiological analysis of raw and finish products. The pre-sterilized plates contained culture medium and a cold water-soluble gelling agent. Two pieces (min 50 g) of sucuk samples were handled as food handlers at same conditions at school canteens. Twenty five gram of minced samples were diluted and homogenized with 125 ml of sterilized distil water. After waiting for 30 min 1 ml diluted sample inoculated onto center of the self-diffusible medium. Samples were incubated for 24 h at 35 °C as given in the manual. On the basis of the presence or numbers of *E. coli* (blue colonies at medium) sucuks were categorized as acceptable or unacceptable and potentially hazardous. Sixteen percent of sucuks were graded as unacceptable and potential hazardous because of presence of numbers of *E. coli* colonies. According to this study it was shown that Compact Dry method is a rapid and safe technique for determining *E. coli* at food stuff. This study showed that presence of pathogenic microorganisms in raw sucuk can be related to processing conditions at school canteens and occurrence of foodborne illnesses at primary schools.

DETERMINATION OF MICROBIOLOGICAL CONTAMINATION SOURCES DURING TURKISH WHITE CHEESE PRODUCTION

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This study has been conducted to determine the microbiological contamination sources during production of white cheese in a local dairy plant in Bursa (Turkey). Twenty nine different control points or material samples e.g. raw material (raw milk, pasteurized milk, milk in cheese vat, curd, brine and potable water), additives (starter culture, rennet and calcium chloride solution), products (moulded cheese before salting, moulded cheese after salting, cheese at cold holding and vacuum packaged cheese), process surfaces (cheese vat, cheese cloth, polyethylene separator sheet, milk stirrer, curd cutting knife, side pressure plate, upper pressure plate, moulded cheese cutting knife, cheese tray and packaging material used during production) as well as workers and process environment (workers' hands, cold room and production room air, floor and wall) have been examined for the enumeration of total aerobic mesophilic bacteria, staphylococci, coagulase positive staphylococci, *Enterobacteriaceae*, enterococci, coliforms, *Escherichia coli*, psychrophilic bacteria, and yeast and moulds. The starter culture was held as a possible contamination source of coagulase positive staphylococci, enterococci and psychrophilic bacteria; brine and upper pressure plate as the contamination source for staphylococci; floor and packaging material as the contamination source of psychrophilic bacteria; cheese vat, cheese cloth and curd cutting knife as the contamination source of total aerobic mesophilic bacteria; cold room and production room air as the contamination sources for yeast and moulds. The data showed that the quality of incoming raw milk was poor. It is important to initiate Good Hygiene Practice (GHP) applications in farms to produce high quality dairy products. The starter cultures were possible causing contamination with coagulase positive staphylococci, enterococci and psychrophilic bacteria. Brine and upper pressure plate were sources of staphylococci. Floor and packaging material were probable sources of psychrophilic bacteria, and the cheese vat, cheese cloth and curd cutting knifes were sources of total aerobic mesophilic bacteria. Production and cold room air is the primary source of yeasts and moulds in the white cheese.

THE PREVALENCE OF *SALMONELLA* SPP. IN TURKEYS AND IN SLAUGHTERED PIGS IN THE SLOVAK REPUBLIC

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The EU food safety policy includes the whole food chain and aims at ensuring a high level of human life and health, taking into account the protection of animal health and welfare, plant health and environment. In order to set the Community target, comparable data on the prevalence of *Salmonella* spp. in populations of farm animals in the Member States needs to be available. Such information was not at hand. Lately therefore were carried out two special surveys to monitor the prevalence of *Salmonella* spp. in turkeys and in slaughter pigs. The surveys were performed over a suitable period in order to take account of possible seasonal variations from 1 October 2006 to 30 September 2007.

The first survey was carried out to assess the prevalence of *Salmonella* spp. in flocks of fattening turkeys sampled within three weeks of leaving the selected holding for slaughter and in flocks of breeding turkeys within 9 weeks before depopulation. Sampling of sock swabs was performed by the competent authority. The second survey was carried out to assess the prevalence of *Salmonella* spp. in slaughter pigs. Samples of ileocaecal lymph nodes were taken to reflect the *Salmonella* status of the pig sent to slaughter and was performed by the competent authority. An evaluation the effect of contamination of the pigs during transport and lairage, and contamination of the carcase during the slaughter process by taking carcase swabs was not performed in Slovakia. Serological tests were also performed.

Prevalence of *Salmonella* spp. in turkeys was 51.1% – 23 positive from 45 samples. In two samples were isolated 2 serotypes. From 25 isolated strains were 22 strains *S*. Saintpaul, 2 strains *S*. Kiambu, 1 strain *S*. Senftenberg. Prevalence of *Salmonella* spp. in slaughter pigs was 7.8% – 30 positive from 385 samples. From 30 isolated strains 3 strains were *S*. Enteritidis (2 strains DT 8, 1 strain DT

21C), 4 strains *S*. Typhimurium (all PT U302), 5 strains *S*. Derby, 3 strains *S*. Choleraesuis, 3 strains *S*. Abony, 2 strains *S*. Bovismorbificans, 2 strains *S*. Montevideo, 1 strain *S*. Havana, 1 strain *S*. Agona, 1 strain *S*. Infantis and 4 strains *S. enterica* subspecies Enterica (6,7;-;1,5). These strains without H 1 phase were confirmed at CRL for *Salmonella* in Bilthoven too. An incidence of strains without certain H phases according to Kauffmann-White scheme is still increased. Serotyping, phagetyping and antimicrobial resistance were performed to trace a contamination source. Molecular typing techniques provided more precise information on bacterial identification and typing of strains e.g. tracing and confirmation of contamination source and relationship of similar strains with or without certain antigen.

MICROBIAL RISKS WITH SHELLFISH OBTAINED FROM ADRIATIC SEA

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In Laboratory for food of animal origin microbiological analysis of food samples are performed. The following analyses are performed *Salmonella, Escherichia coli* and *Vibrio parahaemolyticus* in shellfish. Shellfish are potential hazard for human consumption, especially eaten raw. In Slovenia we have three places for evaluation of shellfish. Sometimes there is a problem with increased number of *E. coli*, which is indicator of faecal contamination, which is due to spreading of sewage in the Adriatic Sea. There are many factors influencing the spreading of sewage, that contribute to dispersion of bacteria in the sea. With molecular analyses we would like to investigate genetic relationship among different serotypes of bacteria and thus understand the source and the dynamics of the bacteria.

SALMONELLA MONITORING IN PIG AND POULTRY BREEDING IN SLOVENIA

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In a cooperation with Veterinary Faculty at the University of Ljubljana, a serological status of *Salmonella* at pig feeding over a year is accomplished. Panvita is the only farm in Slovenia, who actively accomplish monitoring of *Salmonella* at pig feeding. Every month 30 blood samples of pigs in feeding are taken and sent to the University of Ljubljana, where they carry out the tests. Furthermore, bacteriological examination of strewing for *Salmonella* spp. in broiler is also performed. A preventional vaccination against *Salmonella*, which consists of death vaccine against *S*. Enteritidis, is used to rise the antibodies in the broilers in the main flock and thus protect these broilers.

Participant Abstracts

Antibiotic Resistance and Antimicrobial Activities

PREVALENCE OF ERYTHROMYCIN AND CIPROFLOXACIN RESISTANCE IN *CAMPYLOBACTER JEJUNI* AND *CAMPYLOBACTER COLI* FROM RETAIL POULTRY MEAT, FARM CHICKENS AND HUMANS

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Campylobacter jejuni and *C. coli* are the leading cause of human bacterial enteritis worldwide. Additional problem to high incidence of campylobacteriosis is the emergence and spread of antimicrobial resistance of campylobacters. In this work, the prevalence in resistance against erythromycin and ciprofloxacin, antibiotics of choice in human and veterinary medicine, in *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry, farm chickens and humans in Zenica-Doboj Canton, Bosnia and Herzegovina during 2001–2002 was investigated. **Methods:** Antibiotic resistance phenotypes of *Campylobacter* spp. isolated from retail poultry meat (n=50), farm chickens (n=15) and human clinical isolates (n=52) were determined by disc diffusion and E-test methods, and with reference broth microdilution method with CellTiter-Blue® reagent and automated fluorescence signal detection. **Results:** The high proportion of *C. coli* from poultry meat (29/50), farm chickens (8/15) and humans (15/52) were found, 58%, 53.3% and 28.3%, respectively. Erythromycin resistance of poultry, farm chicken and human isolates was 30.6%, 38.5% and 30.2%, respectively. Ciprofloxacin resistance was 26.5%, 30.8% and 32.1%, respectively. Erythromycin resistance rates of poultry, farm chicken and human isolates were high in both species, 31.8%, 50%, 31.6% (*C. jejuni*) and 26.9%, 28.6% and 26.7% (*C. coli*), respectively, which is unusual finding. All ciprofloxacin resistant isolates, with one exception of human isolate, have shown high level of resistance to ciprofloxacin (MIC≥32 μg/ml), and 89.4% of erythromycin-resistant isolates have shown high level resistance to erythromycin (MIC≥128

μg/ml). Moreover, MIC≥512 μg/ml was noted in 59.6% of erythromycin-resistant isolates, including four human isolates. **Conclusions:** The distribution of *C. jejuni* and *C. coli* species in isolates from poultry and farm chickens were similar to that seen in humans. Unusually high prevalence of resistance to erythromycin in both *C. jejuni* and *C. coli* isolated from humans, poultry and farm chickens was noted. Uncontrolled use of antimicrobials in humans and food animals might contribute to the emergence and dissemination of resistant bacteria. Monitoring antimicrobial resistance of zoonotic bacteria from human, animal, food and environmental origins at the local, regional and international level will help to understand its epidemiology and assure safety food.

ANTIMICROBIAL ACTIVITY OF ROSEMARY EXTRACTS (*ROSMARINUS OFFICINALIS* L.) AGAINST DIFFERENT SPECIES OF *LISTERIA*

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To reduce or eliminate chemically synthesized additives from foods is a current demand worldwide. A new approach to prevent the proliferation of microorganism or protect food from oxidation is the use of essential oils or plant extracts as natural additives in foods. We have studied antimicrobial activity of rosemary extracts (*Rosmarinus officinalis*) against *Listeria* sp. and *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella* Infantis. We have used two extracts of rosemary, Ros.con and Ros.conh, containing different levels of carnosic acid. We wanted to proof an antimicrobial activity of selected rosemary extracts with two most commonly used methods: disc diffusion method and broth dilution method. All tested cultures were prepared in a TSB broth. By all methods, the cultures were incubated for 24 to 48 hours on TSA agar with addition of plant extract at 37 °C. Solutions of extracts were prepared in absolute ethanol; the concentrations of extracts were prepared in accordance with the studied method. With the disc diffusion method we have obtained the zone of inhibition diameter after the incubation and the lowest concentrations, where no visible bacterial growth was recorded, were assumed as minimal inhibitory concentration values (MIC). We determined MIC values in the ranges from 625 μg extract/ml EtOH to 5000 μg extract/ml EtOH for Ros.con and from 312.5 μg extract/ml EtOH do 2500 μg extract/ml EtOH for Ros.conh in the medium. We have established that the resistance of *Listeria* species against rosemary extracts depends on: selected extract, selected concentration, various species and strain of *Listeria*. The results showed that the extracts had no antimicrobial effect on bacteria *Salmonella*. With broth dilution method we have determined minimal bactericidal concentration (MBC), as the concentration giving 0.1% bacterial survival. With this method we have tested two different strains of *L. monocytogenes* and in the most cases, we determinate MBC values in the range from 15.63 μg/ml TSB to 98.5 μg/ml TSB Ros.con and Ros.conh in the medium. Results have confirmed

our assumption that resistance of *Listeria* against rosemary extracts depended on the selected strain.

The other part of my research work consisted of applying PCR for detection foodborne pathogens. I have been working with classical- and real-time PCR with *L. monocytogenes*, *Salmonella* sp. and *S. aureus*. Different PCR techniques for detecting *S. enterica* and *L. monocytogenes* were introduced and applied to food samples together with different methods of DNA preparation for PCR. Now we are working on PCR optimization for detection Staphylococcal enterotoxins (sea, seb, sec and sed) as *S. aureus* is considered the third most important cause of foodborne illnesses in the world.

THE ANTIBACTERIAL EFFECTS OF PLUM SAUCE, POMEGRANATE SAUCE AND CITRIC ACID TO CHOPPED AND MINCED MEAT

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In this research, it is aimed to reduce microbial contamination due to wrong application during cutting, chopping, processing and storage of meat and meat products by using plum sauce, pomegranate sauce and citric acid which are known as antibacterial agents. For this purpose, chopped meat and minced meat were treated with plum sauce, pomegranate sauce and citric acid for reducing *Enterobactericeae*, *Staphylococcus* spp., coliforms, *Escherichia coli* and total aerobic mesophilic bacteria. The results showed that the numbers of *Enterobactericeae*, coagulase (-) *Staphylococcus* spp., total aerobic mesophilic bacteria, coliforms and *E. coli* were found in the range of $1 \times 10^3 – 2.25 \times 10^3$ cfu/g, $9.5 \times 10^2 – 1.41 \times 10^3$ cfu/g, $5.25 \times 10^5 – 9.6 \times 10^5$ cfu/g, 21 – 43 MPN/g, and 9.2–43 MPN/g, respectively, in chopped meat control samples. The counts of *Enterobactericeae*, coagulase (-) *Staphylococcus* spp., total aerobic mesophilic bacteria, coliforms and *E. coli* were found in the range of $1.1 \times 10^4 – 2.5 \times 10^4$ cfu/g, $4.2 \times 10^2 – 7.1 \times 10^2$ cfu/g, $1.04 \times 10^6 – 1.19 \times 10^6$ cfu/g, $1.04 \times 10^6 – 1.19 \times 10^6$ cfu/g, 21 – 460 MPN/g, and 15 – 240 MPN/g, respectively, in minced meat control samples. The numbers of total ererobic mesophilic bacteria, *E. coli*, *Enterobactericeae*, coagulase (-) *Staphylococcus* spp. decreased approximately 1–4 log in the chopped meat and minced meat treated with plum sauce, pomegranate sauce and citric acid, so it is shown that these agents have antibacterial effect on chopped meat and minced meat.

ANTIBIOTIC RESISTANCE OF CLINICAL *CAMPYLOBACTER* STRAINS AND OTHER FOODBORNE PATHOGENS

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The aim of my master thesis was to study the antibiotic resistance and genotyping of *Campylobacter* spp. A collection of 53 clinical strains, which were resistant to 11 different antibiotics were examined using disc diffusion susceptibility methods. The MIC of resistant strains was detected by agar dilution method and E-test. Simultaneously the occurrence of the *tet(O)* in all tetracycline resistant strains and the occurrence of the mutation of *gyrA* in all ciprofloxacin resistant strains was confirmed by using a PCR-based method. The resistant strains were genotyped by the AFLP method with using restriction enzymes *Bgl*II and *Mfe*I.

These studies will be continued using molecular biological methods to control food safety with the pathogenic bacteria *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes* and *Enterobacter sakazakii*. The genotyping of *Campylobacter* sp. was started by ERIC, REP and AFLP methods and we plan to extend it to the other bacteria and introduce new methods as MLST. In the field of the physiology we have started to study biofilm formation of the pathogens.

INVESTIGATION ON POSSIBLE EFFECT OF BACTERIAL ENZYMES ON THE *BACILLUS SPHAERICUS* CRYSTAL TOXIN PROTEIN

Canan Usta
Gaziosmanpasa University, Tokat, Turkey

Bacterial insecticides are especially valuable because their toxicity to nontarget animals and humans is extremely low. Compared to other commonly used insecticides, they are safe for both the insecticide user and consumers of treated crops and they are also known as microbiological pathogens. There is a vast number of studies addressing the problem of effect of applied toxin on non-target organisms, but there is no study that has been reported investigating the possible effect of proteases produced by aquatic microorganisms on the *Bacillus sphaericus* toxin (Bs toxin) since the toxin is a protein susceptible to extracellular proteases of non-target organisms. In this study, 520 bacterial isolates from some mosquito habitats in Turkey were obtained and screened for their putative proteolytic extracellular protease(s) against *B. sphaericus* strain 2362 mosquitocidal toxin. Here we addressed the question on the possible roles of proteases produced by bacteria on the Bs toxin. In order to do this, our goals included the followings:

1. To isolate microorganisms from mosquito habitats.
2. To obtain extracellular enzyme extracts of these bacterial isolates.
3. To select isolates of these bacteria exhibiting high protease activity. (For this, each of the enzyme extracts from the isolates was incubated with toxin solution of the *B. sphaericus* in eppendorf tubes for 24 h that were later applied to the SDS-PAGE. A total of 12 organisms were detected as effective.)
4. To test possible proteolytic activities of enzyme extracts of these isolates against Bs toxin. (They decreased the toxic effect of the Bs toxin tested on 3rd instar mosquito larvae of the genus *Culex* spp.)
5. To obtain time course of action of any Bs toxin-active proteolytic effect of these proteases. **(**Preparations of Bs toxin and isolates' extracts were

incubated in three different time course of 12, 24 and 48 h and applied to SDS-PAGE in order to realize the effect of time on the interaction of the extra-cellular enzyme extracts and the Bs toxin. It was found out that 48 h period of incubation was necessary for total degradation of the binary toxin observed as the disappearance of both of the 51 and 42 kDa bands on corresponding SDS-PAGE gels. These results were as well confirmed by bioassays made on the larvae of field collected *Culex* spp.)

6. To get the extracellular enzyme profile of these toxin-effective isolates in order to single out a protease having that proteolytic activity. (In order to discover the toxin inactivating proteolytic enzyme(s), extra-cellular enzyme mixture of bacterial isolates were applied to Native-PAGE. Each of the isolates produced seven to eleven protein bands some of which might be responsible for the degradation of the Bs toxin. To further determine which ones of these protein bands were actually affecting and/or degrading the toxin, each band was separately treated with the Bs toxin and analyzed on SDS-PAGE.)
7. To test the effect of protease-treated Bs toxin on larvae of mosquitoes of the *Culex* spp. (Bioassay applications were performed.) I have done all the steps until item 7 written above. After these experimental studies, the identification of the 7 bacterial isolates is necessary to to study their toxin-effective proteases. The step 8 is very necessary to establish the 9^{th} and 10^{th} steps. They would be performed if the isolates are identified.
8. To identify and characterize these toxin-effective bacterial isolates having higher extracellular protease activity on the Bs insecticidal toxin protein.
9. To determine molecular weights of proteases from effective isolates
10. To analyze the data to be accumulated to test our hypothesis that proteases of our bacterial isolates from mosquito habitats can inactivate Bs toxin.

Our results presented in this research study indicate that both of the protein subunits of pathogenic *B. sphaericus* strain 2362 can be degraded at the same time by the extra-cellular proteolytic activity of bacteria isolated from mosquito environments. This result necessitates that microbial content of the mosquito habitats should be taken into account when bio-control agents such as *B. sphaericus* formulated larvicides is to be used.

EXAM

QUESTIONS IN THE EXAM

1) Epifluorescence microscopy is a method that:
   A) needs detachment of the sample before analysing
   B) detects both dead and live cells
   C) is used in both in practical and research applications
   D) detects multilayered biofilms two-dimensionally

2) Detachment of surface-bound microorganisms on fatty surfaces is enhanced by sampling:
   A) using surface-active solution
   B) dry
   C) using enzyme solution
   D) with water

3) Ultrasonication is:
   A) a mechanical action detaching surface-bound matrix incl. microbes
   B) mainly used on surfaces soaked in liquid
   C) applicable as such for assessing the surface hygiene
   D) used to improve detachment from soft surfaces e.g. textiles

4) Which three steps are required for the polymerase chain reaction?
   A) Denaturation of single stranded DNA, annealing of the oligonucleotide primers to a complementary site, and extension of the primers at the 3´OH end by successive additions of dNTPs.
   B) Denaturation of double stranded DNA, annealing of the oligonucleotide primers to a complementary site, and extension of the primers at the 3´OH end by successive additions of dNTPs.
   C) Denaturation of double stranded DNA, annealing of the oligonucleotide primers to a non-complementary site, and extension of the primers at the 3´OH end by successive additions of dNTPs.
   D) Denaturation of double stranded DNA, annealing of the oligonucleotide primers to a non-complementary site, and extension of the primers at the 5´P end by successive additions of dNTPs.

5) Concept of ‘real-time quantitative PCR’ is based on the fact that:

A) there is a qualitative relationship between the amounts of target nucleic acid present at the end of a PCR assay and the amount of product amplified during its exponential phase.
B) there is a qualitative relationship between the amounts of target nucleic acid present at the start of a PCR assay and the amount of product amplified during its exponential phase.
C) there is a quantitative relationship between the amounts of target nucleic acid present at the start of a PCR assay and the amount of product amplified during its exponential phase.
D) there is a quantitative relationship between the amounts of target nucleic acid present at the end of a PCR assay and the amount of product amplified during its exponential phase.

6) The most important parameters used in detection of pathogenic microorganisms are sensitivity and specificity. Please decide which definition is correct.

A) Sensitivity is the ability of PCR to amplify only target product and ability of primers to recognise only certain target loci in genome of microorganism. Specificity is a minimal copy number of target sequence which can be amplified to detectable amount of product.
B) Specificity is the ability of PCR to amplify only target product and ability of primers to recognise only certain target loci in genome of microorganism. Sensitivity is a maximal copy number of target sequence which can be amplified to detectable amount of product.
C) Specificity is the ability of PCR to amplify only target product and ability of primers to recognise any target loci in genome of microorganism. Sensitivity is a maximal copy number of target sequence which can be amplified to detectable amount of product.
D) Specificity is the ability of PCR to amplify only target product and ability of primers to recognise only certain target loci in genome of microorganism. Sensitivity is a minimal copy number of target sequence which can be amplified to detectable amount of product.

7) Polymerase chain reaction offers several advantages for rapid and sensitive detection of microbial pathogens in comparison with traditional cultivation and biochemical methods. Select all which are suitable.
   A) The method is able to identify microorganisms that are difficult to culture
   B) The culture and enrichment of pathogens are not necessary for quality control
   C) PCR reagents are cheaper than to those required for serological procedures
   D) Animal models are not needed

8) Indole test demonstrates the ability of certain bacteria to decompose:
   A) phenylalanine
   B) tryptophane
   C) gluconates
   D) arginine

9) Lactose fermentation depends on the production:
   A) permease
   B) β-galactosidase
   C) permease and β-galactosidase
   D) lactulose

10) Oxidase test gives negative reaction with a culture of:
   A) *Aeromonas*
   B) *Salmonella*
   C) *Campylobacter*
   D) *Pseudomonas*

11) Which samples are collected in a compulsory *Salmonella* testing in each cage layer flock?
   A) Cloacal swabs, 60 heads
   B) Feces, pooled sample 150 g, 2 pieces
   C) Dust, pooled sample of 100 g, 1 pieces
   D) Gauze socks, pooled sample (1 pair) 2 pieces

12) Which samples are collected in a compulsory *Salmonella* testing in each layer flock under free range?
   A) Smears from laying nests, 60 samples
   B) Feces, pooled sample of 150 g, 2 pieces

C) Gauze socks, pooled sample (1 pair) 2 pieces
D) Dust, pooled sample of 100 g, 1 piece

13) Mark the correct statements.
A) Multidrug-resistant strain can develop by acquisition of a single R-plasmid to an antibiotic sensitive strain.
B) Multidrug-resistant strains are dangerous because of their high virulence.
C) The conjugation occurs in very low frequencies in nature.
D) Transposons, integrons and R-plasmids are genetic elements associated with antibiotic resistance.

14) Transposons
A) are flanked by insertion sequences
C) code for the integrase protein
C) code for the transposase protein
D) cause the spread of resistance genes

15) Integrons
A) cause the spread of resistance genes from one bacterial cell to another.
B) have the ability to actively acquire resistance gene cassettes.
C) can be located on conjugative plasmids.
D) can be located as a part of transposons.

16) Gold standard of bacterial DNA typing methods is
A) plasmid profile analysis
B) serotyping
C) pulsed-field gel electrophoresis
D) phage typing

17) Genotyping methods for differentiation of bacterial strains and isolates include
A) phage typing
B) antibiotic resistance typing
C) pulsed-field gel electrophoresis
D) plasmid profile analysis

18) Which mycobacterial species are not members of the *Mycobacterium tuberculosis* complex:
   A) *M. tuberculosis*
   B) *M. leprae*
   C) *M. avium*
   D) *M. bovis BCG*

19) Typical hosts of the causative agent of paratuberculosis are:
   A) Ruminants
   B) Birds
   C) Fish
   D) Carnivores

20) Which member of the *Mycobacterium avium* complex is the most common causative agent of human infection?
   A) *M. a. avium*
   B) *M. a. hominissuis*
   C) *M. intracellulare*
   D) *M. a. paratuberculosis*

21) What is the most common way of *M. a. paratuberculosis* transmission in young animals?
   A) Environment (dust, peat, water, soil etc.)
   B) Milk and colostrum
   C) Intrauterine transmission
   D) Faeces

22) Hygiene sampling in food premises should be performed:
   A) with only fixed sampling points
   B) with only randomly chosen sampling points
   C) based on a scheme with both fixed and randomly chosen sampling points
   D) at least on the fixed sampling points according to a commonly known procedure

23) List error sources in microbial determination:

24) List work instructions needed in a microbiological laboratory:

AVAILABLE POINTS FOR THE QUESTIONS IN THE EXAM AND EXAM RATING

1) 2 points
2) 2 points
3) 2 points
4) 2 points
5) 2 points
6) 2 points
7) 3 points
8) 2 points
9) 2 points
10) 2 points
11) 2 points
12) 2 points
13) 2 points
14) 3 points
15) 3 points
16) 2 points
17) 2 points
18) 2 points
19) 2 points
20) 2 points
21) 2 points
22) 2 points
23) 8 points
24) 8 points

Maximum points available: 63 points

Planned rating:	< 39	fail	< 62% of total points
	39–52	pass	62–82.5% of total points
	> 52	pass with excellence	> 82.5% of total points
Actual rating	< 32	fail	< 51% of total points
	32 ≤ x < 47	pass	51–75% of total points
	≥ 47	pass with excellence	≥ 75% of total points

SOLUTIONS TO THE EXAM QUESTIONS

16) C	15) B, D	14) A, D	13) A, D	12) C, D	11) B, C	10) B	9) C	8) B	7) A, B, D	6) D	5) C	4) B	3) A, B	2) A, C	1) B, D
24)	23)	22) C	21) B, D	20) B	19) A	18) B, C	17) C, D								

23) Microbes that do not form visible colonies on the agar; The sterilizing performed insufficiently; Diluents, tools and nutrient solutions and agars have been contaminated; Error in weighing or volumetric measurements; Contaminants from the environment; Insufficient mixing of the sample and its dilutions; Point-sized and spreading colonies in same sample; Occasional errors e.g. More viable colonies in the lower dilutions than in higher ones due to inhibition

24) Instructions in sterilizing tools, diluents and nutrients; Instructions in dressing; Instructions in hand washing; Instructions in aseptic practises; Instructions in controlling aseptic working methods; Instructions in controlling the air quality; Instructions in controlling the microbial cleanliness of working surfaces; Instructions in cleaning aseptic working areas

APPENDIX 1: PARTICIPANT LIST

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37. Helena Hradecka, Veterinary Research Institute, Brno, Czech Republic
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40. Ivo Pavlik, Veterinary Research Institute, Brno, Czech Republic
41. Ivan Rychlik, Veterinary Research Institute, Brno, Czech Republic
42. Satu Salo, VTT Technical Research Centre of Finland, Espoo, Finland
43. Frantisek Sisak, Veterinary Research Institute, Brno, Czech Republic
44. Gun Wirtanen, VTT Technical Research Centre of Finland, Espoo, Finland

APPENDIX 2: SEMINAR PROGRAMME

December 10, 2007 – Detection and identification of pathogens

9.00–9.30	Registration at the Veterinary Research Institute in Brno
9.30–9.40	Welcome address, Prof. Toman, Director of the Veterinary Research Institute (VRI) , Brno, Czech Republic
9.40–10.00	Welcome address, SAFOODNET project coordinator, Dr. Gun Wirtanen, VTT Technical Research Centre of Finland (VTT), Espoo, Finland
10.00–10.20	Sampling for microbiological analysis in food industry, Dr. Gun Wirtanen, VTT, Espoo, Finland
10.20–10.40	Sampling for laboratory detection of pathogenic microorganisms, Dr. Satu Salo, VTT, Espoo, Finland
10.40–11.00	*Coffee/tea break*
11.00–11.20	Sampling for *Salmonella* detection in poultry and pig farms and slaughter houses, Dr. Frantisek Sisak, Veterinary Research Institute, Brno, Czech Republic
11.20–11.40	Immunomagnetic separation of selected bacterial species, Dr. Renata Karpíšková, National Institute of Public Health, Brno, Czech Republic
11.40–12.00	PCR detection of pathogenic microorganisms, Dr. Milan Bartoš, GeneProof, Czech Republic
12.00–13.00	*Lunch*
13.00–17.30	Practical sessions: 3 groups focusing on detection of: 1) *Salmonella* spp., 2) *Mycobacterium* spp. and 3) *Listeria* spp. and *Staphylococcus* spp. The practical sessions on detection of pathogenic microorganism will include: • sample processing for culture detection of specified bacterial species • plating pure cultures on specific selective agars • bacterial species identification and confirmation, serology, PCR etc. • ELISA detection of particular microorganisms (if applicable) • sample processing for PCR detection, PCR design • quantification of bacterial load by quantitative real time PCR
19.00–22.00	*Brno city mini-tour and workshop dinner*

December 11, 2007 – Differentiation of pathogens

9.00–9.20	Characterisation of bacterial species by biochemical properties, Dr. Alois Čížek, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
9.20–9.40	Antibiotic resistance in pathogenic microorganism, Dr. Helena Hradecka, Veterinary Research Institute, Brno, Czech Republic
9.40–10.00	Phage typing of bacterial species, Dr. Renata Karpíšková, National Institute of Public Health, Brno, Czech Republic
10.00–10.30	*Coffee/tea break*
10.30–11.30	Molecular epidemiology of mycobacterial infections, Dr. Ivo Pavlik, Veterinary Research Institute, Brno, Czech Republic
11.30–12.30	*Lunch*
12.30–17.00	The practical sessions continue (see programme given above)
18.30→	*Mendel museum followed by workshop dinner*

December 12, 2007 – Differentiation of pathogens continues

8.00–11.45	Continuation of practical session on differentiation of pathogenic microorganism
11.45–12.05	*Coffee/tea break*
12.05–12.35	Overview of DNA typing methods (plasmid profile analysis, different PCRs, DNA fingerprinting), Dr. Ivan Rychlik Veterinary Research Institute, Brno, Czech Republic
12.35–12.45	Closing the workshop
12.45–14.00	Exam based on multiple choice questions and questions with answers to be listed
15.00–15.30	Possibility to listen to the correct answers
14.00→	*Lunch and departure of participants*

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Author(s)
Wirtanen, Gun & Salo, Satu (Eds.)

Title

DETECTION AND IDENTIFICATION OF HARMFUL MICROBES

Abstract

Harmful microbes and even pathogens may enter the manufacturing process and reach the end product in several ways e.g. through raw materials, air, water and chemicals employed in the process, process surfaces or factory personnel. The microbes entering the process can under suitable conditions grow very quickly and consequently cause drastic changes in the microbial population in vulnerable process stages and sites. Occasional proliferation of microbes from stagnant areas e.g. from biofilm formations in dead ends can contaminate the entire system and must be detected rapidly to avoid human suffering due to illnesses and financial losses due to product withdrawals. The availability of reliable microbial monitoring methods enables detection of developing microbial problems in the food processing area as early as possible. The main requirements for good microbial methods are besides reliability also sensitivity, selectivity, rapidity and economy. This workshop entitled *Detection and identification of harmful microbes*, which was held in Brno (Czech Republic) 10th–12th of December 2007, focused on microbiological methods and methodology available for detection, identification and tracking of harmful microbes and pathogens both in industry and research. The workshop also dealt with practical exercises in detecting *Salmonella* spp., *Mycobacterium* spp., *Listeria* spp. and *Staphylococcus* spp. The participant abstracts on detection of microbial contaminations, antibiotic resistance phenomena in microbes and microbial risk management in food processing are published in this publication.

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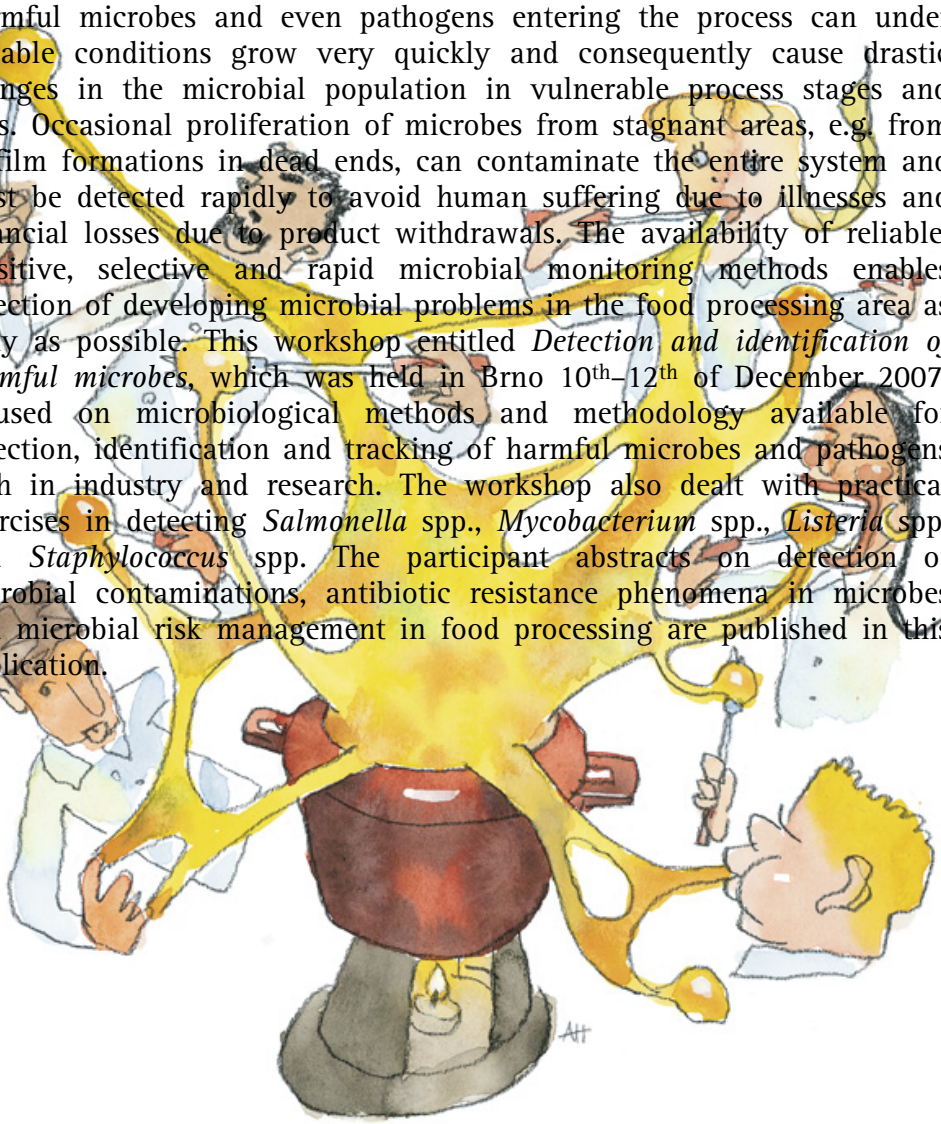
Harmful microbes and even pathogens entering the process can under suitable conditions grow very quickly and consequently cause drastic changes in the microbial population in vulnerable process stages and sites. Occasional proliferation of microbes from stagnant areas, e.g. from biofilm formations in dead ends, can contaminate the entire system and must be detected rapidly to avoid human suffering due to illnesses and financial losses due to product withdrawals. The availability of reliable, sensitive, selective and rapid microbial monitoring methods enables detection of developing microbial problems in the food processing area as early as possible. This workshop entitled *Detection and identification of harmful microbes*, which was held in Brno 10th–12th of December 2007, focused on microbiological methods and methodology available for detection, identification and tracking of harmful microbes and pathogens both in industry and research. The workshop also dealt with practical exercises in detecting *Salmonella* spp., *Mycobacterium* spp., *Listeria* spp. and *Staphylococcus* spp. The participant abstracts on detection of microbial contaminations, antibiotic resistance phenomena in microbes and microbial risk management in food processing are published in this publication.



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