Lisbon School of Health Technology | Park of Nations

TEMPH 2014

TRENDS IN ENVIRONMENTAL MICROBIOLOGY FOR PUBLIC HEALTH

18 - 21 SEPTEMBER 2014

WWW.TEMPH2014.COM

EXECUTIVE COMMITTEE

João Brandão
Carla Viegas
Susana Viegas
Raquel Rodrigues
Ferry Hagen

LOCAL SCIENTIFIC COMMITTEE

Manuela Cano
Cristina Veríssimo
Raquel Sabino
Paulo Pereira
Jorge Machado
Manuela Caniça
Cristina Belo Correia
Laura Rosado
Leonor Falcão
José Calheiros
Paula Alvito
Benilde Mendes
João Brandão
Carla Viegas
Susana Viegas
Raquel Rodrigues

INTERNATIONAL SCIENTIFIC COMMITTEE

Helena Solo-Gabriele
Andrew Wither
Richard Whitman
Robert Samson
Mike Sadowsky
Valerie Harwood
Huw Taylor
Ariane Vettorazzi
Anne Hyvärinen
Martin Täubel
Tom Edge
Sandra McLellan
Ferry Hagen

COMMITTEE

Lisbon School of Health Technology | Park of Nations

PROGRAM

18 - 21 SEPTEMBER 2014

18TH SEPTEMBER 2014

*** AMPHITHEATER ESTESL**

13:00 | 14:00 **WELCOME AND REGISTRATION**

14:00 | 18:00 **WORKSHOP ON MICROBIAL TOXINS IN PUBLIC HEALTH**

14:00 | 14:30 **Freshwater Phycotoxins**
- "Freshwater phycotoxins" - Elsa Dias (PT)

14:30 | 15:00 **Mycotoxins**
- "Occupational exposure to aflatoxin B1 in different occupational settings from Portugal" - Susana Viegas (PT)

15:00 | 15:30 **Marine Phycotoxins**
- "Marine phycotoxins" - Vitor Vasconcelos (PT)

15:30 | 16:00 **BREAK**

16:00 | 16:30 **Toxic Bacterial Contaminants**
- "Aquatic Algae as a Source and Sink of Bacterial Toxins in Waterways" - Michael Sadowsky (USA)

16:30 | 17:00 **Risk Assessment**
- "Microbial exposure of people living near livestock farms. Is there a need to regulate endotoxin emissions and environmental exposure?" - Dick Heederik (NL)

17:00 | 18:00 **Discussion**

Moderators: Elsa Dias (PT), Susana Viegas (PT), Vitor Vasconcelos (PT), Mike Sadowsky (USA) and Dick Heederik (NL)

18:00 | 18:30 **OPENING CEREMONY**

18:30 | 19:30 **WELCOME DRINK | PORTO D'HONRA**

19TH SEPTEMBER 2014

*** AUDITORIUM ESTESL**

08:30 | 19:30 **SEMINAR DAY 1**

INDOOR AIR QUALITY - MICROBIOLOGICAL CONTAMINATION

Chair: Robert Samson (NL) and Manuela Cano (PT)

09:00 | 09:30 **Keynote Speaker**
- "New findings on the indoor mycobiota: will it change our concept of indoor health?" - Robert Samson (NL)

09:30 | 09:50 **Invited Speaker**
- "QPCR determination of microbes in relation to mould and dampness observations in homes" - Martin Täubel (SF)

09:50 | 10:30 **Study Cases (2 x 20 min)**
- "Indoor microbiological contamination in children day care centres: the ENVIRH study" - Manuela Cano (PT)
- "Biological indoor air assessment in elderly care centers: the GERIA project" - Ana Mendes (PT)

10:30 | 11:00 **Free Communications (2 x 15 min)**
- "ARIA Project: Indoor Air Biological Assessment in Primary Schools" - Lívia Aguiar (PT)
- "Microbiological contamination assessment in settle dust: case-study in elderly homes" - Tiago Faria (PT)

11:00 | 11:30 **BREAK**

OCCUPATIONAL EXPOSURE

Chair: Stephan Mayer (DE) and Carla Viegas (PT)

11:30 | 11:50 **Keynote Speaker**
- "Occupational exposure to mould and microbial metabolites during onion sorting" - Stephan Mayer (DE)

11:50 | 12:20 **Study Cases (2 x 15 min)**
- "Fungal studies in Archives: a double concern" – Catarina Pinheiro (PT)
- "Fungal load in highly contaminated settings: how to assess the real occupational exposure scenario" – Carla Viegas (PT)

12:20 | 13:00 **Free Communications (3 x 15 min)**
- "Occupational exposure to biological agents in wastewater treatment plants" – Fátima Aguiar (PT)
- "Bioaerosol in occupational settings: a possible application of QMRA" – Annalaura Carducci (IT)
- "Assessment of bioaerosols in urban and rural primary schools using passive and active sampling methodologies" - Nuno Canha (PT)

13:00 | 14:00 **LUNCH BREAK AND POSTER SESSION**

PATHOGENS IN THE ENVIRONMENTAL: DISPERSION AND EMERGENCE
Chair: Aida Duarte (PT) and Ferry Hagen (NL)

14:00 | 14:20 **Keynote Speaker**
- "Cryptococcus gattii: the emergence of a tropical pathogen in temperate climates" - Ferry Hagen (NL)

14:20 | 14:40 **Invited Speaker**
- "Potential of touch screens as reservoir of multiresistant bacteria" - Aida Duarte (PT)

14:40 | 15:10 **Study Cases (2 x 15 min)**
- "Anthrax: a rare disease in Portugal?" - Rita Cordeiro (PT)
- "Legionnaires' disease situation in Portugal" - Raquel Rodrigues (PT)

15:15 | 16:00 **Free Communications (3x15 min)**
- "MRSA reservoirs outside the hospital: a public health concern" – Teresa Conceição (PT)
- "Distribution of opportunistic fungal pathogens in well and drinking water" - Monika Novak Babič (SL)
- "Bacterial biofilms: a story of persistence and invasion" – Luísa Jordão (PT)

16:00 | 16:30 **BREAK**

ROUND TABLE: "MICROBIAL RESISTANCE IN HOSPITAL AND OTHER ENVIRONMENTS"

16:30 | 17:30 **Opening Lectures (2 x 30 min)**
- "Concerns on antibiotic resistance: cross-talk between different environments" - Manuela Caniça (PT)
- "Fungi in hospital environment and antifungal resistance" - Raquel Sabino (PT)

17:30 | 18:00 **Discussion Panel**
Manuela Caniça (PT), Raquel Sabino (PT), Huw Taylor (Moderator, UK) and Elaine Pina (PT)

18:30 | 19:30 **MICROAREIAS NETWORK MEETING**

20TH SEPTEMBER 2014

*** AUDITORIUM ESTESL**

09:00 | 19:30 **SEMINAR DAY 2**

RECREATIONAL WATER MICROBIOLOGY: PARAMETERS AND METHODS
Chair: Valerie Harwood (USA) and Mike Sadowsky (USA)

09:00 | 09:30 **Keynote Speaker**
- "Recreational water quality in the age of molecular biology: new US regulations, culture vs. qPCR, QMRA and the potential of microarray" – Valerie Harwood (USA)

09:30 | 09:45 **Study Case**
- "Towards a risk assessment for Giardia sp. and Cryptosporidium sp. in portuguese fluvial beaches" - Claudia Júlio (PT)

09:45 | 10:30 **Free Communications (3 x 15 min)**

- "Recombinant adenovirus as a model to evaluate the efficiency of free chlorine disinfection in filtered water samples" – Célia Barardi (BR)
- "An assessment of the suitability of MST methods to determine human and non-human faecal inputs into the river Tejo, Portugal" – Sílvia Monteiro (PT)
- "The role of E. moraviensis as a faecal indicator" – Maja Taučer – Kapteijn (NL)

10:30 | 11:00 **BREAK**

RECREATIONAL WATER MICROBIOLOGY: EMERGENCE, DISPERSION AND REGULATIONS
Chair: Huw Taylor (UK) and Andrew Wither (UK)

11:00 | 11:20 **Keynote Speaker**
- "Addressing regulation at a multinational level" - Andrew Wither (UK)

11:20 | 11:40 **Invited Speaker**
- "RiskManche: the transport and fate of enteric organisms in catchments and coastal waters" - Huw Taylor (UK)

11:40 | 12:00 **Invited Speaker**
- "Recreational water microbiology: science and the regulatory challenge" - David Kay (UK)

12:00 | 13:00 **Free Communications (3 x 20 min)**
- "Temporal variability of microcystin (mcyA) genotypes in a toxic cyanobacterial bloom" - Catarina Churro (PT)
- "Fungal diversity in indoor swimming pools" – Sílvia Monteiro (PT)
- "Occurrence of bacterial enteric pathogens and discrimination of faecal sources in shellfish-harvesting areas and their catchments in France" – Michèle Gourmelon (FR)

13:00 | 14:00 **LUNCH BREAK AND POSTER SESSION**

MICROBIOLOGY AND METAGENOMICS OF SAND
Chair: João Brandão (PT) and Richard Whitman (USA)

14:00 | 14:30 **Keynote Speaker**
- "E. coli stains may be endemic in soils and sands" - Richard Whitman (USA)

14:30 | 14:50 **Latest Trends**
- "Public health risk assessment and regulations for beach sand and water" - João Brandão (PT)

14:50 | 15:05 **Invited Speaker**
- "Microbiological quality assessment of sand from beaches in Portuguese coast: fifteen years of experience" – Helena Barroso (PT)

15:05 | 15:20 **Study Case**
- "A. caninum, E. granulosus, Toxocara sp and T. gondii in sand?" - Maria João Gargaté (PT)

16:00 | 16:30 **BREAK**

ROUND TABLE: "MICROBIOMES, BEACH SAND RENURISHMENT, SANDBOXES AND PARKS, CONSTRUCTION – FUNGI, BACTERIA, VIRUSES, INSECTS AND PARASITES"

16:30 | 17:30 **Opening Lectures (2 x 20 min)**
- "Methods to assess and reduce risk of microbes in sands" - Helena Solo-Gabriele (USA)
- "The use of metagenomics and microbiomes to understand host, environmental, and land use contributions to the microbiota of aquatic systems" - Michael Sadowsky (USA)

17:10 | 18:30 **Discussion Panel**
Mike Sadowsky (USA), Richard Whitman (USA), Valerie Jody Harwood (USA), João Brandão (PT), Raquel Rodrigues (PT), Roger Fujioka (USA) and Helena Solo-Gabriele (Moderator, USA)

20:30 **CONGRESS DINNER**

21ST SEPTEMBER 2014

09:00 | 16:15

* AUDITORIUM ESTESL

SEMINAR DAY 3

FOODBORNE PATHOGENS: GEOGRAPHICAL DISTRIBUTION, CHARACTERIZATION AND EPIDEMIOLOGY

Chair: Mike Sadowsky (USA) and Jorge Machado (PT)

09:00 | 09:20

Keynote Speaker

09:20 | 09:50

- "Bacteriology of forborne diseases in Portugal" - Jorge Machado (PT)

Study Case

09:50 | 10:30

- "Surveillance of listeria monocytogenes in food catering establishments" – Carla Maia (PT) and Maria João Barreira (PT)

Free Communications (2 x 20 min)

- "Characterization of campylobacter jejuni and campylobacter coli isolated from broiler meat along the slaughtering line and in the final product" – Alexandra Duarte (PT)
- "The use of quantitative RT-PCR techniques of E.coli and enterococci for fast detection of fecal pollution in drinking water" - Gerhard Wubbels (NL)

10:30 | 11:00

BREAK

ASSESSMENT AND MANAGEMENT OF EMERGING RISKS: A TOOL TO INSURE FOOD SAFETY

Chair: Ariane Vettorazzi (ES) and Cristina Belo Correia (PT)

11:00 | 11:30

Keynote Speaker

11:30 | 12:00

- "Ochratoxin A: new approaches for its toxicity characterization" - Ariane Vettorazzi (ES)

Study Cases (3 x 15 min)

- "1st case of infant botulism" - Conceição Bonito (PT)
- "MYCOMIX: exploring the toxic effects of MYCOtoxins MIXtures in infant food and potential health impact" - Paula Alvito (PT)
- "Are Portuguese children exposed to mycotoxins through infant foods? A preliminary approach" - Ricardo Assunção (PT)

12:00 | 14:00

LUNCH BREAK AND POSTER SESSION

ANTIBIOTIC RESISTANCE IN FOODBORNE PATHOGENS

Chair: Mónica Oleastro (PT) and Manuela Caniça (PT)

14:00 | 14:30

Keynote Speaker

14:30 | 14:50

- "Current perspectives of emerging antibiotic resistance in foodborne bacteria" - Manuela Caniça (PT)

Invited Speaker

14:50 | 15:30

- "Reducing the risks of resistance development by pathogenic fungi. Multitarget fungicides" - Ricardo Boavida Ferreira (PT)

Study Cases (2 x 20 min)

- "Antimicrobial drug resistance of Campylobacter spp and Salmonella enterica: national data in food producing animals and food of animal origin" – Lurdes Clemente (PT)
- "Mobile genetic elements associated to antibiotic resistance in Salmonella enterica isolates collected in food-chain" – Vera Manageiro (PT)

15:30 | 16:00

Technical Session

- "Metagenomics as a tool in microbiology" – Mike Sadowsky (USA)

16:00 | 16:15

CLOSING CEREMONY

ABSTRACTS ORAL PRESENTATIONS

ABSTRACTS ACCORDING TO SEQUENTIAL ORDER IN THE SCIENTIFIC PROGRAM

TEMPH 2014
TRENDS IN ENVIRONMENTAL MICROBIOLOGY FOR PUBLIC HEALTH

Workshop on microbial toxins in public health

Freshwater phycotoxins

Elsa Dias

Instituto Nacional de Saúde Dr. Ricardo Jorge, Dep. Saúde Ambiental, Lisboa, Portugal

elsa.dias@insa.min-saude.pt

Cyanobacteria are ubiquitous phytoplanktonic organisms in freshwater environments, where they exert fundamental ecologic roles, such as oxygen production and nitrogen fixation. Under favourable environmental conditions, particularly in eutrophic waters, they growth massively, forming the well-known water blooms. These blooms are a concern for public health given the ability of cyanobacteria to produce secondary metabolites that are toxic for humans and animals. Human exposure to cyanotoxins can occur mainly from improper-treated drinking water and during recreational activities in water bodies containing cyanoblooms. This presentation will give an overview of the freshwater cyanotoxins, considering their occurrence, their toxicology and their laboratory diagnosis. The aspects related to risk assessment and regulation will also be discussed. The new trends in cyanobacteria and cyanotoxins research will also be addressed.

Occupational exposure to aflatoxin B_1 in different occupational settings from Portugal

Susana Viegas , Luísa Veiga , Paula Figueiredo , Ana Almeida , Elisabete Carolino & Carla Viegas

1 Environmental Health RG, Lisbon School of Health Technology, Polytechnic Institute of Lisbon, Lisboa, Portugal.

2 Center for Malaria & Tropical Diseases (CMDT), Public Health and Policy, Escola Nacional de Saúde Pública, Universidade Nova de Lisboa, Lisboa, Portugal

Aflatoxin B_1 (AFB_1) is the most prevalent aflatoxin and is associated with carcinogenicity, teratogenicity, genotoxicity and immunotoxicity and only a small number of studies examined exposure in occupational settings.

A study was developed aiming to know exposure to AFB_1 in three occupational settings: poultry, swine production and waste management.

A biomarker of internal dose that measures AFB_1 in serum was used. For AFB_1 quantification, the RIDASCREEN Aflatoxin B_1 30/15 enzyme-linked immunosorbent assay (ELISA; R Biopharm) was used, and was calibrated with aflatoxin standards from 1 to 50 ng/ml. 84 workers were enrolled: 34 from poultry farms, 11 from swine production farms and 40 from waste management industry. A control group (n=30) was also included.

In the control group, the AFB_1 values were all below 1 ng/ml. Eighteen workers (58.6%) from poultry showed detectable levels of AFB_1 with values ranging from <1 ng/ml to 4.23 ng/ml and with a median value of 1.36 ng/ml. In swine, six workers (54.5%) had detectable levels with values ranging from <1 ng/ml to 8.94 ng/ml and with a median value of 1.05 ng/ml. In waste management, all the workers had detectable levels of AFB_1, ranging from 2.52 ng/ml to 25.99 ng/ml with a median value of 9.75 ng/ml.

Data showed that occupational exposure to AFB_1 occurs in the three settings with the waste management being the most problematic. Safety measures need to be developed to avoid exposure to this carcinogenic agent.

Keywords: Aflatoxin B_1; occupational exposure; poultry farms; swine farms; waste management

TEMPH 2014

Bioaccessibility and changes on cylindrospermopsin concentration in edible mussels over storage and processing time

Marisa Freitas[1,2,3], Joana Azevedo[1], Vera Mendes[4], Bruno Manadas[4], Alexandre Campos[1], António Paulo Carvalho[1,2], Vitor Vasconcelos[1,2]

[1] CIIMAR/CIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Rua dos Bragas 289, P 4050-123 Porto, Portugal

[2] Faculty of Sciences, Porto University, Rua do Campo Alegre, 4169-007 Porto, Portugal

[3] Polytechnic Institute of Porto. Department of Environmental Health, Escola Superior de Tecnologia da Saúde do Porto. CISA/Research Center in Environment and Health, Rua de Valente Perfeito, 322, 440-330 Gaia, Portugal

[4] Center for Neuroscience and Cell Biology, University of Coimbra, Portugal

The cyanobacterial alkaloid cylindrospermopsin has been recognized of increased concern to public health due to the global proliferation of its main producer, *Cylindrospermopsis raciborskii*. Previous studies have shown that aquatic organisms, especially bivalves, can accumulate high levels of cylindrospermopsin without lethal effect. Based on the potential for human health risks, a provisional tolerable daily intake of 0.03µg/kg-body weight has been established. However, human exposure assessment has been based on the cylindrospermopsin concentration in raw products. Cylindrospermopsin is highly water-soluble and stable to extreme temperatures and pH, thus the knowledge of the influence of storage and cooking practices as well as human digestion is required to a more accurate risk assessment. This study aimed to assess the changes on cylindrospermopsin concentration in edible mussels over storage and processing time as well as cylindrospermopsin bioaccessibility. *Mytilus galloprovincialis*, fed cylindrospermopsin-producing *Cylindrospermopsis raciborskii*, were refrigerated, frozen, boiled, steamed and subjected to microwave radiation over different periods of time and then analyzed by LC-MS/MS. Cylindrospermopsin bioaccessibility was assessed in uncooked and steamed mussels, which were *in vitro* digested with saliva, gastric and duodenal juices. Mussels stored frozen for 48h and one week showed a significantly higher concentration of cylindrospermopsin, 52.5% and

57.7%, respectively. Cylindrospermopsin was also found in the water in which mussels were cooked. Bioaccessibility of cylindrospermopsin was reduced to levels below limit of quantitation; however, digestion of pure toxin suggests that gastric and intestinal juices degrade cylindrospermopsin.

Keywords: Bioaccessibility, cylindrospermopsin, food storage, food processing, *in vitro* human digestion.

Contact author: Marisa Freitas, CIIMAR/CIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Rua dos Bragas 289, P 4050-123 Porto, Portugal; Faculty of Sciences, Porto University, Rua do Campo Alegre, 4169-007 Porto, Portugal; Polytechnic Institute of Porto. Escola Superior de Tecnologia da Saúde do Porto. CISA/Research Center in Environment and Health, Rua de Valente Perfeito, 322, 440-330 Gaia, Portugal, mfreitas@ciimar.up.pt; maf@estsp.ipp.pt

Contact author: Vitor Vasconcelos, CIIMAR/CIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Rua dos Bragas 289, P 4050-123 Porto, Portugal; Faculty of Sciences, Porto University, Rua do Campo Alegre, 4169-007 Porto, Portugal, vmvascon@fc.up.pt

Abstract

Aquatic Algae as a Source and Sink of Bacterial Toxins in Waterways

Michael Sadowsky and Chanlan Chun

The reemergence of avian botulism caused by *Clostridium botulinum* type E has been observed across the Great Lakes in recent years. Avian botulism, is a paralytic disease of birds, and is initiated by ingesting neurotoxins produced by *Clostridium botulinum.* This often occurs on a yearly cycle and is increasingly becoming more common in the Great Lakes. We hypothesize that *C. botulinum* grows in the macrophytic alga *Cladophora* spp. and produces toxins which can be subsequently transferred to fish and birds, directly or via other vectors. In this study, free-floating algal mats were collected from shorelines of four Great Lakes in the U.S. between June and October 2011 and 2012. The abundance of *C. botulinum* in algal mats was quantified and the type of botulism neurotoxin (*bont*) gene associated with each organism was determined. About 74 and 90% of samples collected in 2011 and 2012 contained *bont* type E genes, respectively. This toxin has been shown to be responsible for avian botulism. The population densities were up to 15,000 most probable numbers (MPN) per dried gram of *Cladophora*. In addition, *bont*-type A and B genes, which are commonly associated with human diseases, were detected in a few algal samples. Mouse toxin assays of the supernatants from enrichment of *Cladophora* showed that *Cladophora*-borne *C. botulinum* were toxin-producing species. Moist heat treatment was effective in reducing or eliminating *C. botulinum* type E in Cladophora mats and steam treatment is suggested for environmental applications to limit animal and human exposure to *C. botulinum* bacteria and toxins. Our results indicate that *Cladophora* is a habitat for *C. botulinum* throughout the Great Lakes, warranting additional studies to better understand the relationship between this toxic bacterium and this alga. Our findings will serve as important information to ultimately manage this problem, and reduce bird mortality and human health risks.

Microbial exposure of people living near livestock farms. Is there a need to regulate endotoxin emissions and environmental exposure?

Prof Dick Heederik, Institute for Risk Assessment Sciences, Utrecht University, The Netherlands

Potential health effects of livestock farm emissions are at present of great interest in relation to public health in the Netherlands. A heated debate is ongoing about potential health risks which arise from livestock operations. This is the result of a major Q-fever outbreak and recent emergence of resistant microorganisms (MRSA, ESBLs) in livestock farms. Apart from zoonoses and resistant micro-organisms, some studies indicate that livestock farm emissions can also affect respiratory health of local residents, but only few studies have addressed this issue. Exposure to microbial toxins, in particular endotoxin, is expected to play a role an important role. Exposure levels around livestock operations can be in the range were acute respiratory effects have been observed. An overview will be given of health effects among individual living near livestock farms. Exposure studies which considered exposure to microbial agents and toxins will be discussed. The recent developments in the Netherlands, which consists of proposing an exposure standard for endotoxin exposure for the outdoor environment near livestock operations, including approaches considered to evaluate whether is it feasible to set an exposure standard will be discussed.

Abstract Session Indoor air quality – Microbiological contamination.

New findings on the indoor mycobiota: will it change our concept of indoor health?

Robert A. Samson

CBS-KNAW Fungal Biodiversity Centre, Utrecht the Netherlands, Agriculture Canada

The fungal genera and species which produce mycotoxins or cause spoilage of food have been well – studied. This is also true for the fungi occurring in indoor environments causing biodeterioration of buildings and have also regarded as causal agents of health hazard. Identification of these fungi up to species level is essential because it can explain the ecological and physiological conditions these fungi can occur. Our current knowledge of the total mycobiota of food and indoor environments shows that it consists of a total of 100-150 taxa. However recent polyphasic taxonomic studies have shown that the diversity of taxa is much greater than was previously thought. In addition data are presented by the analyses of house dust collected in various continents, where many new taxa were found.

Particularly in the well-known genera *Penicillium* and *Aspergillus* a much greater biodiversity was discovered by applying the polyphasic approach which combines phenotypic and molecular analyses. As an example the biodiversity of *Aspergillus* section *Circumdati* (yellow aspergilli) will be discussed. Some common species such as *Aspergillus versicolor* proved to be cryptic taxa and the recently described *Aspergillus creber* proved to be more common indoors than *A. versicolor*. In other genera it appears that there are certain species specific for indoor environments. The example of the genus *Cladosporium* is illustrated where indoor species proved to be more xerotolerant. The common xerophily might be an important factor for indoor growth and that implies that the role of this group of fungi have been underestimated in view of their health implications.

QPCR determination of microbes in relation to mould and dampness observations in homes

Martin TÄUBEL[1,*], Caroline SHORTER[2], Nevil PIERSE[3], Anne HYVÄRINEN[1], and Julian CRANE[2]

[1]Department Environmental Health, National Institute for Health and Welfare, Kuopio, Finland
[2]Department of Medicine, University of Otago, Wellington, New Zealand
[3]Department of Public Health, University of Otago, Wellington, New Zealand

[*]*Corresponding email: martin.taubel@thl.fi*

Moisture damage and visible mould in buildings are consistently linked with ill health, and microbes are suggested to be a key factor in the adverse exposure. Practical tools to reliably assess moisture related microbial contamination in buildings and to potentially predict health hazardous situations are lacking. The aim of this study was to evaluate a simple passive sampling approach in combination with quantitative PCR as a tool to objectively assess moisture damage and mould contamination in homes.

Airborne settled dust passively collected over four weeks on electrostatic wipes placed in 93 residential homes in New Zealand were analysed for fungal and bacterial content using quantitative PCR (qPCR). The log transformed microbial measurement data were analysed against a home mould score using Pearson's correlation test and home characteristics using Student's T-Test, with a focus on researcher and parent reported observations of moisture damage, dampness and mould.

Levels of total fungi and *Penicillium*/*Aspergillus* spp. groups were significantly elevated in homes in which mould odour, visible dampness and leaks or moisture damage were reported, and moreover correlated with a mould score calculated based on the extent of visible mould observed. Both Gram-positive and negative bacterial levels were significantly higher in homes where leaks or moisture damage and mould odour were observed.

Quantitative PCR analyses in combination with a standardized passive sampling approach for settled dust is a promising tool to objectively measure mould contamination in residential homes, both in epidemiological study settings and to support building inspections for moisture and mould damage in practical situations.

INDOOR MICROBIOLOGICAL CONTAMINATION IN CHILDREN DAY CARE CENTRES – THE ENVIRH STUDY

Manuela Cano[1], Susana Nogueira[2], Ana Luísa Papoila[3,4], Fátima Aguiar[1], Nuno Rosa[1], Ana Mendes[1], João P. Teixeira[1], Carmo Proença[1] and Nuno Neuparth [5,6,7]

[1]Instituto Nacional de Saúde Dr. Ricardo Jorge IP, DSA, Portugal
[2]Laboratório Nacional de Engenharia Civil, Lisboa, Portugal
[3]Departamento de Bioestatística e Informática, FCM UNL, CEAUL, Lisboa, Portugal
[4]Centro de Investigação do Centro Hospitalar de Lisboa Central, EPE, Lisboa, Portugal
[5]Serviço de Imunoalergologia, Hospital de Dona Estefânia, CHLC, EPE, Lisboa, Portugal
[6]Centro de Estudos de Doenças Crónicas – CEDOC, Lisboa, Portugal
[7]Departamento de Fisiopatologia, Faculdade de Ciências Médicas da Universidade Nova de Lisboa, Portugal

*Corresponding author: manuela.cano@insa.min-saude.pt (Tel.: +351 217 526 448)

In developed countries, after their homes, the children day care center (CDCC) is the place where children spend most of their time, so it is important to understand how environmental factors influence children respiratory health. The aim of ENVIRH (Environment and Health in children day care centres) study is to gather information on indoor environment in CDCC in order to correlate it with both ventilation and children's health.

This paper presents field measurements of indoor microbiological parameters, performed in 19 CDCC during spring and winter and aims to characterize the indoor environment.

Bacteria and fungi were collected using a MAS-100 impactor with Trypticase Soy Agar, MacConkey Agar and Malt Extract Agar as culture media for total bacteria, Gram-negative bacteria and fungi, respectively. Concentrations were calculated and fungi were identified.

Dust samples were collected on filters using a vacuum cleaner with a Dustream™ collector. Concentration of *Der p1* and *Der f1* dust mite allergens were assayed separately by ELISA quantitative kit.

Most of the CDCC revealed bacterial levels above the reference levels defined by the Portuguese legislation. Nurseries presented lower bacterial concentrations than activity rooms. Indoor fungal concentrations were above outdoor levels in 50% of the studied rooms and the predominant mould genera detected in both seasons were Penicillium and Cladosporium.

In spring were obtained higher concentrations of house dust mites in dust with 16% of the rooms surpassing the sensitization threshold.

BIOLOGICAL INDOOR AIR ASSESSMENT IN ELDERLY CARE CENTERS: THE GERIA PROJECT

Ana Mendes[1]*, Lívia Aguiar[1], Cristiana Pereira[1], Manuela Cano[1], Paula Neves[1], Diana Mendes[1], Maria do Carmo Proença[1], João Paulo Teixeira[1,2]

[1] Environmental Health Department, Portuguese National Health Institute Doutor Ricardo Jorge, Porto and Lisbon, Portugal.

[2] Institute of Public Health (ISPUP), Porto University, Portugal.

*Corresponding author: asestevao@gmail.com (Tel.: +351 964 780 242)

Airborne bacterial and fungal exposures have been documented to induce allergic diseases, toxicoses, irritation, infections and their components are linked to development and exacerbation of chronic respiratory illness. This paper presents results which have been produced within the GERIA ongoing project 'Geriatric study in Portugal on Health Effects of Air Quality in Elderly Care Centers', by measuring and characterizing biological indoor air assessment in 22 elderly care centers (ECC) in Porto and 18 in Lisbon, in winter and summer seasons, and its health effects particularly hazardous for individuals with underlying respiratory disease, such as the elderly.

After a building walk-through survey the microorganism air sampling was conducted using a microbiological air sampler and two agars, tryptic soy agar for bacteria and malt extract agar for fungi. Both indoor and outdoor samples (250 L of air) were collected in duplicate and with one field blank per culture medium per day. Results were expressed as colony-forming units per cubic meter of air (CFU/m^3).

Most of the evaluated ECC presented condensations and infiltrations along walls and roofs inside the buildings. Bacteria showed significantly higher indoor levels compared to outdoor, in both seasons, as well as, indoor significant differences between seasons. Moreover, bacteria concentration show significant variation between ECC rooms. Although fungi main species found were *Cladosporium* and *Penicillium*, considered to be common in indoor air, *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger* were also identified, pathogenic species that produce mycotoxins and therefore may produce several adverse health effects.

ARIA Project – Indoor Air Biological Assessment in Primary Schools

Authors: Lívia Aguiar[1], Cristiana Pereira[1], Ana Mendes[1], Paula Neves[1,2], João Paulo Teixeira[1,3]

1 Environmental Health Department, Portuguese National Health Institute Doutor Ricardo Jorge, Porto, Portugal
2 Research Centre of Health, School of Allied Health Sciences of Polytechnic of Porto, Portugal
3 Institute of Public Health (ISPUP), Porto University, Porto, Portugal

Presenting Author: E-mail: livia7_aguiar@hotmail.com Tel. no.: +351 912 720 263

Children are considered a susceptible group due to their particularly vulnerability to the development of respiratory diseases, such as asthma, and also the amount of time they spend inside classrooms, reasons why they deserve priority attention in indoor air quality studies.
Indoor air biological assessment took place during winter season, in 10 schools within 35 classrooms. These were evaluated regarding their biological contamination, during their normal occupancy, through the analysis of total bacteria count, fungi count and identification. Air sampling was carried out with a microbiological air sampler, using *Tryptic Soy Agar* for total bacteria and *Malt Extract Agar* for fungi. Results were expressed as colony-forming units per cubic meter of air (CFU/m^3) and compared with recently revised Portuguese standards.
Mean bacteria concentration is above the reference value in 9 out of 10 schools evaluated. Regarding primary schools mean fungi concentrations, only in one school the value is according with the reference value, nonetheless being very close to the established limit. If the previous Portuguese legislation was still ruling (500 CFU/m^3 for bacteria and fungi), 5 primary schools were above the reference value concerning fungi concentrations, and for bacteria values all schools were above this same reference.
Cladosporium sp. was the prevalent species found in 3 primary schools, while *Penicillium* sp. was predominant in 5. In one school, *Aspergillus fumigatus*, a known potential pathogenic/toxigenic species, was the prevalent specie identified (40%). The presence of toxin-producing fungi like *Aspergillus fumigatus* indoors should be a cause for concern considering the potential risk of mycotoxicosis.

Our current research is supported by ARIA Project: PTDC/DTP-SAP/1522/2012 from Foundation for Science and Technology (Fundação para a Ciência e Tecnologia - FCT).

MICROBIOLOGICAL CONTAMINATION ASSESSMENT IN SETTLE DUST: CASE-STUDY IN ELDERLY HOMES

T. Faria[1,2], M. Menezes[1,2], S. Cabo Verde[1], C. Viegas[2], M. Almeida-Silva[1,2]

[1] Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, EN 10, km 139.7, 2695-066 Bobadela LRS, Portugal

[2] Environmental and Health RG - Lisbon School of Health Technology/Institute Polytechnic of Lisbon, Lisbon, Portugal

Presenting author: tiagofaria@ctn.ist.utl.pt

According to the United Nations the proportion of total population aged 60 years or over in Europe was 22% for the year 2012 and prospects 34% for 2050 (UN, 2012). People spend 80–90% of their time indoors and elderly people are likely to spend even more time (more than 90%) (Almeida-Silva et al., 2014). Thus, indoor microflora may have special impact for this age group. The sampling and analysis of airborne microorganisms has received attention in recent years due to concerns with mould contamination in indoor environments, the threat of bioterrorism and the occurrence of associated health effects. However, these microorganisms can also be deposited and, even so, be responsible for human health effects.

A total of 7 dwellings (3 situated in an urban area and 4 in a rural area) covering 12 elders where selected. The ISAAC questionnaire was adapted and applied to all participants. Settle dust samples were retrieved from vacuum cleaner bags collected in each dwelling and after a laboratory process fungi and bacteria load were characterized by conventional methods.

The aim of this study was to assess fungal and bacterial contamination in elderly homes, in order to understand its deposition in settle dust.

United Nations. 2012. *Population Ageing and Development.* New York: United Nations.

Almeida-Silva, M., Wolterbeek, H.T., Almeida, S.M. (2014) Elderly exposure to indoor air pollutants. *Atmos Environ*, 85: 54-63.

Occupational exposure to mould and microbial metabolites during onion sorting

Abstract for oral presentation at the congress Trends in Environmental Microbiology and Public Health

Authors
Stefan Mayer
Berufsgenossenschaft Handel und Warendistribution
M5, 7
D 68161 Mannheim
Email: S.Mayer@bghw.de
Phone: 0049 621 183 5937
Fax: 0049 621 183 6 5937

Magdalena Twarużek
Kazimierz Wielki University, Faculty of Natural Science, Institute of Experimental Biology, Division of Physiology and Toxicology
Chodkiewicza Street 30
PL 85-064 Bydgoszcz

Anna Błajet-Kosicka
Kazimierz Wielki University, Faculty of Natural Science, Institute of Experimental Biology, Division of Physiology and Toxicology
Chodkiewicza Street 30
PL 85-064 Bydgoszcz

Jan Grajewski
Kazimierz Wielki University, Faculty of Natural Science, Institute of Experimental Biology, Division of Physiology and Toxicology
Chodkiewicza Street 30
PL 85-064 Bydgoszcz

The outer shell of onions is naturally colonized by moulds, yeasts and bacteria. During sorting of onions the microorganisms, cell wall fragments and/or microbial metabolites are released to the ambient air and may represent a health hazard for the sorting workers. First measurements have revealed an airborne mould exposure in the range of 10^4 to 10^7 cfu/m³. Beside the mould exposure onion sorting is also associated with an endotoxin exposure to concentrations in the range of 10^2 bis 10^4 EU/m³.
According to the directive 2000/54/EC on biological agents and the corresponding German Biological Agents Ordinance employers have to assess inter alia the potential health hazard due to mycotoxin exposure. In a preliminary study onions shells from different onion sorting plants and of different onion origins were analysed with respect to mould species, mould counts and secondary metabolites of moulds. For assessment of the airborne exposure, the inhalable fraction of airborne dust was determined. Additionally cytotoxicity of the isolated moulds has been tested using the MTT assay with swine kidney cells.
For a rough estimation of the possible mycotoxin exposure the mycotoxin concentrations on the onion skins were compared to the maximum limits which have been set by the EU for some foodstuffs. The advantages and limitations of this approach will be discussed.

Acknowledgments
Authors are grateful to Ms. Ewelina Soszczyńska and Mr. Robert Kosicki for the technical assistance.

“Fungal studies in Archives: a Double Concern”

Fungal problems are normally associated with human health and indoor air quality. However, in Archives, Museums and Galleries they assume another important role, especially for the organic cultural heritage. It is the case of paper based heritage, prone to attack whenever optimal conditions (and fungus) are present. Several studies have been performed abroad but in terms of fungal contamination the Portuguese scenario was still unknown. And once we know the fungal flora present, can we establish safety limits for both the documents and the staff/visitors attending the premises? For both health and conservation, the recent study in four Portuguese Archives allowed for some conclusions to be drawn and comes to reinforce the need for fungal surveillance while standing as a stepping stone for the definition of the quality values still lacking in the field of Cultural Heritage.

catarina pinheiro.

FUNGAL LOAD IN HIGHLY CONTAMINATED SETTINGS: HOW TO ASSESS THE REAL OCCUPATIONAL EXPOSURE SCENARIO

C. Viegas[1,] A. Quintal Gomes [1,2] R. Sabino[1, 3]

[1] Environmental and Health RG - Lisbon School of Health Technology/Institute Polytechnic of Lisbon, Lisbon, Portugal

[2] Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon, Lisbon, Portugal

[3] Mycology Laboratory, Infectious Diseases Department, National Health Institute Dr. Ricardo Jorge, Lisbon, Portugal

Presenting author: carla.viegas@estesl.ipl.pt

Although viability is crucial in the study of infectious organisms, cultural analysis may not accurately reflect the real fungal community present in a given sample. This situation can be even more severe in settings with higher fungal load, since the fast growth of some species prevents the finding of the real scenario regarding fungal contamination. Several occupational settings (e.g. poultries, waste-sorting and cork industries) were assessed using not only conventional methodologies but also molecular detection tools, in order to overcome the mentioned limitations. Molecular methodologies also enable the detection of toxigenic strains from common fungal species isolated from each occupational setting studied, allowing the adequate risk assessment. The referred settings were assessed for fungal contamination in the air by the use of impactors and impingers and also in surfaces, by swabbing the surfaces from the same indoor spaces. Regarding poultries, through molecular biology, we were able to detect the presence of aflatoxigenic strains of *A. flavus* that were not able to grow in culture. In the waste-sorting plant, it was possible to amplify DNA from the *A. fumigatus* complex in all culture-positive sampling sites plus one other sampling site that was negative by conventional culture analyses. Regarding the cork industry, the lack of detection by conventional methods of *Penicillium glabrum* could be explained by the overgrowth of the other prevalent species, such as *Chrysonilia sitophila*, which did not affect *P. glabrum* detection by molecular-based PCR methods. Thus, we were able to detect 11 samples (91.6%) with *P. glabrum.* The use of molecular methods in the detection of fungi should be reinforced, since cultural and molecular

OCCUPATIONAL EXPOSURE TO BIOLOGICAL AGENTS IN WASTEWATER TREATMENT PLANTS

Fátima Aguiar[1], Nuno Rosa[1] and Manuela Cano[1]
[1]Instituto Nacional de Saúde Dr. Ricardo Jorge IP, DSA, Portugal

*Corresponding author: fatima.aguiar@insa.min-saude.pt (Tel.: +351 217 519 262)

Poor risk assessment is one of the emerging issues related with the occupational exposure to biological agents. The principles for the management of biological risks are described in the Directive 2000/54/EC and employers are responsible for ensuring risk assessment.

However, a correct exposure assessment is difficult due to the lack of information on biological risks, lack of validation and harmonization of detection and measurement methods for biological agents.

In the mean time it is necessary to assess the risks and to identify higher risk tasks in order to protect workers.

Aiming to establish the risks associated with routine tasks, culturable microorganisms were collected using a MAS-100 impactor with Trypticase Soy Agar, MacConkey Agar and Malt Extract Agar as culture media for total bacteria, Gram-negative bacteria and fungi, respectively. The assessment was based on comparison of microorganism concentrations with reference environments taking into account exposure data.

Possiblerole of *Acanthamoeba polyphaga* in Human Adenovirus protectionagainstwater chemicaldisinfection

Carducci A.[1*],Di Giuseppe G.[2], Verani M.[1]

[1]*Laboratory of Hygiene and EnvironmentalVirology, Department of Biology, Universityof Pisa, Pisa, Italy*
[2]*Protistology-Zoology Unit, Department of Biology, Universityof Pisa, Pisa, Italy*

** Prof. Annalaura Carducci – email: annalaura.carducci@unipi.it - Tel. +390502213646*

Introduction. Free-living amoebaehavebeenrecovered in aquaticenvironments and theymayactasreservoirs or vehicles of variousmicroorganisms living in the sameenvironment by phagocytosis, withoutkillthem. In this work itwasstudiedthe interactionin water between*Acanthamoeba polyphaga* (AP) and Human Adenovirus(HAdV), proposedasindicators of water viralcontamination for their high environmentalresistance, in order to highlight the role of protection from chemicaldisinfection of internalized virus.
Methods. In the first part of the study a seriesofexperiments were performed to standardize a methodology for virus-amoeba "co-cultivation": a series of solutionsformed by water, AP and HAdV were co-culturedtogether and the viraluptakewasassessed by directimmunofluorescence.In asecondseries of experiments, the disinfectionefficacy of 3 differentconcentration (5; 2.5 and 1 mg/L) of sodiumhypochloriteagainst AP and HAdVeithersingly or when co-culturedwasassessed.
Results. The data obtained by the co-culture trials demonstratedthatHAdVwasincorporatedinto the hostamoebaconfirmingmoreover a preference of preysize for it.In singlydisinfectiontests APresulted more resistantthanHAdV to chemicaldisinfection: amoebastillremainedalive with 5 mg/Lsodiumhypochloritewhile virus loss infectivity. In co-cultured trials, atthisdisinfectantconcentration, wefoundHAdV in AP cytoplasm.
Conclusion. The results of the studyconfirm and underline the possiblerole of protectionof Acanthamoeba polyphaga for Human Adenovirus typeagainstchemicaldisinfection in water environment,revealing a new system of viralresistance.

Assessment of bioaerosols in urban and rural primary schools using passive and active sampling methodologies

Canha, N.[1,2,*], Almeida, S.M.[1], Freitas, M.C.[1], Wolterbeek, H.Th.[2]

[1]C2TN – Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Bobadela LRS, Portugal
[2]Department of Radiation, Radionuclides and Reactors, Faculty of Applied Sciences, Delft University of Technology, Delft, The Netherlands

** Corresponding author: Email: nunocanha@ctn.ist.utl.pt | Phone: +351 219946155*

People spend most of their time in indoor environments and, consequently, these environments are more significant for the contribution of the daily pollutant exposure than outdoors. In case of children, a great part of their time is spent at school. Therefore, evaluations of this microenvironment are important to assess their time-weighted exposure to air pollutants.

The aim of this study was to assess the children exposure to bioaerosols at schools from two different types of areas, urban and rural. A methodology based upon passive sampling was applied to evaluate fungi, bacteria and pollens, simultaneously with active sampling for fungi and bacterial assessment. Results showed very good correlations between sampling methods, especially for summer season. Passive sampling methodologies presented advantages such as no need of specific and expensive equipment, and they allow achieving important qualitative information.

The study was conducted in different periods of the year to study the seasonal variation of the bioaerosols. Fungi and pollen presented higher levels during the summer time whereas bacteria did not present a seasonal variation. Indoor to outdoor ratios were determined to assess the level of outdoor contamination upon the indoor environment. Levels of fungi were higher outdoor and bacteria presented higher concentrations indoors.

methodologies when used in parallel can provide additional information useful in the evaluation of occupational exposure to fungi.

Ferry Hagen[1]

[1] Department of Medical Microbiology, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands

During the past years our knowledge of causative agents of fungal diseases has considerably changed, this is mainly caused by the introduction of high-throughput molecular tools to study the genetic diversity. Within the field of medical and veterinary mycology the use of molecular techniques has led to major changes in the taxonomy of a plethora of fungi, including those that are causing disease among humans and animals. Changes in fungal taxonomy were –and are– frequently driven by the fact that the conventional method of microscopic and physiological identification do not match with the molecular based phylogenetic data, and the latter is assumed to be the golden taxonomic standard. On the other hand, the taxonomy of fungi was further fine-tuned by using molecular techniques to confirm that groups within a pathogenic species belong to specific hosts or are related to certain geographic regions, for example within the genera of *Coccidioides, Cryptococcus* and *Sporothrix*. One of the most studied pathogenic fungi are *Cryptococcus gattii* and *C. neoformans*, the former is involved in several ongoing outbreaks in North America, as well as that this 'tropic' species has now been found in more temperate climate zones were it infects otherwise healthy individuals. The largest *C. gattii* outbreak until present, also known as the Vancouver Island *C. gattii* outbreak, was first identified by veterinarians who saw an increase in cryptococcosis cases among pet animals, subsequently followed by an increase in human cases. Large-scale environmental screening identified that soil and trees were heavily contaminated with this tropical fungus, and that not *Eucalyptus* trees but rotting wood in general is the niche for this pathogen. Ongoing environmental research, in combination with veterinary and medical reports of cryptococcosis cases, identified more geographic localities where this fungal pathogen can be found. This presentation will focus on how molecular techniques can be of help to trace the source of infection, in terms of geographical origin as well as in terms of the source of infection.

Potential of touch screens as reservoir of multiresistant bacteria

Aida Duarte[1], Helena Barroso[2]
[1]iMed.UL, Faculdade de Farmácia da ULisboa, Av das Forças Armadas, 1649-019 Lisboa
[2]CiiEMandLMAEM, Instituto Superior de Ciências da Saúde Egas Moniz, Campus Universitário, Qta. da Granja, Monte de Caparica, 2829-511 Caparica

Computer technology has become an essential part of all aspects of our lives. Touch screens in modern medical devices and mobile phones are becoming increasingly common and are used routinely all day long. These devices may act as reservoir for microorganisms and contribute to the dissemination of pathogens to patients, as well as transfer of microorganisms to susceptible population in community. Isaacs et al (1998) not found resistant isolates in computer keyboards, leading to conclude that were not a significant source of the spread of resistant bacteria. Two years later Bures et al. found Methicillin-Resistant *Staphylococcus aureus* (MRSA) from computer keyboards and in cultures from patients. Ulger et al (2009) showed that healthcare workers hands and their mobile phones were contaminated with various types of microorganisms. Narciso et al (2009) described an outbreak in a hospital ICU, Lisboa, in which the ventilator touch monitor was the source of transmission of ESBL-producing *Klebsiella pneumoniae* isolates. The aim of our study was to verify if mobile phones and students handlers serve as a reservoir of multi resistant bacteria. Samples from thirty students were collected twice with a thirty days interval. Specific culture media were used for identification, quantification and antibiotic susceptibility testing. PCR amplification was performed for genetic characterization.

It was found 28% and 7.5% of *Bacillus* spp. into phones and hands, respectively. In contrast *Staphylococcus* spp. were detected in less phones (37.7%) than in student hands (96.5%), of which 82% were identified as *S. aureus* and 6.5% were methicillin resistant (MRSA). The gram negative strains, only *Escherichia coli* were isolated from hands and showed susceptibility to all antibiotics. However, *S. aureus* strains were multiresistant to antibiotics, including erythromycin (44.7%). The isolates representing *S. aureus* MRSA were assigned to SCCmec type II and IIIB, both were related to highly epidemic MRSA clones, such as the New York/Japan and Brazilian clones, respectively. Our study demonstrated that the type of microorganisms isolated from hands and phones were similar.

Anthrax: a rare disease in Portugal?

Cordeiro R.[1], Antunes W.[2], Pelerito A.[1], Alonso C.[2], Freitas J.[2], Lopes de Carvalho I.[1], Gomes I.[2], Carvalho J., Lopes-João A.[2] and Núncio M.S.[1]

[1]Unidade de Resposta a Emergências e Biopreparação, Departamento de Doenças Infecciosas, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal.

[2]Laboratório de Bromatologia e Defesa Biológica do Exército, Lisboa, Portugal.

Email: rita.cordeiro@insa.min-saude.pt; Telephone: +351 217 519 207

Bacillus anthracis is responsible for the transmission of an acute infectious disease-anthrax common in herbivores, wild or domestic animals, and can affect humans when exposed to animals and/or tissues of infected animals.

B. anthracis have the ability to produce spores which are responsible for the long persistence of this organism in the environment for long periods of time. The high resistance of *B. anthracis* spores associated with high pathogenicity, easily production and relative simplicity of disseminating through improvised devices, make this organism a biological agent of choice for bioterrorism attacks.

Currently in Portugal, anthrax is a rare disease. Only sporadic cases in rural workers have been reported, indicating that the disease is still endemic due to the presence of pathogenic strains in natural reservoirs, particularly in soils. Although there are records of anthrax and a high prevalence in the past, there are no current and accurate data of the endemic areas, the contamination degree of soils and molecular information of autochthonous *B. anthracis* strains. In 2012, it was possible to isolate *B. anthracis* strain from a human sample.

Current investigations allowed first localizations of national endemic areas for *B. anthracis* and the characterization of autochthonous strains that are in circulation. This study will benefit defense, security and public health, since *B. anthracis* is in the top of the list of potential agents able of being used as biological weapons in acts of bioterrorism and by the fact that anthrax it is an endemic zoonosis in worldwide.

Legionnaires' disease situation in Portugal

Raquel Esaguy Rodrigues
Instituto Nacional de Saúde Dr. Ricardo Jorge, Departamento de Saúde Ambiental, Unidade de Água e Solo, Laboratório de Microbiologia de Águas.
Email: raquel.rodrigues@insa.min-saude.pt ; Telephone: +351 21 752 64 83

Legionnaires' disease is an acute pneumonia caused by *Legionella.*
It was first described in Portugal in 1979 on an WHO Bulletin. It is still considered nowadays as an under-reported and under-diagnosed disease.

In the last few years, Portugal made several efforts on monitoring Legionnaire's disease, including integration in the Compulsory National Reported Diseases (DDO) system, in January of 1999. In the year of 2004, the General Directorate of Health created the Program for Integrated Epidemiological Surveillance of Legionnaires 'Disease (VigLab - Legionnaires' disease), which binds together clinical, laboratory and epidemiological data.

Sporadic cases are reported, some of which directly from ELDSnet (travelers' cases) whilst others are considered as local cases. Epidemics have also been identified.

MRSA reservoirs outside the hospital: a public health concern

T. Conceição[1], C. Coelho[1], H. de Lencastre[1,2], M. Aires-de-Sousa[3]

[1]Laboratory of Molecular Genetics, Instituto de Tecnologia Química e Biológica da Universidade Nova de Lisboa, Oeiras, Portugal; [2]Laboratory of Microbiology and Infectious Diseases, The Rockefeller University, New York, USA; [3]Escola Superior de Saúde da Cruz Vermelha Portuguesa, Lisbon, Portugal.

Nosocomial prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in Portugal is among the highest in Europe (54.3%). The aim of the present study was to search MRSA reservoirs in the community, namely in public buses and environmental surfaces in the vicinity of major hospitals.

Between 2011 and 2013, handrails of 199 public buses circulating in Lisbon and 53 environmental surfaces in the surrounding area of three hospitals (ATM machines, crosswalks and parking meters buttons, public telephones, bus stops, and stairs handrails) were screened for MRSA. All isolates were tested for antimicrobial susceptibility and for the presence of *mecA*, Panton-Valentine leukocidin (PVL) and arginine catabolic mobile element (ACME). Molecular characterization included pulsed-field gel electrophoresis (PFGE), staphylococcal cassette chromosome (SCC) *mec* typing, *spa* typing and multilocus sequence typing (MLST).

Seventy-three (37%) out of the 199 buses showed MRSA contamination. The majority of the isolates belonged to three clones: clone A (29%) - PFGE A, *spa* types t2357/t747/t379/t025/t910, ST22, SCC*mec* type IVh; clone B (33%) - PFGE B, t002/t214/t535, ST5-II/IV and clone C (29%) - PFGE C, t008, ST8-IV/VI. Clones A and B are currently the two major lineages in Portuguese hospitals, while clone C corresponds to the international community-acquired USA300 or related. Two MRSA isolates recovered from an ATM machine (ST8-IV) and from a bus stop (ST105-II) belonged to clones C and B, respectively, both predominant in buses.

Public buses constitute a major MRSA reservoir and particular environmental surfaces should not be neglected, representing a major public health concern that needs careful monitoring.

(250 words-máx 250 words)

Distribution of opportunistic fungal pathogens in well and drinking water

Novak Babic M.[1]*, Zupancic J.[1], Zalar P.[1], Gunde-Cimerman N.[1,2]

[1]Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana, Slovenia,

[2]Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins (CIPKeBIP), Jamova 39, SI-1000 Ljubljana, Slovenia

* Email address: monika.novakbabic@bf.uni-lj.si

Safe drinking water is essential for everyday human life. The European legislations for drinking water require microbiological analyses for the detection of bacteria like coliforms, *Escherichia coli, Clostridium* sp. and for bottled water also *Pseudomonas* sp. Regarding fungi no genera are mentioned. To meet the requirements, underground water sources are filtered, treated with disinfectants, ozone or UV light. Many fungi which can form biofilms inside the water supply systems can survive treatments, propagate and reach the consumers via water. In our study, we focused on the occurrence of human opportunistic pathogenic fungi in well and drinking water in Ljubljana, the capital of Slovenia. Water samples were filtered and membrane filters cultivated on DRBC agar supplemented with chloramphenicol. The isolated filamentous fungi were identified based on ITS rDNA sequences, while yeasts were identified based on D1/D2 domain of LSU rDNA. In addition, metagenomic analysis of fungal communities from tap water based on ITS2 was performed. The majority of fungal isolates represented causative agents of cutaneous, subcutaneous infections, catheter-related infections and infections of respiratory and urinary tract. Of special concern was the detection of black yeasts *Aureobasidium pullulans* var. *melanogenum*, *Exophiala dermatitidis*, *Rhinocladiella similis*, white yeast *Candida parapsilosis* and red yeast *Rhodotorula mucilaginosa*, isolated from 36, 10, 9, 13, and 21 % of drinking water samples, respectively. Our results indicate that the presence of opportunistic pathogenic fungi, presently almost completely overlooked in the drinking water legislations, should be changed accordingly, particularly due to the risk for infections with fungi in immuno-compromised people.

Bacterial biofilms: A story of persistence and invasion.

Sousa S.[1,2], Bandeira M[3]., Carvalho P.A.[3], Duarte A.[4] and Jordao L.[2]

[1]Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal.

[2]Instituto Nacional de Saúde Dr Ricardo Jorge, Lisboa, Portugal.

[3]Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal.

[4]Faculdade de Farmácia da Universidade de Lisboa, Lisboa, Portugal.

maria.jordao@insa.min-saude.pt, Tel: 217519200 ext 1465; Fax: 217519200

Biofilms are described as colonies of microorganisms that are attached to each other and to a surface, in an irreversible mode. These structures are virtualy everywhere: natural and humanized environments, as well as within living beings (humans and animals). For a long time biofilms where regarded as a bacterial survival strategie. Nowadays, in the industrialized world, the impact of acute bacterial infections caused by rapidly proliferating planktonic cells have gradually decreased in comparison to chronic infections owing to environmental organisms growing as biofilms. The major concern in this field is the healthcare-associated infections (HAIs). In 2012, HAIs estimated cases reached 6.7 million either in long-term care facilities or acute-care hospitals from which result 37,000 deaths configuring a serious public health problem [1].

The etiological agents are diverse being often resistant to antimicrobials and able to assemble biofilms both in abiotic and biotic surfaces. Here we evaluated the ability of different bacteria to assemble biofilms on a model surface and materials mimicking surfaces present either on healthcare units or medical devices. All bacteria were able to assemble biofilmls although following different kinetics and exhibiting different structural features assessed by electron microscopy. Additionally a link was established between bacteria ability to assemble biofilms and increased antibiotic resistance [2].

1. ECDC Europe; (2012), Annual epidemiological report 2011
2. Bandeira M; (2014), *Pathogens* (doi:10.3390/pathogens3030720)

Round Table: Microbial resistance in hospital and other environments

Opening lecture entitled: **Concerns on antibiotic resistance: Cross-talk between different environments**

Author: Manuela Caniça

National Reference Laboratory of Antibiotic Resistances and Health Associated Infections (NRL-AMR-HAI), Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge

Abstract:

The arisal of antibiotic resistance is a major public health problem, at national and at international level. It has serious consequences in the control of infections, in hospital and in the community, as some classes of antibiotics are often the last resort to treat bacterial infections. Among others, in last years, it has been seen an explosion of extended-spectrum beta-lactamases, namely those from CTX-M lineage, which have become particularly widespread, as well as beta-lactamases from non-ESBL families, such as carbapemenases (KPC and metallo-beta-lactamases). We also emphasize the problem of methicillin-resistant *Staphylococcus aureus* isolates in different environments, and the appearance of vancomycin-resistantce in *Staphylococcus aureus*, a resistance mechanism recently identified in Portugal. The factors involved in the emergence of antibiotic resistance are numerous, and the constant adaptation of microorganisms to the selective pressure exerted by antibiotics is impressive. However, the success of spread of certain resistant clones remains sometimes difficult to determine. Thus, it is manifest that action must be taken, and research in this area should be enlarged, not only to better understand the dynamics of spread of resistance between different bacteria and different ecosystems, but also to enlarge the pharmaceutical pipeline of antibacterials against multidrug resistant pathogens.

Fungi in hospital environment and antifungal resistance

Raquel Sabino[1,2], Viegas C[2], Parada H[1], Brandão J[1], Veríssimo C[1]

[1]Mycology Laboratory - Infectious Diseases Department, National Health Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal

[2]Environmental Health RG, Lisbon School of Health Technology, Polytechnique Institute of Lisbon, Lisbon, Portugal.

Nosocomial infections are a major issue of concern, especially the ones caused by fungi due to high mortality rate and very often difficult management.

Several risk factors such as chemotherapy, neutropenia, organ transplantation, indwelling catheters and devices, burns, antimicrobial therapy, severe surgery, and radiotherapy, are amongst the most relevant to acquire an invasive fungal infection. Hospital environment is one of the major concerns in nosocomial fungal infections. Invasive fungal infections depend on the interplay between host susceptibility and environmental exposure. The level of fungal contamination in a hospital environment can increase dramatically in the combination of several factors such as the presence of construction work in the hospital or close to it. The easy transmission of fungi in the air, water or even by direct or indirect contact with other people, contaminated surfaces or objects, lead to a growing concern about infections acquired by immunocompromised patients. *Aspergillus* and *Candida* species account for most fungal infections in these patients. The mortality rate attributed to candidemia varies between 8 to 53%, while in disseminated infections caused by filamentous fungi like *Aspergillus*, *Fusarium* and *Mucormicotina* species this rate varies between 56% and 95%. Despite intrinsic resistance found in some species of these genera, exposure of fungi to antifungal agents via medical or agricultural use of these compounds commonly used for plant protection appears to have a major impact on acquisition of resistance to azoles by *Aspergillus fumigatus*.

Due to these risk factors appropriate environmental control practices are important in preventing or arresting an outbreak of nosocomial fungal infection.

Raquel Sabino
raquelsabino@hotmail.com
Phone number: (00351)217519247
Mycology laboratory – Infectious Diseases Department
National Health Institute Dr. Ricardo Jorge, Av. Padre Cruz,
1649-016 Lisboa, Portugal

Recreational Water Quality in the Age of Molecular Biology - New US Regulations, Culture vs. qPCR, QMRA and the Potential of Microarray

Valerie J. Harwood, Ph.D.

University of South Florida, Tampa, FL USA

We have used bacteria of fecal origin (fecal indicator bacteria, or FIB) as the primary warning system for fecal contamination of water since the late 19th century. For over a century, culture methods have been used to enumerate FIB in order to assess drinking, recreational (bathing) and environmental water quality. Measurements of all FIB, including *Escherichia coli, Enterococcus, Clostridium perfringens,* or a coliform group, by culture methods share several crucial shortcomings. Culture methods are slow; therefore warning of contamination is delayed. Culture methods may not detect bacteria that are physiologically stressed, but still viable, therefore environmental conditions such as water salinity can greatly influence measurements. FIB are widely distributed in the feces of most animals, including humans, therefore their levels provide no indication of contamination source, and incomplete information about human health risk that might arise from exposure to the water. 2012 saw the rollout of the first new federal regulations for recreational water quality in decades, as the U.S. Environmental Protection Agency quality criteria included a quantitative PCR (qPCR) method for *Enterococcus* spp. While same-day notification of water quality via qPCR analyses alleviates the long lag between sampling and warning, new approaches are required for the other issues. Microbial source tracking (MST) can identify sources of contamination, from which one can infer which pathogens are most likely to be present. Such inferences can inform experimental design (which pathogens to test for) and/or quantitative microbial risk assessment (QMRA). QMRA estimates infection risk from particular pathogens based on pathogen levels, dose/response parameters, and exposure. However, testing for individual pathogens continues to be expensive, time-consuming, and a “shotgun” approach. Even when the contamination source(s) are known, the set of pathogens that may be present is typically quite large. Microarrays of oligonucleotides specific for pathogens, FIB, and MST markers have the potential to revolutionize QMRA and water quality testing; however, the drawback of small sample size must be overcome by effective upstream concentration and amplification steps. The second half of this decade may well witness a sea change in water quality testing methodology, improved public health protection, and more effective environmental protection.

Towards a risk assessment for *Giardia* sp. and *Cryptosporidium* sp. in Portuguese fluvial beaches

Julio C.[a], Oleastro M.[a], Ferreira[a] I., Martins S.[a], Sá C.[c], Ângelo H.[a], Guerreiro J.[b], Tenreiro R.[c]

[a] Instituto Nacional de Saúde Dr. Ricardo Jorge, Departamento de Doenças Infecciosas, LNR de Infecções Gastrentestinais, Av. Padre Cruz, 1649 – 016 Lisboa, Portugal.
[b] Universidade de Lisboa, Faculdade de Ciências, Centro de Oceanografia, Lisboa, Portugal.
[c] Universidade de Lisboa, Faculdade de Ciências, Centro de Biodiversidade, Genómica Integrativa e Funcional (BioFIG), Lisboa, Portugal.
Claudia.julio@insa.min-saude.pt

Background: Waterborne outbreaks of diarrhoeal illness reported worldwide are mostly associated with *Cryptosporidium* sp. and *Giardia* sp. Lake and river waters contaminated with (oo)cysts are major routes of human exposure making essential the development of preventive strategies for water safety. Since monitoring of water contamination with (oo)cysts is not routinely performed in Portugal, this study aims to unveil the possible associations between Portuguese fluvial beach characteristics and risk for public health caused by different genotypes of *Giardia* sp. and *Cryptosporidium* sp.
Methods: Nineteen beaches were selected according to land use and environmental parameters and sampled, on winter and summer, for the presence of *Giardia* sp. and *Cryptosporidium* sp., as well as faecal indicators and physicochemical parameters. Immunomagnetic separation was performed according the US EPA Method 1623 with Dynal procedure (Dynabeads), followed by detection of (oo)cysts by immunofluorecence microscopy. Cysts viability was also confirmed by nucleic acid dye (DAPI) staining.
Results: The results pointed to a wide distribution of these protozoa in the Portuguese river beaches studied, although at low concentrations. The estimated risk of exposure to parasites, appeared low for both parasites, suffering promptly increase values, related with peaks of rainfall. It was also noted that there is high correlation between levels of coliform termotolerantes and *Giardia*, the *E. coli* and enterococci.
Conclusion: Although the risk to public health is low, the correlation found between faecal indicators and *Giardia* leads us to highlight the importance of these parasites search whenever the values of the faecal indicators reach the maximum values indicated in the policy (20067EC).

RECOMBINANT ADENOVIRUS AS A MODEL TO EVALUATE THE EFFICIENCY OF FREE CHLORINE DISINFECTION IN FILTERED WATER SAMPLES

Nascimento, M.A.; Magri, M. E.;, Schissi, C. D. and Barardi, C.R. M*.

Laboratório de Virologia Aplicada, Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de Santa Catarina, 88040-900 Florianópolis, Santa Catarina, Brazil..

*celia.barardi@ufsc.br /Tel: +55 48 37215207/ Cel: +55 48 91930124

Abstract

In the present work, recombinant adenovirus (rAdV) that expresses green fluorescent protein (GFP) when cultivated in HEK 293A cells was chosen as a model to evaluate the efficiency of chlorine for AdV disinfection in filtered water samples from two Water Treatments Plants from Florianopolis (pHs 6.5 and 6.9). Buffered demand free (BDF) (pH 6.9 and 8.0) was used as control. Two free chlorine concentrations and two temperatures were assayed for all samples (0.2 mg/L, 0.5 mg/L, and 15° C, and 20° C). Fluorescence microscopy (FM) was employed to check viral infectivity *in vitro* and qPCR as a molecular method for viral genome copies. The time required to inactivate $4\log_{10}$ of rAdV was less than 1 min (for 0.2 mg/L and 0.5 mg/L) using FM, except for BDF pH 8.0 (up to 2.5 min for $4\log_{10}$). The qPCR assay was not able to provide information regarding rAdV inactivation. The data were modeled (Chick-Watson) and it was observed that the Ct values for $4\log_{10}$ inactivation was less than 0.25 for all experimental conditions, except for the BDF pH 8.0, with Ct over 1.249. The Ct values were comparable to those reported in the literature. Real samples of treated water from WTP (at the distribution network) were also evaluated for and human adenovirus (HAdV) was detected (average of $2.75.10^3$ PFU/L). Finally, it was possible to prove that adenoviruses were rapidly inactivated in surface water treated with chlorine and that recombinant adenovirus expressing GFP proved to be a good model for this evaluation.

AN ASSESSMENT OF THE SUITABILITY OF MST METHODS TO DETERMINE HUMAN AND NON-HUMAN FAECAL INPUTS INTO THE RIVER TEJO, PORTUGAL

Monteiro, S.[1,2,*], Ebdon, J.[2], Santos, R.[1], and Taylor, H[2].

[1] Laboratório Análises, Instituto Superior Técnico, Lisbon, Portugal

[2] School of Environment and Technology, University of Brighton, Brighton, United Kingdom

***Presenting author: silvia.monteiro@tecnico.ulisboa.pt, +351 218417110**

Introduction

In urban areas, the main source of faecal pollution to the aquatic environment is partially treated wastewater discharges from municipal WWTW. Recent improvements to municipal sewage discharges have highlighted the impact of diffuse pollution associated with agriculture, wild animals and urban run-off. Although individual inputs may be small, collectively they can have a significant impact on water quality.

Aim

To determine the suitability of bacteriophages of *Bacteroides* GB-124, viral and mitochondrial DNA (mtDNA)-based Microbial Source Tracking (MST) as tools for the assessment of sources of faecal pollution in the Tejo River.

Methods

Samples of Tejo river water were collected for the detection of GB-124 bacteriophages, and somatic coliphages (SC) by standardized phage-lysis methods, faecal indicator bacteria (FIB) using Most Probable Number (MPN) technique, Norovirus, human and porcine Adenovirus (HAdV and PAdV, respectively), Bovine Polyomavirus (JCPyV and BPyV, respectively) by qPCR, and mtDNA markers by qPCR.

Results

Results of the surveillance performed showed that human and animal contamination were detected river water samples. Samples presented low levels of FIB. Samples were negative for GB-124 bacteriophage with low counts for SC.

Positive samples for HAdV also tested positive for the HmtDNA marker. During rainy events the percentage of positive samples for cat, dog, and pigeon mtDNA markers increased. The results obtained for NoV were correlated with those obtained for HAdV during colder months.

Conclusion

These preliminary findings are significant as they suggest that the methods may have a role in monitoring the microbial quality of water bodies in Europe and thereby supporting human health protection.

The role of *E. moraviensis* as a faecal indicator

Maja Taučer-Kapteijn [a, b] , Wim Hoogenboezem [a] , Gertjan Medema [b, c].

[a] *The Water Laboratory, J.W. Lucasweg 2, 2031 BE Haarlem, the Netherlands.*

[b] *Delft University of Technology, Faculty Civil Engineering & Geosciences, Department of Water Management, Stevinweg 1, 2628 CN Delft, the Netherlands.*

[c] *KWR Watercycle Research Institute, Groningenhaven 7, 3433 PE Nieuwegein, the Netherlands.*

Corresponding author: Maja Taučer-Kapteijn, maja.taucer@hetwaterlaboratorium.nl, The Water Laboratory, J.W. Lucasweg 2, 2031 BE Haarlem, the Netherlands.

ABSTRACT

Because *Enterococcus moraviensis* has occasionally been detected in large volume samples of recharged water (after dune infiltration) in one of the infiltration areas in the Netherlands, several aspects of possible environmental growth were studied. Growth experiments using membrane filtration were performed on: sediment from recharge mains, biofilm formed in recharged water, humic substances and plant material from the dunes. *E. moraviensis* was not able to grow on sediment suspensions. Die-off rate was higher at 25˚C than at 15˚C. Although *E. moraviensis* was able to attach to the biofilm, no growth was observed. Growth experiments using boiled plant extracts in different concentrations at 15˚C showed growth maxima after 4 days. Plant extracts (50 g l-1, 5 g l-1 and 0.5 g l-1) prepared using filtration (0.45 µm) also promoted growth of *E. moraviensis* yielding $5.3*10^3$ cfu g^{-1}, $8.3*10^3$ cfu g^{-1} and $2.1*10^4$ cfu g^{-1} respectively. Extracts of plant material promotes the growth of *E. moraviensis* at 15 ˚C. Although the biofilm was colonised by *E. moraviensis,* no growth of was observed in biofilm at 15 ˚C. Also sediments from recharge mains did not contribute to the growth of *E. moraviensis.* The ability of *Enterococcus moraviensis* to multiply on plant extract at 15 ˚C has been shown, which means that *E. moraviensis* does not meet all conditions of good faecal indicator.

TEMPH 2014

Session: Recreational water microbiology: emergence, dispersion and regulations

Addressing regulation at a multinational level

Andrew Wither
National Oceanography Centre (UK)

Some degree of control and regulation of the microbial quality in recreational waters has been applied in many parts of the world for the past 50 years. However there has been little consistency in the choice of microbial parameters or in the methods of reporting contamination levels. National and international standards and legislation has developed steadily during this period, yet there is still a poor understanding of the response of the beach user to microbial contamination and a wide variation in the parameters and protocols for reporting recreational water quality.

This introductory talk will chart the diverse development of control parameters in different countries, consider their strengths and failings and suggest those areas where a much improved understanding of the health implications of recreational waters is needed.

RiskManche: The transport and fate of enteric organisms in catchments and coastal waters

Huw Taylor, Sarah, Purnell, Adewale Olalemi and James Ebdon
School of Environment and Technology, University of Brighton, UK

Abstract:

With financial support from the European Regional Development Fund's Interreg IV(A) Programme, RiskManche brings together a partnership of universities, research institutions and environmental protection agencies in the France (Channel) England region to develop new thinking in the field of waterborne disease transmission, so as to provide practical tools for human health and environmental protection.

Pressures on water resources in the region continue to grow and climate change-related storm events are challenging how we maintain effective barriers to the transmission of infectious disease within the water cycle. RiskManche takes both a tiered and a trans-disciplinary approach to the problem, first bringing together scientists from France and England to develop a better understanding of the behaviour of specific pathogens associated with 'critical control points' of human water-related disease (e.g., viral disease transmission through the consumption of shellfish). This reductive approach is complemented by a broader methodology in which innovative catchment mapping and modelling methods are used to predict how waterborne pathogen levels in river and coastal water respond to changes in land-use and weather patterns. The new science is providing valuable 'expert knowledge' to help protect human health, but importantly, this is complemented and informed by local 'non-expert knowledge', which is shared through regular stakeholder participation events.

The project has demonstrated the value of integrating research efforts at a cross-border level to tackle common issues of environmental and human health protection. Importantly, the active participation of 'non-expert' stakeholders from both France and England, at all stages of the project, has challenged the partners to focus on the real needs of local stakeholders and to present new knowledge in ways that support the development of practical tools that are readily applicable throughout the cross-border region.

Keywords: Waterborne; disease; watershed; pathogen; Water Framework Directive; Water Safety Planning; shellfish; bathing waters; modelling.

‘Recreational water microbiology: science and the regulatory challenge’

Mark Wyer[1], Carl Stapleton[1], David Kay[1]

Recreational water management is at the cutting edge of regulation since publication of the first WHO guidelines for recreational water management in 2003. This paper present results of current UK investigations designed to deliver reliable real-time forecasts of bathing water quality which are communicated to the bathing population through internet postings and physical signage on the beach. High explained variance (75-85%) in the resultant models has been achieved but only through generation of a bespoke and intensive model calibration data resource. This model calibration data has highlighted patterns in faecal indicator concentrations at UK sites which have significant implications for the design and application of regulatory standards. Perhaps the most significant observation has been the diurnality in faecal indicator concentrations which is so marked that the time of day when compliance samples are collected has been shown to influence beach compliance by two classes when assessed against the EU Bathing Water Directive (2006) standards. In effect, therefore, a beach may pass or fail depending on sample timing during the bathing day. It would clearly be inappropriate to manipulate any sampling programme simply to ensure compliance of a bathing water. But these observations also cast doubt on the assumption inherent in most modern regulatory sampling programmes and attempts to model water quality using bathing beach compliance data: i.e. that a single regulatory compliance sample can be used to characterize water quality on the ‘bathing day’.

[1] Centre for Research into Environment and Health, Aberystwyth University, UK (dave@crehkay.demon.co.uk)

Temporal variability of microcystin (*mcy*A) genotypes in a toxic cyanobacterial bloom.

Churro C.[1,3], Silva B.[2], Pereira P.[1], Valério E.[1], Vasconcelos V.[3,4]

(1) Laboratório de Biologia e Ecotoxicologia, Departamento de Saúde Ambiental, Instituto Nacional de Saúde Dr. Ricardo Jorge, Avenida Padre Cruz, 1649-016 Lisboa, Portugal.

(2) Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge, Avenida Padre Cruz, 1649-016 Lisboa, Portugal.

(3) Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, 4069-007 Porto, Portugal.

(4) Centro Interdisciplinar de Investigação Marinha e Ambiental, CIIMAR/CIMAR, Universidade do Porto, Rua dos Bragas 280, 4050-123 Porto, Portugal.

Email: catarina.churro@insa.min-saude.pt, Phone number: (+351) 217 519 200.

The cyanobacterium *Planktothrix agardhii* forms persistent blooms in water reservoirs and is often associated with the production of microcystins (hepatotoxins). However, high cell densities of *P.agardhii* are not always accompanied with high levels of microcystins and contrariwise. The genotype abundance and composition can be an important driver of toxicity in these natural blooms. In this work a perennial bloom was monitored during two years (2012-2014) in order to characterize the genotype structure and succession. A real-time PCR protocol was developed to quantify *P.agardhii* using the *rpoC*1 gene and to quantify potential microcystins producers using the microcystin syntethase gene (*mcy*A). The *mcy*A was quantified by targeting two gene regions: a generic region for all microcystin producers and a specific region for *Planktothrix.* Phytoplankton diversity and abundance was quantified by direct cell inspection and counting. The total microcystin concentration in water was measured using ADDA-ELISA kit. The results showed that two different *mcy*A genotypes are present in this water reservoir. The temporal variability accessed by cyanobacteria cell density, real-time PCR and microcystin concentration will be discussed. Furthermore, the real-time PCR protocol developed in this study enabled to determine and quantify genotype bloom composition, thus representing a promising tool in cyanobacteria bloom monitoring.

Fungal diversity in indoor swimming pools

Freire M.[1,*], Dias A.[1], Macieira F.[1], Monteiro S.[1], Santos R.[1]

[1] Laboratório Análises, Instituto Superior Tecnico, Lisbon, Portugal

*Presenting author: mario.freire@tecnico.ulisboa.pt, +351 218417110

Due to its warm and moist atmosphere, indoor swimming pools provide the suitable conditions for fungal growth. The large spectra of usage (recreational, sports, rehabilitation, etc) makes the population attending these spaces very heterogeneous, ranging from very young children to advanced elderly, normally the more susceptible hosts for infections. In this study, water samples were collected from indoor swimming pools, in the Lisbon area. Samples were then processed by membrane filtration and incubated into specific media. Fungal colonies were isolated and identified by macroscopic and microscopic observation, and when necessary, by additional molecular methods. Results showed that the most common fungi isolated were from the genus *Cladosporium*, *Penicillium*, *Aspergillus* and *Rhizopus.* Yeast from the genus *Rhodotorula* and *Candida* were ubiquitous to all samples. Fungi are known to be highly resistant to disinfection procedures such as chlorine and so the presence of these saprophytes might constitute an indication that better and improved disinfection processes are required. Since bathers have direct contact with the water, and the subsequent aerosol produced, the presence of some fungi might pose serious health problems, thus being necessary a more effective and rigid analysis of these microorganisms.

Occurrence of bacterial enteric pathogens and discrimination of faecal sources in shellfish-harvesting areas and their catchments in France.

Gourmelon M.[1], Balière C.[1], Quenot E.[1], Cozien J.[1], Lozach S.[1], Caprais M.P.[1], Hervio-Heath D.[1], Le Saux J.C.[1], Balière Cl.[2], Bruey Q.[2], Giard J.C.[2], Rincé I.[2], Jardé E.[3], Le Hello S. [4], and Rincé A[2] .

[1] Ifremer, Département RBE,Unité SG2M, Laboratoire Santé Environnement Microbiologie, Plouzané, France
[2] Université de Caen Basse-Normandie, Unité de Recherche Risques Microbiens, Caen, France
[3] CNRS, UMR 6118 Géosciences, Rennes, France
[4] Institut Pasteur, Centre National de Référence *E. coli/Shigella/Salmonella*, Unité de Recherche et d'Expertise des Bactéries Pathogènes Entériques, Paris, France

Contact: Michele.Gourmelon@ifremer.fr

Three shellfish-harvesting areas in France (one in Brittany and two in Normandy) were selected to evaluate the presence of *E. coli*, host-associated *Bacteroidales* markers and bacterial enteric pathogens (*Campylobacter*, *Salmonella*, and Shiga-toxin-producing *E. coli* (STEC)) in shellfish (88 batches; cockles, oysters, and mussels) and in the upstream water (99 samples) during a one year-study.
Concentrations of faecal indicators (*E. coli*) were high in water and cockles while oysters and mussels were less contaminated.
Human-, ruminant- and pig-associated *Bacteroidales* markers were frequently detected in water samples, but hardly detected in shellfish.
Campylobacter spp. were investigated using an enrichment method based on NF ISO10272 and rt-PCR detection of 16S rRNA. They were detected in 45.6% of shellfish and 83.8% of water samples. Among the 795 strains, *C. jejuni* and *C. coli* were most frequently isolated in water whereas *C. lari* was frequently observed in shellfish.
Salmonella spp. were detected according to the protocol based on NF EN ISO 6579 and detection of *invA* and *ttrBCA* genes by rt-PCR after an enrichment step. 29.3% of water samples and only six shellfish enrichments (5.5%) were positive for both genes. Twenty-four strains of *Salmonella* were isolated and corresponded to 8 different serotypes.
The *stx* genes were detected from 92.9% and 47.7% of water and shellfish samples, respectively. 3,950 *E. coli* strains including 38 STEC were isolated and have been subject to further characterizations.
Overall results provide important information on the origin of pollution and for hazard evaluation.

E. coli stains may be Endemic in Soils and Sands

Richard Whitman*, Muruleedhara Byappanahalli, and Meredith B. Nevers
U.S. Geological Survey, Great Lakes Science Center
Lake Michigan Ecological Research Station
1100 N. Mineral Springs Road, Porter, Indiana, USA

Studies begun in 1985 revealed large populations of *E. coli* in sands at beaches of Indiana Dunes along Lake Michigan. Initially attributed to human input (e.g., septic leaks), it soon became clear that fecal indicator bacteria(FIB) populations (*E. coli*, enterococci) were present at beaches throughout the Great Lakes, including uninhabited islands of pristine Lake Superior. These baseline findings bolstered other studies to indicate that FIB are ubiquitous in sands and soils throughout the world. In investigations of distribution in watershed ecosystems, early studies demonstrated that *E. coli* was generally limited to sands above the water table and decreased above and below the foreshore berm. Seasonally, *E. coli* occurred throughout the year in both moist soils and sands independent of animal input. Further, *E. col*i was shown to grow in sterile forest soils independent of any nutrient or carbon augmentation; soil *E. coli* strains were genetically different from *E. coli* commonly found in human and wildlife fecal sources. These populations rose from 10^2 to as high as 10^6 and persisted for 18 months without augmentation or disturbance. In experiments with nutrient addition, this growth could be significantly stimulated with plant detritus, plankton, filamentous algae, and field debris. Later genomic studies confirmed that *E. coli* populations in soil and sand comprise numerous genotypes not observed in animals. These accumulated of these findings suggest the E.coli may be ubiquitous in sand and soils worldwide with many strains endemic within these natural substrates.

*psammonman@gmail.com, 1088 N 350 East, Chesterton, IN 46304 USA 219-406-0924.

Public Health Risk Assessment and Regulations for Beach Sand and Water

J. Brandão

Beach water is often monitored for microbiological quality to detect the presence of indicators of human sewage contamination so as to prevent public health outbreaks associated with water contact. However, despite popular belief that sun light sterilizes surfaces, beach sand harbors microbes harmful to human health, often in concentrations greater than the beach water. Currently, there are no standards for monitoring, sampling, analyzing, or managing beach sand quality. Growing evidence has identified pathogenic bacteria, viruses, and fungi in a variety of beach sands worldwide. Regulatory agencies need to address this problem: Quality needs to be monitored, contaminations need to be controlled and the public should be made aware of where to rest and what children play with on a day spent at the beach.

Microbiological quality assessment of sand from beaches in Portuguese coast: fifteen years of experience

Helena Barroso1, Aida Duarte2
1CiiEMandLMAEM, Instituto Superior de Ciências da Saúde Egas Moniz, Campus Universitário, Qta. da Granja, Monte de Caparica, 2829-511 Caparica,2iMed.UL, Faculdade de Farmácia da ULisboa, Av das Forças Armadas, 1649-019 Lisboa

Beach sands can be a source of pathogenic and potentially pathogenic microorganisms and bathers spend more time in contact with sand than in the water. The water is subject to regular microbiological analysis, monitoring only water and not sand contributed for a gap in assessing the overall public health quality of recreational areas. In order to monitor the weakly cleaning treatment applied to the beach sand, during the bathing season, municipalities have promoted a microbiological control of beach sand. Our experience over the last fifteen years showed that a great variety of microorganism could be present in the sand and it was verify a relationship between indicator bacteria and harmful microorganisms. Among these, opportunistic bacteria were found such as Staphylococcus aureus and Pseudomonas aeruginosa, responsible by skin, eye, ear and mucous membrane infections. Also, the filamentous fungi of clinical relevance, such as: species of Aspergillus, Fusarium, Scopulariopsis, and dermatophytes Trichophyton, may be considered an infectious hazard factor for the population bathing. During this process variations of the micro flora occurred probably related with environmental pressure including climacteric conditions. Also, differences in presence of pathogens and the level of their detection may differ significantly because of technical limitations and the representativeness of sample. Our results obtained over fifteen years has shown a reduction in microbial burden associated with the treatment process and hygiene measures implemented in times of high population density, during the bathing season. Sanitary management of beaches can play a very important role with regard to public health.

A. caninum, E. granulosus, Toxocara sp and *T. gondii* in sand?

Maria João Gargaté

Reference Unit for Parasitic and Fungal Infections - Department of Infectious Diseases
National Institute of Health Dr. Ricardo Jorge

In Portugal as around the world, the beaches and shorelines are some of the more highly valued areas for recreation, responsible for significant tourism industry revenue. Over the past decade, there has been a significant increase in the number of beachgoers visiting these areas, resulting in simultaneous build-up of housing, hotels and restaurants, along the entire coast. Water/ sands continue to be a major reservoir and conduit for transmission of many parasites. Waterborne parasitic organisms of human concern, associated with beaches and sand, are generally disseminated into the environment in the feces or urine of infected animals or humans. This is especially the case of the protozoan parasites, such as *Giardia duodenalis*, *Cryptosporidium* sp., and *Toxoplasma gondii*, and the helminths *Toxocara* sp. *Ancylostoma caninum* and *Echinococcus granulosus* .

Very little information exists concerning the presence of parasites in beach sand and the presence of these microorganisms transmitted by water that have not been investigated in recreational sand areas may be potentially significant.

In this communication the biology, some biologic behaviour, epidemiology and significance of this group of organisms for the public health in beach and recreational sand are reviewed. Potential management actions can be taken and are also addressed.

Methods to Assess and Reduce Risk of Microbes in Sands

Helena Solo-Gabriele, Ph.D.
Professor and Associate Dean for Research
University of Miami, Coral Gables, FL

In the U.S. the safety of recreational beach use from disease is monitored through measures of fecal indicator bacteria (FIB) in water. Fecal indicator bacteria are used to indicate the presence of potential disease causing microbes and are well suited for predicting impacts from offshore sewage sources. However, as local governments implement improvements in wastewater treatment and sewer outfall designs, the primary source of FIB shifts from offshore sources to onshore sources. Onshore sources, referred to as non-point sources, include beach bathers, animals, stormwater, and sand. Sand, especially sand within the intertidal zone, will tend to accumulate and retain onshore sources of microbes. This is of significance for two reasons. First beach goers, especially children, are in frequent contact with sand in this zone. Second, sand quality will impact water quality and ultimately cause beach closures. This presentation will focus on describing the microbial quality of sand including multiple classes of organisms including FIB, pathogenic bacteria, viruses, protozoa, and fungi. Methods to assess risks from microbes in sands will be described along with an emphasis on information gaps in terms of assessing risks from fungi. A case study will be presented in terms of how to reduce risks from beach sands through beach mitigation efforts.

ABSTRACT

The Use of Metagenomics and Microbiomes to Understand Host , Environmental, and Land Use Contributions to The Microbiota of Aquatic Systems

Michael Sadowsky and Chris Staley (USA)

Small and large scale associations between bacterial communities and the concentration of nutrients and chemicals were determined in the Upper Mississippi River in Minnesota to determine if community structure was associated with discrete types of chemical inputs associated with different land cover. Bacterial communities were characterized by Illumina sequencing of the V6 region of 16S rDNA and compared to > 40 chemical and nutrient concentrations. Bacterial community structure at the local level was shaped mostly by associations among bacterial orders. However, the abundances of orders were correlated regionally with nutrient and chemical concentrations, and were very related to major land coverage types. The primary abiotic factors associated with local community composition were total organic carbon and total dissolved solids, and these co-varied with land cover. The concentration of *Escherichia coli* was poorly related to community composition or nutrient concentrations. Up to 14 bacterial orders were related to land coverage type, and 7 showed significant differences in abundance ($P \leq 0.046$) between forested or anthropogenically-impacted sites. This study identifies specific bacterial orders that were associated with specific chemicals and nutrients derived from land cover types. This may be useful in assessing water quality. Results of this study reveal the need to investigate community dynamics at both the local and regional scales and to identify shifts in taxonomic community structure and these will be useful in determining sources of pollution in the Upper Mississippi River.

Abstract

Surveillance of *Listeria monocytogenes* in food catering establishments

BARREIRA Maria João, MAIA Carla

National Health Institute Doutor Ricardo Jorge.Av. Padre Cruz, 1649-016 Lisbon, Portugal

Listeria monocytogenes (Lm) is the causative agent of human listeriosis, a potentially fatal foodborne infection. The main route of transmission is through consumption of contaminated food being pregnant women, newborn infants, the elderly and immunocompromised individuals the most vulnerable population groups.

Lm is a cause of concern to food processing establishments, as it is an ubiquitous microorganism, capable of growing under refrigeration temperatures and to form biofilms on food processing surfaces. Its persistence in food-associated environments represents a key factor in food contamination.

One of the Microbiology Laboratories activities is the evaluation of the quality of ready-to-eat (RTE) food in catering establishments. The presence of Lm in RTE food samples is evaluated. In Lisbon area and surroundings, 4% of the samples analyzed, between 2009 and 2013, were positive for Lm.

In 2013, in two different visits to a Hospital, RTE food collected were positive for Lm. In order to identify the cause of contamination, surface swabs were collected and analyzed. Results showed that 29% of samples were positive for Lm.

Lm strains were characterized by Pulse-Field Gel Electrophoresis (PFGE). Five different PFGE profiles were distinguished. The same PFGE profile was identified in one of the RTE food and in two surfaces.

After cleaning and disinfection of the canteen surface, swab samples were collected and Lm was recovered. This indicates that sanitation programs must be reviewed and proven effective with appropriate verification, through the analysis of environmental swabs for Lm detection.

Characterization of *Campylobacter jejuni* and *Campylobacter coli* isolated from broiler meat along the slaughtering line and in the final product

A. Duarte [12], T. Seliwiorstow [3], S. Denayer [2], K. Dierick [2], N. Botteldoorn [2$*], M. Uyttendaele [1$]

[1] Laboratory of Food Microbiology & Food Preservation, Dept. Food Safety & Food Quality, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B - 9000 Gent, Belgium
[2] Institute of Public Health, Section Food-borne pathogens, Juliette Wytsman Street 14, 1050 Brussels, Belgium
[3] Dept. Veterinary Public Health & Food Safety, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
[$] Equally contributors

Campylobacter is the most reported zoonotic infection in EU since 2005 and the broiler meat is his principal source. Guillain Barré Syndrome (GBS), a form of paralysis that can be fatal, has been associated to a previous *C. jejuni* infection. The mimicry between gangliosides of human peripheral nerves and the *Campylobacter* sialylated lipooligosaccharides (LOS) may be in the origin of the syndrome. To decrease the presence of *Campylobacter* on broiler a better understanding of contamination trough the slaughtering procedure is needed.

In this study, *Campylobacter* strains isolated along the slaughterline of 5 slaughterhouses and collected under the Belgian boiler monitoring plan, were characterized in terms of antibiotic resistance and presence of potential virulence factors. For the antibiotic resistance, the Minimum Inhibitory Concentration (MIC) assay based on a microdilution method was used. For the *C. jejuni* virulence factors presence, five LOS locus classes (A, B, C, D and E) were discriminated by PCR.

As results, the origin of the broiler meat contamination is fecal, since the strains found in the cecum and duodenum were the same as the ones found in the subsequent sampling points.
A combined resistance between ciprofloxacin, nalidixic acid and tetracycline and a sensitivity to gentamicin and chloramphenicol were shown in *C. jejuni and C. coli*.
LOS locus classes A, B and C are the most associated to GBS, with A as the more prevalent. In this study, no class A was found and classes B and C represented 41% of the characterized strains.

The use of quantitative RT-PCR techniques of E.coli and Enterococci for fast detection of Fecal pollution in drinking water.

Gerhard H. Wubbels, Marsh v.d. Wiel, Teo Lijzenga and Auke Douma.

Waterlaboratorium Noord (WLN), The Netherlands

Keyword：RT-PCR, Fecal contamination

Recently new methods are developed to detect within four hours fecal pollution in drinking water . These methods are RNA-based and specially designed for the fecal target-organisms. For both E.coli and the Intestinal Enterococci a detection limit is achieved of approximately 1 living cell/100 ml. This low level detection in real water samples is very different from other publications and makes it unique in the field of Polymerase chain Reaction techniques . Experiments wherein a comparison is made with standardized ISO culture methods showed that the Rerversed Transcriptase RNA methods for E.coli and Enterococci are more sensitive and accurate than the culture techniques. By choosing RNA instead of DNA sequences for targeting both bacteria the focus is laid on potentially surviving organisms instead of dead or not-culturable. This makes it possible to use it in both situations with or without using chlorine in distributing drinking water.

Experiences in practical situations in real fecal polluted drinking water situations confirmed the former findings with laboratory experiments.

Nowadays the methods are being used in the north of the Netherlands to detect a fecal pollution in early stage and is used to build up a database with information about the effect of working on hygienic base in difficult situations.

Corresponding author:
Drs. Gerhard H. Wubbels
E g.wubbels@wln.nl
T (+)31504022115
M(+)620412578

Plenary Lecture

Session ¨Assessment and management of emerging risks: a tool to insure food safety”

Title:

Ochratoxin A: new approaches for its toxicity characterization

Authors:

Vettorazzi A; Corcuera LA, López de Cerain, A

Department of Pharmacology and Toxicology. University of Navarra, Irunlarrea 1, 31008 Pamplona, Spain

Contact details presenting author: Ariane Vettorazzi. E-mail: avettora@unav.es,
TF : +34 948425600 ext 803324

Abstract (250 words):

Ochratoxin A (OTA) is a mycotoxin to which humans are continuously exposed via food, mainly through cereal-based foods, spices, nuts, coffee, cocoa, grapes and wine. The main concern regarding OTA contamination in food is its potential carcinogenicity; strong scientific evidences on the ability of the mycotoxin to cause tumors in rodent bioassays have been obtained. However, even if widely studied, its mode of action as a carcinogen is still under continuous debate.

One practically unexplored aspect when evaluating toxicity of many xenobiotics, is the sex-dependent toxic response to chemical carcinogens in laboratory animals. This might be a key question for OTA as sex-specific differences in OTA-mediated carcinogenesis have been described in rodents: rat males being more sensitive than females to kidney tumor formation.

On the other hand, it is known that humans are exposed to more than one mycotoxin through the variety of different foods ingested every day or by consuming foodstuffs contaminated with more than one mycotoxin. Unfortunately, the majority of the toxicological studies carried out until date, have been performed with individual mycotoxins.

In the present lecture, the results obtained in our laboratory regarding these two research approaches will be presented. More concretely, results regarding the influence of sex on OTA kinetics in F334 rats and the combined toxicity of OTA with the well-known and carcinogenic aflatoxin B1 will be presented.

Abstract

The first case of infant botulism in Portugal

Infant botulism is a very rare neuroparalytic disease that can occur in babies under two years of age, caused by ingestion of *Clostridium botulinum,* an ubiquitous gram positive bacilli that grows better under anaerobic, low salt and low acid conditions. The spores formed by this bacterium are not inactivated, unless the food is heated under high pressure to 121ºC, for at least 20 minutes.

This rare occurrence is due to the toxin produced by the bacteria, after ingestion of the spores and their germination in the infant's intestine. Those neurotoxins interfere with the presynaptic release of acetylcholine at the neuromuscular junction.

The first case detected in Portugal occurred in 2009. The patient was a boy aged one month

The child was breastfed, but sometimes his parents used to give him wild herbal chamomile infusion and honey. After refusing to eat for three days, the boy was taken to the Hospital, with the symptoms overlapping with botulism.

The laboratory identification was performed using mouse bioassay, according to standard procedures issued by the Center for Disease Control (CDC), Atlanta, USA. Type B botulinum toxin was detected in the infant´s faeces sample. *Clostridium botulinum* type B was isolated from the faeces as well as from honey and chamomile herbs samples.

In contrast to foodborne botulism, associated to the ingestion of botulinum toxin pre-formed in foods, infant botulism occurs after ingestion of the spores and subsequent production of the toxin in the children's intestine because of their still weak defences.

One way to prevent this disease is not to give honey and wild herbal tea to the infants under two years of age.

MYCOMIX: Exploring the toxic effects of MYCOtoxins MIXtures in infant food and potential health impact

Alvito P.[1,2], Arruda N.[1], Assunção R.[1,2,3], Barreira M.J.[1], Borges T.[4], Leal S.[5], Louro H.[1], Martins C.[1], Martins V.[4], Nunes B.[1], Silva M.J.[1], Vasco E.[1], Tavares A.[1], & Calhau M.A.[1]

[1]Instituto Nacional de Saúde Doutor Ricardo Jorge I.P., Lisboa; [2]Centre for Environmental and Marine Studies (CESAM), Faculty of Sciences, Universidade de Lisboa; [3]Universidade de Évora; [4]General Health Directorate, Lisboa; [5]Primary Health Care Unit, Cidadela, Cascais.PT

*paula.alvito@insa.min-saude.pt

There is growing concern within public health about mycotoxin involvement in human diseases particularly related to children´s exposure through contaminated food[1] . The natural co-occurrence of mycotoxins is an increasing concern which could be expected to exert greater toxicity and carcinogenicity than exposure to single mycotoxins[2]. The present project aimed to study the occurrence of multiple mycotoxins and toxicity interactions in baby foods and cereals consumed by Portuguese children. Scarce data are available in the literature concerning the co-occurrence of mycotoxins in infant food and their combined toxicity and no data exists in Portugal concerning this issue. This project gathered a multidisciplinary team in order to answer to several questions: 1) Are children exposed daily to mycotoxins through food? 2) What are the quality and quantity that characterize this exposure? 3) Can this exposure bring harm to children? Will it put them on risk? The co-occurrence of several mycotoxins were evaluated in different infant foods (cereal based) marketed at Portugal (Lisboa). These results, combined with the consumption data from a food consumption survey performed at the Primary Health Care Unit from Cidadela, Cascais (children aged until 3 years old) were used to estimate the intake of mycotoxins in Portugal, through a probabilistic approach using @Risk software. Toxicological studies including bioavailability and absorption of mycotoxins[3] and its interactive effects[4] were also performed.

Key words:

mycotoxins, children, food toxicology, human health

References:

Are Portuguese children exposed to mycotoxins through infant foods? A preliminary approach

R. Assunção[1,2,3*], E. Vasco[1], B. Nunes[1] & P. Alvito[1,3]

[1]Departamento de Alimentação e Nutrição, Instituto Nacional de Saúde Doutor Ricardo Jorge I.P., Lisboa, Portugal; [2]Universidade de Évora, Évora, Portugal; [3]Centre for Environmental and Marine Studies (CESAM), Faculty of Sciences, Universidade de Lisboa, Lisboa, Portugal.

*ricardo.assuncao@insa.min-saude.pt

Mycotoxins are a wide group of fungal secondary metabolites that cause toxic and carcinogenic outcomes in humans exposed to them. The major foodborne mycotoxins of public health interest are the aflatoxins, fumonisins, trichothecene and ochratoxin A (OTA)[1,2]. Infants have a more restricted diet and they generally consume more food on a body weight basis than adults thus they are a particularly vulnerable population group to food contaminants as mycotoxins. In Portugal, Alvito *et al*. had reported the presence of aflatoxin B_1 (AFB_1) and M_1 (AFM_1) and OTA in baby foods[3] and no data are available until now concerning the exposure assessment of Portuguese children to these mycotoxins.

Exposure assessment, which evaluates the degree of intake of a certain contaminant, is one of the four steps included in risk assessment. Although several scientific reports have been published in order to propose the best methodologies for the exposure assessment framework, to date harmonization is far from being achieved[4].

The aim of the present study was to estimate the exposure of Portuguese children to mycotoxins due to infant foods ingestion, based on a probabilistic approach, using a risk analysis software (@Risk 6 for Excel, Palisade). The mycotoxin occurrence[3] of AFB_1, AFM_1 and OTA in infant foods and consumption data for children until 3 years old were modeled. Consumption data were based on preliminary results recently obtained during a pilot study performed at Primary Health Care Unit from Cascais under the Mycomix Project. Different strategies had been considered to treat the left censored data.

Key words:

Mycotoxins, exposure assessment, probabilistic analysis, Monte Carlo simulation

Antimicrobial drug resistance of *Campylobacter* spp and *Salmonella enterica*: national data in food producing animals and food of animal origin

Clemente, L.[1], Correia, I.[1], Ferreira, E.[2,3], Manageiro, V.[2,3], Jones-Dias, D.[2,3], Albuquerque, T.[1], Themudo, P.[1], Rocha, T.[1], Tavares, A.[4], Geraldes, M.[4], Barahona, M.J.[1], Caniça, M.[2,3]

[1]INIAV – Instituto Nacional de Investigação Agrária e Veterinária. Estrada de Benfica, 701, 1549-011, Lisboa, Portugal.

[2]Instituto Nacional de Saúde Dr. Ricardo Jorge. Av. Padre Cruz, 1649-016, Lisboa, Portugal

[3]Centre for the Studies of Animal Science, Institute of Agrarian and Agri-Food Sciences and Technologies, University of Oporto, Oporto, Portugal

[4]INIAV – Instituto Nacional de Investigação Agrária e Veterinária. R. dos Lagidos 4485-655 Vairão, Portugal

lurdes.clemente@iniav.pt
Tel: +351 217115247

Campylobacter spp and *Salmonella enterica* are the two most common causes of bacterial foodborne illnesses in humans in developed countries, being food producing animals one the main reservoirs.

Antimicrobial susceptibility testing was determined through Minimum Inhibitory Concentration, in 448 isolates of *Campylobacter* spp recovered from broiler ceca at slaughter (*n*=351) and broiler carcasses (*n*=97); and 1600 isolates of *S. enterica* recovered from poultry (*n*=868), swine (*n*=101), other animal species (*n*=61), animal feed (*n*=43) and food products of animal origin (*n*=527). Screening and identification of beta-lactamase and plasmid-mediated quinolone resistance (PMQR) genes were performed by PCR and sequencing.

The highest level of resistance in *Campylobacter spp* isolates recovered from broilers and carcasses, was recorded to ciprofloxacin, followed by tetracycline, erythromycin and streptomycin. Four isolates of *Campylobacter coli* were resistant to gentamicin.

Table: Antibiotic resistance in foodborne pathogens

Keynote entitled: **Current perspectives of emerging antibiotic resistance in foodborne bacteria**

Author: Manuela Caniça

National Reference Laboratory of Antibiotic Resistances and Health Associated Infections (NRL-AMR-HAI), Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge

Abstract:

The increasing occurrence of antibiotic resistance in primary and processed food products, it is of the greatest importance, where the horizontal gene transfer of antibiotic resistance determinants is of huge concern. Indeed, common inhabitants of the human and animal gut of food animals may be disseminated through the food chain. The widespread use of drugs in veterinary can also contribute to the selection of antibiotic resistance mechanisms in pathogenic and non-pathogenic isolates. The common mode of plasmid-mediated resistance (one gene for one class of antimicrobials) requires that an organism harbor and express an array of genes in order to maintain multidrug-resistance; however, for example, at fluoroquinolone resistance, bacteria have an innovation that is a pleiotropic drug-modifying enzyme providing resistance to two structurally and functionally different classes of antibiotics by acquisition of a single gene. But other resources are available to the bacterium confronted with the challenge of antibiotics that is the ability to acquire resistance genes, but not express them; such “nonexpressing” bacteria would remain sensitive to the antibiotic while carrying a potentially transmissible resistance gene. Biofilm formation is also an important phenomenon in the food process. A new dimension in microbial adaptability is taking serious proportions, thus the Council Recommendation “on the prudent use of antimicrobials agents in human medicine” (2002/77/EC), highlight that the “*coordination between human, veterinary and environment sectors should be ensured and the magnitude of the relationship between the occurrence of antimicrobial resistant pathogens in humans, animals and the environment should be further clarified”.* In fact, a “One Health” approach is being encouraged.

Reducing the risks of resistance development by pathogenic fungi. Multitarget fungicides

R. B. Ferreira[1,2,3,*], M. Pinheiro[1,3], S. Monteiro[1,3]

[1]Instituto Superior de Agronomia, University of Lisbon, Lisboa, Portugal
[2]Instituto de Tecnologia Química e Biológica, New University of Lisbon, Oeiras, Portugal
[3]CEV SA, Cantanhede, Portugal
[*]Presenting author: Ricardo Boavida Ferreira, rbferreira@itqb.unl.pt, tel. +351.213653416, fax: +351.213653195

Pathogenic fungi are a major threat in modern society:

- They claim hundreds of thousands of lives worldwide each year. Once inside the human body, death rates by *Aspergillus fumigatus* may reach 90%;
- Damage to agricultural crops and fungicide costs each run in the multibillion dollar range per year;
- Amazing similarities in recognition and defense mechanisms occur between humans/animals and plants; however, human and plant fungal pathogens have been treated as separate fields of science;
- There is a shortage of efficient fungicides in agriculture and human health; those showing higher efficacy are toxic. Current estimates indicate one new chemical fungicide per 100,000 novel compounds tested;
- Tighter legislation and increasing public concern over the use of chemical fungicides;
- Commercially available fungicides are typically short-lived; many become obsolete as deleterious side-effects to man and/or environment are discovered or because of fungal resistance development –e.g. resistance of *A. fumigatus* to azole developed during long-term azole therapy in clinical settings or due to considerable use of azole fungicides in agriculture.

Solution:

- Switch the search from Chemical to Biological and exploit the myriad biomolecules involved in the chemical warfare which takes place between host and pathogenic fungus to establish pathogenesis.

Case-study: Blad.

- It is a stable, edible, 173-amino-acid-residue polypeptide which occurs in the cotyledons of germinated *Lupinus* plants;
- It cleaves catalytically chitin and chitosan;
- It is a lectin, binding strongly to cell wall chitin and to oligosaccharide moieties of membrane glycoproteins;
- Micro-array data showed that Blad interferes with divalent cation absorption;
- Other bioactivities are currently under investigation.

These bioactivities explain Blad's wide-open spectrum of potent fungicidal activity.

Antimicrobial drug resistance of *Campylobacter* spp and *Salmonella enterica*: national data in food producing animals and food of animal origin

Clemente, L.[1], Correia, I.[1], Ferreira, E.[2,3], Manageiro, V.[2,3], Jones-Dias, D.[2,3], Albuquerque, T.[1], Themudo, P.[1], Rocha, T.[1], Tavares, A.[4], Geraldes, M.[4], Barahona, M.J.[1], Caniça, M.[2,3]

[1]INIAV – Instituto Nacional de Investigação Agrária e Veterinária. Estrada de Benfica, 701, 1549-011, Lisboa, Portugal.

[2]Instituto Nacional de Saúde Dr. Ricardo Jorge. Av. Padre Cruz, 1649-016, Lisboa, Portugal

[3]Centre for the Studies of Animal Science, Institute of Agrarian and Agri-Food Sciences and Technologies, University of Oporto, Oporto, Portugal

[4]INIAV – Instituto Nacional de Investigação Agrária e Veterinária. R. dos Lagidos 4485-655 Vairão, Portugal

lurdes.clemente@iniav.pt
Tel: +351 217115247

Campylobacter spp and *Salmonella enterica* are the two most common causes of bacterial foodborne illnesses in humans in developed countries, being food producing animals one the main reservoirs.

Antimicrobial susceptibility testing was determined through Minimum Inhibitory Concentration, in 448 isolates of *Campylobacter* spp recovered from broiler ceca at slaughter (*n*=351) and broiler carcasses (*n*=97); and 1600 isolates of *S. enterica* recovered from poultry (*n*=868), swine (*n*=101), other animal species (*n*=61), animal feed (*n*=43) and food products of animal origin (*n*=527). Screening and identification of beta-lactamase and plasmid-mediated quinolone resistance (PMQR) genes were performed by PCR and sequencing.

The highest level of resistance in *Campylobacter spp* isolates recovered from broilers and carcasses, was recorded to ciprofloxacin, followed by tetracycline, erythromycin and streptomycin. Four isolates of *Campylobacter coli* were resistant to gentamicin.

Antimicrobial susceptibility profiles in *Salmonella* isolates differed according with the serotype and the origin of the isolates. Overall, in poultry, the higher frequency of resistance was observed towards nalidixic acid (33.5%) and ciprofloxacin (32.5%), whereas in swine was to tetracycline (61.4%), sulfamethoxazole, streptomycine (55.4%), and ampicillin (44.5%). A higher multidrug-resistance was recovered from food of swine (62.6%) and bovine (59.4%) isolates.

Twelve *Salmonella* isolates recovered from broilers (*n*=7), broiler carcass (*n*=1), partridge (*n*=1) and swine meat (*n*=3), were resistant to cefotaxime. The $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-32}}$, $bla_{\text{SHV-12}}$, $bla_{\text{CTX-M-type}}$, $bla_{\text{TEM-type}}$ and $bla_{\text{CMY-2}}$ genes were identified in these isolates. No PMQR-encoding genes were detected.

Measures must be taken to control the escalating spread of antimicrobial resistance in foodborne pathogens.

Mobile genetic elements associated to antibiotic resistance in *Salmonella enterica* isolates collected in food-chain

V. Manageiro,[a,b,†] E. Gomes-Neves,[b,c,†] J. M. Correia da Costa,[a,b] E. Ferreira,[a] M. Caniça[a]

[a] *Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge, Lisboa and Porto, Portugal*

[b] *CECA-ICETA, Centro de Estudos de Ciência Animal, Universidade do Porto, Porto, Portugal*

[c] *ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal*

[†] These authors contributed equally to the work.

Presenting author: Vera Manageiro, National Reference Laboratory of Antibiotic Resistances and Health Associated Infections (NRL-AMR-HAI), Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge (NIH)
Tel.: +351 217519248, Email: vera.manageiro@insa.min-saude.pt

Abstract: In this study, we assessed the presence of IS*26* elements in 60 multidrug resistant (R-type ASSuT phenotype) *Salmonella enterica* isolates collected in slaughtered swine samples in Portugal, investigating its role in the dissemination of antimicrobial resistance among different reservoirs. PCR-mapping allowed us to describe a new genetic organization, in the emergent *Salmonella* Rissen, including a peptidase C14 caspase catalytic subunit P20 gene between two IS*26* elements. Furthermore, we detected the same genetic structures in *Salmonella* isolates from different serotypes in samples from slaughtered swine, carcasses/meat, and meat handlers, namely a resistance region harboring bla_{TEM-1} genes also flanked by 2 copies of IS*26* elements in *Salmonella* Typhimurium (n=17) and *Salmonella* 4,[5],12:i:- variants (n=2). This study highlights that IS*26* mobile genetic elements might play an important role in the mobilization of antibiotic resistance genes, which can constitutes a problem in terms of veterinary medicine and public health in general.

Keywords: *Salmonella*, *IS*26, swine, Portugal

[1]Etzel R.A. (2006). *Current Problems in Pediatric and Adolescent Health Care*, 36, 282-305. [2]Bouaziz et al. (2008). *Toxicology*, 254(1-2): 19-28. [3]Assunção et al. (2014). *Journal of Toxicology and Environmental Health, Part A*, 77: 14-16, 983-992. [4]Tavares et al. (2013). *World Micotoxin Journal*. 6, 4, 375-388.

Acknowledgments:
This research was performed under the MycoMix project (PTDC/DTP-FTO/0417/2012), funded by the Fundação para a Ciência e Tecnologia (FCT), Portugal.

ABSTRACTS POSTER PRESENTATIONS

THE ABSTRACTS ARE ORGANIZED ACCORDING TO THE DAY OF PRESENTATION

TEMPH 2014
TRENDS IN ENVIRONMENTAL MICROBIOLOGY FOR PUBLIC HEALTH

P1
POSTERS
19TH
SEPTEMBER

TEMPH 2014
TRENDS IN ENVIRONMENTAL MICROBIOLOGY FOR PUBLIC HEALTH

Cytotoxicity of the most frequently occurring and emerging mycotoxins

Błajet-Kosicka A., Twarużek M., Soszczyńska E., Grajewski J.

Kazimierz Wielki University, Faculty of Natural Sciences, Institute of Experimental Biology, Division of Physiology and Toxicology, Bydgoszcz, Poland

Several classes of fungal secondary metabolites are considered to be highly toxic. Mycotoxins commonly contaminating food and raw materials can occur simultaneously. Although the toxic properties of single compounds are rather well defined, the toxicity of mycotoxin mixtures still needs evaluation.

Thus the aim of this project was to examine the cytotoxicity of different food and feed relevant mycotoxins individually and in combinations.

The *in vitro* studies were performed on a swine kidney (SK) cell line as well as kidney epithelial cells from an African green monkey (Vero). Cytotoxicity of citrinin (CIT), ochratoxin A (OTA), deoxynivalenol (DON) and T-2-toxin was evaluated with MTT test.

The SK cells were more sensitive to T-2 toxin (IC_{50} = 3,9 ng/ml) compared to Vero cells (IC_{50} = 62,5 ng/ml), whereas the latter cell line was more sensitive to DON ($IC_{50\ Vero}$ = 1250 ng/ml, $IC_{50\ SK}$ = 2500 ng/ml). The cytotoxicity of OTA and CIT was similar in case of both cell lines. The relations of cytotoxicity has been evaluated as follows: T-2 > DON > OTA > CIT – independent from the cell line. Binary mixtures testing revealed possible synergistic effects of mycotoxins of interest.

Financial support: “Toxicity studies of molds and their secondary metabolites contaminating human and animal habitats”: ROP of Kujawsko-Pomorskie Voievodship in the years 2007-2013, Task 5.4. Strengthening regional capacity for research and technology development.

Detection of *Campylobacter* spp. and *Clostridium difficile* in biogas plants

S. Huber[1]*, C. Höller[1], M. Lebuhn[2], U. Messelhäusser[1]

[1] Bavarian Health and Food Safety Authority, Veterinärstr. 2, 85764 Oberschleissheim, Germany

[2] Bavarian State Research Center for Agriculture, Department for Quality Assurance and Analytics, Lange Point 6, 85354 Freising, Germany

* Contact details: stefanie.huber@lgl.bayern.de
tel.: +49 9131 6808 5266
fax: +49 9131 6808 5458

Studies concerning the occurrence and fate of human and animal pathogens like *Campylobacter* spp. and *Clostridium difficile* in biogas plants are rather scarce. Pathogenic microorganisms might be present in different substrates for biogas plants like corn silage and poultry or cattle manure. To what extent these microorganisms are usually reduced during the different stages in the biogas production, is not yet clear. It would also be possible that some microorganisms will multiply if conditions are favourable for bacterial growth in these plants. It is important to gain more information on these topics to evaluate the hygienic quality of the digested residues which are often used as fertilizers.

Nine Bavarian biogas plants were sampled at different stages of biogas production - from original substrates to digested residues. *Campylobacter* spp. and *Clostridium difficile* were detected with combined cultural and real-time-PCR methods (and additionally with an ELFA test for *Campylobacter spp.*).

None of the 94 tested samples was positive for *Campylobacter spp.* in PCR analysis. One swine manure sample gave a positive result with the ELFA method but cultivation was not successful. *C. difficile* was detected in 43 of 94 samples. 73 % of fermenter content and 42 % of residues samples were positive for *C. difficile*. Moreover toxin genes of the *C. difficile* isolates were identified (toxin A + toxin B: 51 %, toxin A + binary toxin: 5 %, toxin A + toxin B + binary toxin: 14 %, no toxin genes: 30 %).

These results suggest a low risk for biogas plant residues to be contaminated with *Campylobacter spp*.. In contrast *C. difficile* was detected in a high percentage of fermenter and residues samples. If these residues are used as fertilizers *C. difficile* may be transferred to feed and food.

Seasonal variation of bacterial communities in shellfish harvesting waters: preliminary study before applying phage therapy

Pereira C[1]*, Santos L [1], Silva AP[1], Cunha A[1], Romalde JL[2], Nunes ML[3], Almeida A[1]

[1]Department of Biology and CESAM, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal.

[2]Departamento de Microbiología e Parasitología e CIBUS e Facultad de Biologia, Universidade de Santiago de Compostela e Campus Universitario Sur.,15782 Santiago de Compostela (A Coruña), Spain

[3]National Institute of Biological Resources (INRB, IP/L-IPIMAR), Av. Brasília, 1449-006 Lisbon, Portugal

*Corresponding author: E-mail: csgp@ua.pt, Tel.: +351234370350, Fax: +351234372587.

Abstract

Shellfish is currently involved in outbreaks of pathogenic bacteria, including multidrug resistant bacteria, even after depuration. Therefore, new efficient strategies to make shellfish consumption safe, such as phage therapy applied during depuration, are urgently needed to minimize the transmission of infections. The success of phage therapy depends on a comprehensive understanding of the natural bacterial communities in harvesting waters. This work aimed to evaluate the seasonal dynamics of the whole and disease-causing bacterial communities, as well as bacterial indicators of sanitary quality in two authorized harvesting zones of Ria de Aveiro (Portugal). This data will allow identifying crucial treatment periods, when phage therapy/depuration will be more effective. The structure of bacterial community was evaluated by Denaturing Gradient Gel Electrophoresis and the Enterobactereaceae family, the genera *Salmonella*, *Vibrio* and *Aeromonas* were quantified by Fluorescent In Situ Hybridization (FISH). *Escherichia coli* was enumerated by the membrane method (ISO 9308-1). The detection of *Salmonella* was done after enrichment, using the media XLD and BGE (ISO 6340) During the warm season, the disease-causing bacteria and the microbiological indicators of water quality were enriched. Nevertheless, the highest values of non-indigenous bacteria occurred during the rainy season, indicating that the risk of humans diseases outbreaks-may occur throughout the year. Still, a higher complexity of indigenous bacterial community allied to the dominance of new populations of shellfish pathogenic bacteria during the summer suggested that the spring/summer season would be the best period to apply phage therapy during depuration.

Bacteriophages with potential to inactivate *Salmonella enterica* in bivalve molluscs

Pereira C.[1]*, Pereira S.[1], Silva Y.J.[1], Costa L.[1], Romalde J.L.[2], Nunes M.L.[3], Almeida A.[1]

[1]Department of Biology and CESAM, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal.

[2]Departamento de Microbiología e Parasitología e CIBUS e Facultad de Biologia, Universidade de Santiago de Compostela e Campus Universitario Sur.,15782 Santiago de Compostela (A Coruña), Spain

[3]National Institute of Biological Resources (INRB, IP/L-IPIMAR), Av. Brasília, 1449-006 Lisbon, Portugal

*Corresponding author: E-mail: csgp@ua.pt, Tel.: +351234370350, Fax: +351234372587.

Abstract

Salmonella enterica is the most frequent causative agent of human gastroenteritis after consumption of contaminated seafood. Bacteriophages are safe bio-controlling agents, and have been recognized in aquaculture for their pathogen reduction properties.

The objective of this work was to isolate new bacteriophages with potential to inactivate *S. enterica* that were used to examine the host-phage dynamic. The isolated phages used in this study, SE-1, SE-2 and SE-5, were tested individually and as cocktails of two and three phages. The host-phage dynamics was characterized in TSB medium, through phage quantification by the soft agar overlay technique and host quantification by incorporation in TSA medium.

The maximum of bacterium inactivation with SE-1 phage was 1.8 log achieved after 4 h of phage therapy. After 12 h of treatment, the rate of inactivation was still considerably high (1.3 log). With SE-2 and SE-5 phages, the maximum of bacterium inactivation was 2.4 and 2.5 log, respectively, after 12 h of phage therapy. The cocktail SE-2/SE-5 was more effective (reductions of 2 log after 4 h and 2.7 log after 12 h) than the SE-2 and SE-5 phages used alone. However, the efficiency of SE-1/SE-2, and SE-1/SE-2/SE-5 cocktails was similar, respectively, to SE-2 and SE-5 phages alone. The efficiency of SE-5 phage alone was higher than the phage cocktail SE-1/SE-5. The three phages are efficient in the inactivation of *S. enterica*, being potential candidates, used individually or in cocktails, as agents for the biological control of infections transmitted to humans by consumption of bivalve molluscs.

MYCOTOXINS – GENOTOXICITY STUDIES AND METHODOLOGIES

Carina Ladeira[1,2]

1- Escola Superior de Tecnologia da Saúde de Lisboa – IPL, Portugal
2- CMDT – Public Health and Polices, Universidade Nova de Lisboa, Portugal

Author address: carina.ladeira@estesl.ipl.pt

Human exposure to mycotoxins can be determined by environmental and biological monitoring. Mycotoxins can assume carcinogenic, mutagenic, teratogenic, oestrogenic, neurotoxic, immunotoxic properties, and biomonitoring instruments can be applied in order to assess their presence, and namely, their effects. Cytokinesis-block micronucleus assay is a method that allows the cytological scoring of nuclear abnormalities due to genotoxic insult, and its use is well established in *in vitro* genetic toxicology testing and has become an accepted standard method to assess the genotoxic hazard of chemicals which led to the development of an Organization for Economic Cooperation and Development. Comet assay has become one of the standard methods for assessing DNA damage, namely in genotoxicity testing, human biomonitoring and molecular epidemiology, studying the mechanisms of action of genotoxic chemicals; monitoring oxidative stress in animals or human subjects resulting from lifestyle factors, or exposure to environmental agents; studying the effects of dietary antioxidants; and others.

Key Words: Mycotoxins, genotoxicity, cytokinesis-blocked micronucleus assay, comet assay.

Metabolomics profile of *Aspergillus niger* through comprehensive two dimensional gas chromatography

Carina Costa[1,*], Sílvia M. Rocha[2], Adelaide Almeida[3]

[1] Departamento de Biologia, Universidade de Aveiro, 3810-193 Aveiro, Portugal, * carina.pedrosa@ua.pt

[2] Departamento de Química & QOPNA, Universidade de Aveiro, 3810-193 Aveiro, Portugal

[3] Departamento de Biologia & CESAM, Universidade de Aveiro, 3810-193 Aveiro, Portugal

Keywords: Microbial metabolomics, Fungal infections, *Aspergillus niger*, HS-SPME/GC×GC-ToFMS

Fungal infections have greatly increased in risk populations, namely in immunocompromised patients,[1-2] and conventional methods are unable to diagnose infections on their early stages. Microbial metabolomics arises as a powerful feature screening the metabolites produced by microorganisms.[3] It provides information regarding the state of biological organisms which can be used as a diagnostic tool for diseases through fungal specific metabolites pattern. This research aims to in-depth study the *Aspergillus niger* exo-metabolome in order to establish a specific metabolites pattern that can be further exploited to fungal diagnosis. Thus, a methodology based on headspace-solid phase microextraction combined with comprehensive two-dimensional gas chromatography coupled to mass spectrometry with a high resolution time of flight analyser (HS-SPME/GC×GC-ToFMS) tandem with multivariate analysis was developed. *A. niger* exo-metabolome was analysed in different growth conditions: temperature (25 and 37ºC), and incubation time (3 and 5 days), and culture medium (solid and liquid media). *A. niger* exo-metabolome includes around 500 metabolites, distributed over several chemical families, being the major ones alcohols, aldehydes, esters, hydrocarbons, ketones and terpenoids. Differences among volatile metabolites produced under different growth conditions were observed, being the major relative abundance determined for 5 days of growth, at 25ºC, using solid medium. These results indicate the high complexity of *A. niger* exo-metabolome, thus, to extract relevant information, Principal Component Analysis was applied, and a set of 30 metabolites were also defined as the *A. niger* metabolomic specific pattern, which is already detected at an early stage of growth (3 days). This information can be useful for diagnosis of fungal infection; even can be further exploited in clinical context.

References:

1- Badiee P, Kordbacheh P, Alborzi A, *et al.* (2009) Study on invasive fungal infections in immunocompromised patients to present a suitable early diagnostic procedure. International Journal Infection Diseases 13: 97-102.

2- Karageorgopoulos DE, Vouloumanou EK, Ntziora F, *et al.* (2011) β-D-Glucan Assay for the Diagnosis of Invasive Fungal Infections: A Meta-analysis. Clinical Infectious Diseases 52: 750-770.

3- Tang, J. (2011) Microbial Metabolomics. Current Genomics 12: 391-403.

Open air children playgrounds: the importance of microbial control of floor

C. Matias[1], A. Fernandes[1], L. Proença[1,2], A. Duarte[3], H. Barroso[1,2,4]

[1]Instituto Superior de Ciências da Saúde EgasMoniz, Campus Universitário, Qta. Da Granja, Monte de Caparica, 2829-511 Caparica, [2]CiiEM, Instituto Superior de Ciências da Saúde Egas Moniz, Campus Universitário, Qta. da Granja, Monte de Caparica, 2829-511 Caparica,[3]iMed.UL, Faculdade de Farmácia da ULisboa, Av das Forças Armadas, 1649-019 Lisboa; [4]Laboratório de Microbiologia Aplicada Egas Moniz, Campus Universitário, Qta. da Granja, Monte de Caparica, 2829-511 Caparica

Playgrounds are part of township kindergartens, used by children, adults and animals that share the environment. Samples were collected in order to identify, quantify and characterize the microbial flora of the soil from 30 playgrounds located in grand area of Lisboa. The samples have been collected near the surface of playground toys and trees and consisted of pebble stone (5mm), sand or synthetic floor. The washing solutions of sand and pebble stone, and the solution where the swabs were immersed from synthetic floor were used to inoculate different culture media. Biochemical tests were used to identify the microorganisms and antimicrobial susceptibility determined among selected bacteria.

According to the results obtained during the study it was possible to verify that climatic changes and the type of floor have a major impact on microbial flora: when there is an increase of temperature, there is a significant decrease in the number of bacteria, also the synthetic floor showed the higher number of isolates with antibiotic resistance.

Among the playgrounds, differences may be noted in the total amount of microorganisms. Low contamination are directly related to the cleaning conditions, animal control and social status of population. Resistance to antibiotics used in clinical practice was detected in bacteria isolated from different playgrounds, and was worrisome the identification of *Staphylococcus aureus* meticilline resistant (MRSA).

The importance of microbiological control was demonstrated in our work, emphasizing the need for cleaning of playgrounds that could serve as the vehicle of transmission of pathogenic microorganisms

Evaluating *mcy*A gene expression in two toxic cyanobacterial species under different light intensities using RT-qPCR

Salvador, D.[1] Churro, C.[1], Valério, E.[1]

[1] Unidade de Água e Solo, Departamento de Saúde Ambiental, Instituto Nacional de Saúde Dr. Ricardo Jorge. Avenida Padre Cruz, 1649-016 Lisboa, Portugal.

E-mail: daniel_fsalvador@hotmail.com; Tel: +351 21 751 92 00; Fax: +351 21 752 64 00

Cyanobacteria are phytoplanktonic organisms widely occurring in freshwaters, being frequently associated with the production of toxins, namely microcystins. Microcystins are produced non-ribosomally, by a multienzyme complex (*mcy* genes). It is believed that environmental factors such light intensity can influence toxin production.

The aim of this study was assess the influence of light intensity, in the transcription of the *mcyA* gene and corresponding production of microcystins in *Microcystis aeruginosa* and *Planktothrix agardhii*. For that purpose, cultures were exposed to three different light intensities (4, 20 and 30 µmol photons m^{-2} s^{-1}) for 18 days at 20 ± 1ºC. The growth was followed daily using absorbance readings. At each growth stage samples were collected for cell counting, microcystins quantification by ELISA and RNA extraction. The level of transcripts was quantified by Real-Time RT-qPCR and the relative expression determined using three reference genes, 16S rRNA, *gltA* and *rpoc1*.

The results showed that the best reference gene was *gltA*, since it remained more stable, independently of the light intensity and exposure time. There were differences in the expression of *mcyA* between the two species. In *M.aeruginosa*, the highest levels of expression occurred at 20 whereas for *P.agardhii* was at 30 µmol photons m^{-2} s^{-1}. It was also observed differences in the *mcyA* expression with the growth stage and light intensity. Furthermore differences between the *mcyA* expression and the toxin content were different among species.

Our results indicate that the light intensity influences microcystin production and *mcy*A expression levels, although differently in *M.aeruginosa* and *P.agardhii*.

Microbiological assessment of Indoor Air Quality in different Hospital sites

Sandra Cabo Verde[1], Susana Marta Almeida[1], João Matos[1,2], Marcia Meneses[1,3], Carla Viegas[3]

[1] Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Loures, Portugal
[2] Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Monte da Caparica, Portugal
[3] Grupo de Investigação Ambiente & Saúde, Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Lisboa, Portugal

Presenting Author: E-mail: sandracv@ctn.ist.utl.pt Tel.no.: +351 219946253

Abstract

Indoor air quality (IAQ) is an important factor in preventing infections in occupants of hospital facilities. Poor hospital IAQ may lead to hospital-acquired infections, sick hospital syndrome, and various occupational hazards. Air-control measures are crucial for reducing dissemination of airborne biological particles in hospitals.

The objective of this study was to perform a survey of bioaerosol quality in different sites of a Portuguese Hospital, namely Operating Theatre (OT), Emergency service (ES) and Surgical ward (SW). Total aerobic counts (TAC) and fungal load (FL) were assessed using a microbiological air sampler (MAS-100 single-stage impactor).

The Emergency service presented the highest airborne microbial concentrations (TAC range 284 - 736 CFU/m^3; FL range 31 – 123 CFU/m^3), exceeding in several sampling sites the limit values set by the national legislation (DL118/2013). Bacterial concentrations in Surgical ward (TAC range 103 – 384 CFU/m^3) and Operating Theatre (TAC range 44 – 170 CFU/m^3) were under the recommended limit. While fungal levels were below 1 CFU/m^3 in OT, in SW (range 9–32 CFU/m^3) there were sites with fungal indoor concentration higher than the detected in the outdoor. Airborne Gram-positive cocci were the most frequent phenotype (88%) detected from the measured bacterial population in all indoor environments. *Staphylococcus* (51%) and *Micrococcus* (37%) were dominant among the bacterial genera identified in the present study. Concerning indoor fungal population the prevalent genera were *Penicillium* (41%) and *Aspergillus* (24%).

TEMPH 2014

Bioaccessibility and changes on cylindrospermopsin concentration in edible mussels over storage and processing time

Marisa Freitas[1,2,3], Joana Azevedo[1], Vera Mendes[4], Bruno Manadas[4], Alexandre Campos[1], António Paulo Carvalho[1,2], Vitor Vasconcelos[1,2]

[1] CIIMAR/CIMAR - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Rua dos Bragas 289, P 4050-123 Porto, Portugal

[2] Faculty of Sciences, Porto University, Rua do Campo Alegre, 4169-007 Porto, Portugal

[3] Polytechnic Institute of Porto. Department of Environmental Health, Escola Superior de Tecnologia da Saúde do Porto. CISA/Research Center in Environment and Health, Rua de Valente Perfeito, 322, 440-330 Gaia, Portugal

[4] Center for Neuroscience and Cell Biology, University of Coimbra, Portugal

The cyanobacterial alkaloid cylindrospermopsin has been recognized of increased concern to public health due to the global proliferation of its main producer, *Cylindrospermopsis raciborskii*. Previous studies have shown that aquatic organisms, especially bivalves, can accumulate high levels of cylindrospermopsin without lethal effect. Based on the potential for human health risks, a provisional tolerable daily intake of 0.03µg/kg-body weight has been established. However, human exposure assessment has been based on the cylindrospermopsin concentration in raw products. Cylindrospermopsin is highly water-soluble and stable to extreme temperatures and pH, thus the knowledge of the influence of storage and cooking practices as well as human digestion is required to a more accurate risk assessment. This study aimed to assess the changes on cylindrospermopsin concentration in edible mussels over storage and processing time as well as cylindrospermopsin bioaccessibility. *Mytilus galloprovincialis*, fed cylindrospermopsin-producing *Cylindrospermopsis raciborskii*, were refrigerated, frozen, boiled, steamed and subjected to microwave radiation over different periods of time and then analyzed by LC-MS/MS. Cylindrospermopsin bioaccessibility was assessed in uncooked and steamed mussels, which were *in vitro* digested with saliva, gastric and duodenal juices. Mussels stored frozen for 48h and one week showed a significantly higher concentration of cylindrospermopsin, 52.5% and

BIOAEROSOL EVALUATION IN AN INDOOR ENVIRONMENT: ASSESSMENT OF FUNGAL AND BACTERIAL LOAD IN FITNESS CENTERS

Ramos, C.A.[1*], Viegas, C.[2], Cabo Verde, S.[1], Wolterbeek, H.T.[3], Almeida, S.M.[1]

[1] Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10, Km 139,7 2695-066 Bobadela LRS, Portugal;

[2] Environmental Health RG - Lisbon School of Health Technology - Polytechnique Institute of Lisbon, Av. D. João II, lote 4.69.01, 1990-096 Lisboa, Portugal;

[3] Faculty of Applied Sciences, Department of Radiation, Radionuclides and Reactors, Technical University of Delft, Delft, The Netherlands.

*Corresponding Author: carla.ramos@ctn.ist.utl.pt, Tel. +351 21994 6156

In fitness centers, moisture, marked human presence, elevated physical activity that promotes the resuspension of dust from the ground and contact between the occupants and surfaces are conditions that promote the microbial growth. The aim of this work is to assess the indoor air contamination in fitness centers, by fungal and bacteria characterization. Three fitness centers in the city of Lisbon were chosen for the sampling campaigns that occurred between October and December of 2012. Samples were performed in two periods of the day (morning and night). Two different media cultures were used (Malt Extract Agar for fungi and the Tryptic-Soic Agar for bacteria). Fungal colonies were grouped by macroscopic colonies characteristics (e.g. color, shape and elevation). The obtained bacterial isolates were characterized based on their macroscopic traits (e.g. pigmentation, texture, and shape), microscopic morphology (cellular morphology, and presence/ absence of endospores) and biochemical characteristics (gram staining, catalase and oxidase activities).

For fungal concentrations, the national legal limit value was never exceeded in any situation, although some insecure situations were found (presence of *Chrysonilia* sp. at 60 $CFU.m^{-3}$ and *Crysosporium* sp. at 148 $CFU.m^{-3}$). Regarding bacteria concentrations, very often, the results show that at the end of the day the bacterial load was higher indoors than outdoors, suggesting the influence of the human presence for indoor bacterial load.

The results of this study manifest importance of maintaining good practices in terms of maintenance of ventilation systems, cleaning spaces and to promote a correct behavior of occupants in preventing microbial spread.

EXPOSURE OF VULNERABLE GROUPS OF PEOPLE TO BIOARESSOLS

Ramos, C.A.[1, 3*], Canha, N.[1, 3], Almeida-Silva, M.[1, 3], Lino, J.[1], Faria, T.[1,2], Cabo Verde, S. [1], Viegas, C.[2], Almeida, S.M.[1]

[1] Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10, Km 139,7 2695-066 Bobadela LRS, Portugal;

[2] Environmental Health RG - Lisbon School of Health Technology - Polytechnique Institute of Lisbon, Av. D. João II, lote 4.69.01, 1990-096 Lisboa, Portugal;

[3] Faculty of Applied Sciences, Department of Radiation, Radionuclides and Reactors, Technical University of Delft, Delft, The Netherlands.

*Corresponding Author: carla.ramos@ctn.ist.utl.pt, Tel. +351 21994 6156

Indoor air is a complex mixture of viable and non-viable particles. The main factors that influence microbial growth are moisture, temperature and nutrients available in a building. Exposure to microbial contaminants is clinically associated with health effects depending upon the nature of the microbiological agent and the state of health of the host. Four microenvironments were selected according to the population that uses them (distinct physical and heath conditions and different activity levels) and considering that these microenvironments gather special conditions to microbial proliferation. Firstly, there are fitness centers that not only are occupied by people that are more exposed to air pollutants once they inhale large quantities of air during exercise but also offer some activities that are practiced on the floor (contact between the occupants and surfaces) and have noticeable perspiration and water condensation. Secondly, children in primary schools are more vulnerable to environmental pollutants compared to adults since they breathe more air relatively to their body weight and also have a lower ability to deal with the toxic chemicals. Thirdly, elderly stay most of their time in elderly care centers and present a weak immunological system, debilitated by other health conditions. And by last, in hospitals there are three main groups of occupants - patients, healthcare workers and visitors – and each one of these groups is different in terms of their health status and susceptibility to airborne microorganisms The quantification of species, the fungal identification and bacterial classification allows a better characterization of these microenvironments in order to generate best practices and procedures to promote public health and safer environments.

New approaches for assessment of potentially-toxic cyanobacteria in recreational waters of the Romanian Black Sea

STOICA E.[1]

[1]NIRDEP - National Institute for Marine Research and Development "Grigore Antipa", Constanta, Romania, E-mail: estoica@alpha.rmri.ro, Tel: +40241540870, Fax: +40241831274

Abstract

The potential for recreational exposure to toxic cyanobacteria in the Romanian Black Sea coast is relatively large. The Black Sea coast (244 km length) is the main touristic area of Romania, especially its southern part with a high average population density and multiple summer recreational facilities. Like many other brackish- and marine eutrophic waters from Europe and worldwide, potentially-toxic cyanobacteria in the Romanian Black Sea coast has occur. Five genera with known toxin producing taxa (*Anabaena*, *Aphanizomenon*, *Microcystis*, *Oscillatoria*, *Phormidium)* were frequently present in Romanian coastal waters during the last five decades. However, toxic cyanobacteria of Romanian recreational seawaters remain under-investigated due to traditional microscopic assessment for more than half a century.

This paper aims to present preliminary results of an on-going study, including the main Romanian recreational coastal areas with history of toxic cyanobacterial blooms, undertaken in our laboratory to explore different molecular approaches (e.g. standard PCR, 454 high-throughput DNA sequencing) for detection and identification of potentially-toxic cyanobacteria. The obtained results have shown that: (i) molecular methods based on PCR amplification of (mcy)genes can be successfully used for early detection of toxic cyanobacterial blooms in the Black Sea surface waters; (ii) next-generation 16S rRNA cyanobacterial gene amplicons sequencing methods assess patterns of cyanobacterial Black Sea diversity in much greater detail than with microscopy or standard molecular techniques; (iii) tools based on the use of advanced molecular methods could be useful for obtaining a rapid assessment of toxic cyanobacteria coverage in coastal Black Sea.

Methanol preservation of filamentous cyanobacteria for molecular and morphological studies.

Churro C.[1,2], Pereira P.[1], Paulino S. [1], Vasconcelos V.[2,3], Valério E.[1]

(1) Laboratório de Biologia e Ecotoxicologia, Departamento de Saúde Ambiental, Instituto Nacional de Saúde Dr. Ricardo Jorge, Avenida Padre Cruz, 1649-016 Lisboa, Portugal.

(2) Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, 4069-007 Porto, Portugal.

(4) Centro Interdisciplinar de Investigação Marinha e Ambiental, CIIMAR/CIMAR, Universidade do Porto, Rua dos Bragas 280, 4050-123 Porto, Portugal.

Email: catarina.churro@insa.min-saude.pt, Phone number: (+351) 217 519 200.

Molecular studies on cyanobacteria often involve filtering and freezing of samples leading to loss of cell morphological features. Methanol is a coagulating fixative often used in cell preservation in association with other fixatives. This study intends to evaluate the application of methanol fixation in the preservation of viable DNA for PCR reactions and also preserve cell morphology for microscopic studies in filamentous cyanobacteria. Several types of samples – cultured isolates, mixed cultures and environmental bloom samples - were fixed using a cold methanol dehydration series (50, 70 and 100%) and stored at -20ºC up to one year. Samples were analyzed at the time of fixation and after 6 and 12 months preservation in cold methanol. The DNA yield and DNA purity extracted from fixed samples was determined spectrophotometrically, while electrophoretic analysis of genomic DNA was carried out to investigate DNA integrity. The DNA template amplification was tested in both conventional and real-time PCR. Base pair alteration was analyzed by comparing the sequences obtained from preserved and unpreserved samples. Microscopic observations and cell measurements were performed in fixed samples. The DNA extracted from samples preserved up to one year was successfully amplified and quantified using conventional and real-time and PCR. The DNA sequence and cell morphology were also maintained during the preservation time. The applicability of methanol preservation in molecular studies is discussed.

Microbiota of a Hospital's environment has a diverse composition far from the expected

Pedro Farias[1], Ana Maria Almeida[1],Eduardo Melo[2], Paula V. Morais[3]

[1]Instituto Piaget-CIIERT, 1900-726 Lisboa, Portugal

[2]Comissão de Controlo de Infeção e Resistência aos Antimicrobianos, Centro Hospitalar Tondela-Viseu, 3504-509 Viseu, Portugal

[3]IMAR-CMA, 3004-517 Coimbra, Portugal and Department of Life Sciences, University of Coimbra, 3001-401 Coimbra, Portugal

Presenting author: Pedro Daniel Geadas Farias

e-mail: pedro.farias101@mail.com

Tel.:+351 239 824 024

Hospitals are environments where both, infected and non-infected people group. How microbial communities persist and change in indoor environments has much interest to public health. Despite the lack of direct evidence to prove that environmental contaminants are responsible for Hospital Acquired Infections (HAIs), there is increasing evidence suggesting that the environment may act as a reservoir for at least some of the implicated pathogens. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are bacterial species related to HAIs that are relatively common in several environmental niches. The aim of the present work was to evaluate the persisting microbiota after daily cleaning in a 672-bed Portuguese hospital, able to grow in selective media for *Pseudomonas* spp. and for *Klebsiella* spp.

The hospital was evaluated for a three month period, in three different wings in a total of 163 samples. Samples were collected in non-critical equipments and surfaces, and inoculated in the selective media.

In all wings, higher levels of contamination were mostly associated to wet surfaces and wet equipment. Most of the strains isolated in *Klebsiella* selective medium belonged to the genera *Staphylococcus* and *Kocuria* and strains from *Klebsiella* species were found sporadically. Ten different species of *Pseudomonas* were isolated. Most of the strains (25.7%) belonged to the species *P. plecoglossicida* and *P. aeruginosa* was mostly isolated in biofilms from taps.

This work showed that many different bacterial species can persist on hospital surfaces. The level of bacterial contamination was related with the presence of humidity on the surface.

P2
POSTERS
20TH
SEPTEMBER

TEMPH 2014
TRENDS IN ENVIRONMENTAL MICROBIOLOGY FOR PUBLIC HEALTH

Identification of potential pathogenic yeasts from garden trees in Lisbon area

A. Vencá[1], A. Pontes[1], A. P. Maduro[1], J. Inácio[1], M. L. Martins[1]

[1] Laboratório de Micologia, UEI Microbiologia Médica, Instituto de Higiene e Medicina Tropical, Lisbon, Portugal.

Pathogenic fungi can be found in urban areas and may represent a risk for the population, particularly for immunocompromised patients.

For this study, the identification of potential pathogenic yeasts, we have collected samples from gardens in the urban areas of Lisbon, Portugal. The characterization of potential pathogenic yeasts had not been done in Lisbon.

The samples were obtained from different gardens and from several species of trees (olive, carruba, pine, almond tree, eucalyptus, platanus and cypress), in a total of 33 trees from seven urban gardens. The identification of the yeasts was carried out molecularly by PCR Multiplex, RFLP of ITS region of rDNA and RFLP of the gene *URA5*. Subsequently, we tested the susceptibility of the identified yeasts to fluconazole and voriconazole using the CLSI standardized disk diffusion method.

A total of 56 yeasts were isolated, of which 38 were *Aureobasidium* sp.(67.8%), 11 were *Rhodotorula* sp. (19.6%), two were *Candida glabrata* (3.6%) and two were *Candida guilhermondii* (3.6%), and the remain three isolates were *Candida lusitaniae, Candida parapsilosis* and *Cryptococcus neoformans* (1.8% each).

The isolates of *C. glabrata* demonstrated resistance to fluconazole and voriconazole, and *C. parapsilosis* was resistant to fluconazole.

The most frequent fungi obtained were *Aureobasidium sp.,* and as the remaining yeasts that were found in this study, have been described has opportunistic human pathogen. The presence of *C. neoformans* in the environment is particularly interesting, since that is the main agent of cryptococcosis.

Assessment of antibacterial resistance levels and presence of pathogenic bacteria in Portuguese wild ungulates

D. Dias[1], R. Torres[1], C. Fonseca[1], S. Mendo[1], T. Caetano[1]

[1]CESAM and Department of Biology, University of Aveiro, Aveiro, Portugal

Wild animals should be rarely exposed to antimicrobial agents and therefore low levels of antibacterial resistance are theoretically expected in their microbiological community. However, their increasing interaction with anthropogenic activities can have dramatic impact in this matter. The present study aims to access the levels of antibacterial resistance of *Escherichia coli* isolated from faecal samples of wild ungulates inhabiting different geographical areas in Portugal (Lousã region, Idanha-a-Nova and Montesinho), that are under the influence of different human and livestock densities. Moreover, the presence of *Salmonella* spp. was also investigated according to ISO 6579:2002 Annex D. To that purpose, a total of 64 faecal samples from red deer (n=39), wild boar (n=21) and roe deer (n=4) were collected. Ten *E. coli* strains were selected from each sample based on growth in agar and amplification of the *uidA* and *gadA/B* genes. Before antibacterial susceptibility testing, these isolates were typed by rep-PCR to select for genetically different strains (n=152). Our results show a low level of *Salmonella* spp. incidence (1,6%), which was only identified in wild boar from Lousã. Regarding the antibacterial susceptibility of the *E. coli* strains already examined, resistance was only detected for ampicillin (10%), amoxicillin/clavulanic acid (1%), cefoxitin (1%), ceftazidime (3%), sulfamethoxazole-trimethoprim (4%), streptomycin (4%; CLSI breakpoints) and tetracycline (8%; CLSI breakpoints). The occurrence of potential pathogenic bacteria such as shiga toxin-producing *E. coli* (STEC) will also be monitored. Wild ungulates can be reservoirs of antibacterial resistant bacteria and may act as a transmission vehicle in the wildlife-livestock-human interface.

Contact details:
E-mail: dianadias@ua.pt
Telephone number: 00351913557432

Hepatitis A immunity in the District of Aveiro (Portugal): an eleven-year surveillance study (2002-2012)

Pereira S.[1*], Baptista I.[1,2], Linhares I.[1], Neves A.F.[3], Almeida A.[1]**

[1] Department of Biology and CESAM, University of Aveiro, 3810-193 Aveiro, Portugal;

[2] Department of Chemistry and QOPNA, University of Aveiro, 3810-193 Aveiro, Portugal;

[3] Clinical Analysis Laboratory Avelab, Rua Cerâmica do Vouga, 3800-011 Aveiro, Portugal;

***** Corresponding author: sarasouto@ua.pt; Tel.: +351234370350, Fax: +351234372587.

****** Presenting author: mines.baptista@ua.pt

Hepatitis A is a common viral liver disease and brings serious health and economic problems as its epidemiologic pattern changes over time. National serosurveys from developed countries have indicated a decline in HAV (hepatitis A virus) seroprevalence over time due to the improvement of economic and sanitation levels. The hepatitis A virus (HAV) immunity rate was surveyed throughout an eleven-year period by sex and age group in Aveiro District. In this retrospective study, blood samples from patients of Aveiro District, in ambulatory regime, collected at the Clinical Analysis Laboratory Avelab between 2002 and 2012 were screened for the presence of antibodies against HAV antigen using a chemiluminescence immunoassay. The global immunity (positive total anti-HAV) was 60% and only 0.3% of the patients presented recent infection by HAV (positive IgM anti-HAV). The HAV immunity was age-dependent ($p<0.05$), but no significant differences ($p>0.05$) between sexes were observed. The immunity was similar throughout the study period ($p>0.05$). The results of this study indicate that young people (especially under 25 years old) from District of Aveiro are susceptible to HAV infection, constituting a high risk group. The elderly should be also a concern in the future of hepatitis A infection.

Evaluation of resistance development and viability recovery by toxigenic and non-toxigenic *Staphylococcus aureus* strains after repeated cycles of high hydrostatic pressure

Inês Baptista[1,2], Rui P. Queirós[2], Ângela Cunha[1], Sílvia M. Rocha[2], Jorge A. Saraiva[2], Adelaide Almeida[1]*

[1]Department of Biology and CESAM, University of Aveiro, 3810-193 Aveiro, Portugal

[2]Department of Chemistry and QOPNA, University of Aveiro, 3810-193 Aveiro, Portugal

*Correspondent author – aalmeida@ua.pt

Staphylococcus aureus is an opportunistic human pathogen that causes a large spectrum of infections including food poisoning due to some strains' ability to produce heat-stable enterotoxins. Thus, *S. aureus* control/inactivation by food industry using preservation methods is socially and economically crucial. High pressure processing (HPP) is an emerging non-thermal food preservation method that uses pressure between 100-1000 MPa to produce microbiologically safe products, maintaining fresh-like appearance with minimal nutritional and organoleptic properties' modification.

This study aimed to assess the development of resistance to HPP by three *S. aureus* strains (a non-toxigenic strain, ATCC 6538, and two toxigenic strains, 2153 MA and 2065 MA) after 10 consecutive pressurization cycles and their ability to recover after cycles applied at 2, 5, 8, 11 and 14 days. The ATCC 6538 strain treatment was 600 MPa/30 minutes while the toxigenic strains 2153 MA (with enterotoxin A) and 2065 MA (with the enterotoxins A, G and I) were treated at 600 MPa/15 minutes, all at 20ºC.

After an initial reduction effectiveness, the ATCC 6538 and the 2153 MA strains were able to resist 10 cycles without significant changes but, the 2065 MA strain was completely inactivated after the fourth cycle. None of the strains was able to recover from HPP treatments after incubation. HPP effectively inactivates non-toxigenic and toxigenic strains of *S. aureus* after a single treatment, without the development of resistance after consecutive cycles of pressurization and their viability recover is not detectable after 14 days of incubation.

Bioaerosol in occupational settings: a possible application of QMRA

Carducci A[1], Donzelli G.[1], Verani M[1]

[1]Laboratory of Hygiene and Environmental Virology, Department of Biology, University of Pisa, Via S. Zeno 35/39, 56127 Pisa, Italy

Abstract

The assessment of occupational biological risk is generally limited to the evaluation of a "potential exposure", without any quantitative estimate. However, the QMRA (Quantitative Microbial Risk Assessment) methodology, already applied to water and food, could be useful also for risk assessment and management at the workplace.

In the present work we have developed a preliminary QMRA model to assess the microbial risk associated to the inhalation of bioaerosol contaminated by HAdV. Then this model has been applied to the air contamination data coming from different settings and to several exposure time.

The virological monitoring showed the presence of HAdVs in all the considered settings, thus confirming their wide diffusion. Nevertheless, the average concentrations of HAdV were different, ranging from 2 Log GC/m^3 ("white" points) to 8 Log GC/m^3 (hospital bathrooms).
The model estimates show that in the most contaminated settings a stay longer than 3 minutes would lead to a probability of infection of 100%, while for the less contaminated areas even after 15 min the probability of infection remains around 1%.

This approach is new and should be faced with caution. However it is worthy of discussion and further investigation.

Extended-spectrum beta-lactamase producing *Escherichia coli* in river water in the North of Portugal

Marta Monteiro[1], Marisa Pinto[1], Daniela Gonçalves [1,2] and Helena Ferreira[1,2]

[1]Microbiology, Biological Sciences Department, Faculty of Pharmacy, University of Porto,
[2] REQUIMTE University of Porto, Portugal

Microbiological quality of recreational waters is addressed by faecal contamination indicators as are coliforms and enterococci. *Escherichia coli* is a relevant representative of coliforms, in terms of river water microbiological quality. *Escherichia coli* is an opportunistic pathogen able to carry antimicrobial resistance mechanisms of clinical relevance, as are extended-spectrum beta-lactamases (ESBLs).

The aim of our study was the detection of antimicrobial resistant bacteria in river water, namely ESBL producing coliforms.

For the purpose, river water was collected in two different points, a recreation area and a free running zone. Water was processed by membrane filtration in Mac Conkey and Mac Conkey with aztreonam, ceftazidime, cefotaxime and meropenem. Colonies of lactose fermenters were randomly selected for antimicrobial susceptibility testing by agar diffusion method, according to the Clinical Laboratory Standards Institute (CLSI) and screened for ESBL production, by the double disc synergy test, clavulanic acid addition and PCR amplification with specific primers. Identification of the selected strains was achieved by ID 32 GN.

Non repetitive ESBL producing *Escherichia coli* isolates were detected in the collected samples, showing three different beta-lactam resistance phenotypes and CTX-M-group 1.

This kind of bacteria may lead to colonization risk in recreational use, leading to increased resistance to treatment in the community and spread of these bacteria in soil, waters and other different environments. Unexpected contamination by this kind of bacteria might reflect direct or indirect anthropogenic impact in natural waters, namely by animal production in the surrounding area or clandestine wastewater runoffs.

Extended-spectrum beta-lactamase producing coliform bacteria on beach sands in the North coast of Portugal

Sofia Lima [1] and Helena Ferreira [1,2]

[1]Microbiology, Department of Biological Sciences, Faculty of Pharmacy University of Porto
[2] REQUIMTE, University of Porto, Portugal

Environmental contamination with antimicrobial resistant bacterial isolates can play an important role in resistance spread. Sand might promote an unexpected contact with antibiotic resistant bacteria of unknown origin.

The aim of this study was the detection of antimicrobial resistant bacteria in sand samples, with special focus on Extended Spectrum Beta Lactamase (ESBL) producing coliforms.

For that purpose, we collected wet sand in three different beaches of the North of Portugal and suspended in Tryptic Soy Broth (TSB) and incubated overnight at 37ºC. Isolates were selected on MacConkey agar and MacConkey agar with ampicilin, ceftazidime, cefotaxime, aztreonam and meropenem. Other approach for experimental work included suspending the samples in sterilized water, followed by membrane filtration. Filters where then placed in the same culture media used for the samples suspended in TSB. After random selection of colonies, we performed an antimicrobial susceptibility test by agar diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI). Screening for ESBL production was done by the double disk synergy test and clavulanic acid addition, according to the CLSI guidelines. Selected strains were identified by ID32GN.

ESBL producing *Escherichia coli* isolates were detected in the collected samples.

Considering the results the presence of these resistant bacteria might represent a colonization risk in recreational use of these beaches and also a threat in terms of antibiotic resistance environmental dissemination.

Microbial analysis of beach sand in Dublin Bay, Ireland

U. Sieira-Novoa, R. Sanchez, K. O'Brien, M. Alonso, L. Deering, W. G. Meijer

UCD School of Biomolecular and Biomedical Science and UCD Earth Institute, University College Dublin, Dublin, Ireland

Presenting Author Email Address: (uxia.sieira-novoa@ucdconnect.ie)

In Ireland, under the EU revised Bathing Water Directive (rBWD), bathing water quality is classified based on the levels of Faecal Indicator Bacteria (FIB), categorising designated bathing waters as "excellent", "good", "sufficient" or "poor". Although the rBWD specifies water quality, it does not include microbial standards for the beach sand area, where people spend the majority of their time when using recreational waters.

This study, funded by Environmental Protection Agency (EPA), aims to detect FIB, pathogenic bacteria and associated viruses present in beach sand; estimate the potential risks to public health and suggest management measures to reduce these risks. To date, faecal indicator intestinal enterococci levels, in beach and water, in conjunction with tides are continuously measured from two beaches around Dublin Bay, with data from Sandymount Strand shown here. Results to date indicate dry sand has the highest levels of intestinal enterococci (levels approximately 100 times higher than wet sand and water). This may have direct implications on public health and therefore promote the case that sand should be included in the guidelines for beach quality. Another technique employed on this project is Microbial Source Tracking (MST). MST is a molecular method used to identify and discriminate between different types of faecal pollution. To date, human pollution is the most dominant source on Sandymount Strand.

In vivo and *in vitro* bactericidal effect of sulphurous thermal water

C. M. Pinheiro[a], C. Moita[a], P. Tavares-Ratado[a,b], A Jorge S. Silva[c], M. A. Almeida[d], C. T. Tomaz[b]

[a] *Laboratory of Clinical Pathology, Sousa Martins Hospital, ULS Guarda, Portugal.*

[b] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, 6200-506 Covilhã, Portugal.*

[c] *Medical Department of Almeida, Caldas de Manteigas, Cró and Longroiva Spas, Portugal*

[d] Department of Biology, University of Aveiro, Portugal.

The main objective of this study was to evaluate the bactericidal effect of sulphurous water both on nasal and oropharyngeal flora of individuals with chronic rhinosinusitis or allergic rhinitis, before and after thermal treatment. *In vitro* activity of sulphurous water was also studied on bacterial isolates grown in culture media prepared with different percentages of thermal water.

For *in vivo* bactericidal studies, nasal and oropharyngeal swabs were collected from 50 patients, before and after sulphurous water treatment and the microbial isolation and identification was performed. For *in vitro* studies, Trypto-Casein-Soy and Pastagar media were prepared with different percentages of thermal water and were used to cultivate 60 bacterial isolates, selected from the local community and hospital patients.

The results showed a global reduction of microbial colonization in nasal and oropharyngeal flora of the patients after thermal treatment. In nasal flora, it was found a decrease in the level of *S. aureus*, coagulase-negative Staphylococcus and α-hemolytic Streptococcus. On the other hand, in oropharyngeal flora a decrease in colonization by coagulase-negative Staphylococcus and Neisseria was observed. *In vitro* studies showed a decrease in meticillin resistant *S. aureus* (MRSA) isolates but no effects were observed in Gram negative bacilli.

Taking into account the role of microorganisms, such as coagulase-negative Staphylococcus and *S. aureus* in the pathogenesis of allergic rhinitis and rhinosinusitis it can be suggested that the thermal treatment with sulphurous water can reduce the colonization of these microorganisms leading to a decrease of the inflammatory process associated with upper tract respiratory diseases.

This work was supported by Regional Operational Programme Center 2007-2013 QREN, project CENTRO-01-CT62-FEDER-002035.

TITLE: Fungi in Water Pools and Its Importance in Public Health

Authors: Costa, C.R.; Brandão, J.; Parada, H.; Sabino, R.; Veríssimo, C.; Rosado, L.

Institution: National Institute of Health Dr. Ricardo Jorge

ABSTRACT

Swimming-pools and spas used for sports, leisure and therapeutic purposes present risk of infection transmission to maintenance staff and general users. Water quality assessment currently requires only bacterial indicators. There's hence a legal void concerning fungal contaminants. The main purpose of this study was to characterize opportunistic and pathogenic fungi in these environments.

During this work, 37 samples of superficial water were collected monthly, covering 7 municipal and 3 therapeutic swimming-pools of Lisbon.

The method of analysis was membrane filtration of 100 ml of water. Membranes were laid on both Mycosel agar, followed by incubation during 15 to 20 days at 27.5ºC and malt agar with chloramphenicol, followed by incubation during 5 days at the same temperature. Morphologic and biochemistry were used during the identification process.

The fungi found more frequently in these samples were *Cladophialophora* sp. (20,4%), Yeast (18,5%), Actinomycetes (9,4%), *Phoma* sp. (8,9%), *Cladosporium* sp. (8,4%) e *Chaetomium* sp. (7,7%). The frequency of fungal presence in therapeutic pools and in Municipal ones were 73% and 25%, respectively. The presence of moulds was comparatively higher than that of yeast, in both municipal and therapeutic pools. No dermatophytes were isolated.

This study showed a low contamination of the pool water, with higher prevalence of black moulds. There was an obvious diversity of the fungal species isolated, some of which, potentially pathogenic, especially for risk groups.

Key words: water; swimming-pools; fungi; potentially pathogenic; public health.

Mer Operon Genes Occurrence among Mercury-resistant Bacteria of Tagus Estuary (Portugal)

Figueiredo NLL, Duarte A, Carvalho CML

[1]*Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal*

Mercury pollution of aquatic system may be critical for human health. For instance, in Minamata Bay (Japan) many children suffered neurotoxicity effects and a large number of people died due to environmental contamination. Mercury-resistant (HgR) bacteria are the main responsible for the critical transformations in mercury biogeochemical cycle, such as reduction, methylation and demethylation. The reduction and demethylation are normally associated to an operon - mer operon, which encodes for enzymes (merA and merB) that confer HgR phenotypes to bacteria.

Tagus Estuary has been shown to have high mercury contamination due to past industrial activities. This study aimed to evaluate mer operon occurrence among HgR bacteria isolated from Tagus Estuary. HgR bacteria were isolated from sediments of four areas with different mercury contamination levels (Barreiro > Cala do Norte > Rosário > Alcochete). Among the isolates, 60 aerobic bacteria were selected and characterized phenotypically and genetically and their susceptibility to mercury compounds (Hg2+ and MeHg) was also evaluated by the determination of minimal inhibitory concentration (MIC). Sequencing of 16S rRNA was used for bacterial identification and the mer operon was searched by PCR, using primers for merA and merB genes. The results revealed resistance to mercury compounds ranging from 0.16 to 10.01 μg/mL Hg2+ and 0.02 to 1.12 μg/mL MeHg and their distribution was related to the contamination levels, i.e. highly resistant bacteria exist in highly contaminated areas. Mer genes were found only in 7% of the isolates, which encompassed Bacillus, Citrobacter and Aeromonas genera. Overall, it was found a low occurrence of mer genes among HgR bacteria despite the high levels of mercury resistance, therefore, suggesting that besides mer operon, resistance may be conferred by others mechanisms, such as methylation. The consequence being that HgR bacteria of Tagus Estuary may be responsible for the formation of the neurotoxicant methylmercury.

This work was funded by FCT (Project PROFLUX - PTDC/MAR/102748/2008) and by iMed.UL (PEst-OE/SAU/UI4013/2011). Figueiredo NL is grateful to FCT for PhD scholarship: SFRH/BD/78035/2011

P3
POSTERS
21st
SEPTEMBER

TEMPH 2014
TRENDS IN ENVIRONMENTAL MICROBIOLOGY FOR PUBLIC HEALTH

Attachment and biofilm formation by spore-forming bacteria isolated from milk powder plants

Maria Cecília E. Ribeiro[1]; Mirna L. Gigante[1], Rafael Jiménez-Flores[2]*

(1) Faculty of Food Engineering, University of Campinas (UNICAMP), SP, Brazil; (2) Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo, CA, USA

* presenting author: maria26@fea.unicamp.br, +55 (17) 98113-2934

The presence of spores in milk powder is due the formation of a stationary biofilm where the spore-forming bacteria grow, sporulate, and slough off a great number of spores in the final product. The structure and composition of biofilms depend on a large variety of intrinsic and extrinsic factors. The aim of this study was to evaluate the effect of the matrices (water or fat free milk), microorganism (six thermophilic spore-forming bacilli: CM12, SL12, SL9, CM3, CH7, and SH6) isolated from milk powder plants in the USA, and time of exposure (5 minutes, 2 or 20 hours) on spore attachment and biofilm formation. Stainless steel coupons were immersed in fat free pasteurized milk (55°C for 20 hours at 200 rpm) to form a natural biofilm. After that, the coupons were exposed to spore either in an aqueous solution or fat free UHT milk. Spore attachment ranged from 3 to 4 log cfu/cm^2 and was observed for all strains after 5 minutes. However, CM12 and SL9 strains showed the lowest average attachment when compared to the other strains and also showed the lowest average attachment on water when compared to the milk. After 20 hours, the spore attachment on fat free milk was higher than in the water solution. The results suggest that spore attachment and biofilm formation may occur early in the milk processing and can contribute intrinsic properties of the bacterial strains, such as toxins and enzymes that may impair product safety and quality.

Keywords: spores, attachment, milk powder

Acknowledgments: CNPq, CAPES

Biofilm formation capacity in *Enterococcus faecalis* and *Enterococcus faecium* isolated from processing ricotta

Fernandes, M. S.[1*]; Kabuki, D. Y. [1]; Kuaye, A. Y. [1]
[1]University of Campinas (UNICAMP) – Department of Food Technology.
Rua Monteiro Lobato, 80 – Cidade Universitária Zeferino Vaz – Campinas, SP, Brazil – CEP 13083-862. P.O. Box 6121.
*Presenting author. Email: meg@fea.unicamp.br. Phone numbers: +55 19 35214011.

Abstract

E. faecalis and *E. faecium* are pathogenic species found in dairy products and have the ability to form biofilms on abiotic surfaces in the food processing environment. In this work, the ability of biofilm formation of *E. faecalis* and *E. faecium* isolates from processing ricotta was evaluated on stainless steel coupons at temperatures of 7, 25 and 39 °C and at 0, 1, 2, 4, 6, and 8 days. The coupons were immersed in a culture medium consisting of 80% cheese whey and 20% whole UHT milk, inoculated with a suspension of approximately 1-5x10^2 cfu/ml of cultures. At each time and temperature the counts of cells adhered to the surface were performed by plating onto BHI agar. At 7 °C, the counts of *E. faecalis* and *E. faecium* did not indicated biofilm formation, remaining below 2 and 1 log cfu/cm^2, respectively. After 1 day of contact, *E. faecalis* was able to form biofilm, with counts of 5.75 and 6.09 log cfu/cm^2 for temperatures of 25 and 39 °C, respectively. *E. faecium* was also able to form biofilm, with counts of 5.96 and 6.07 log cfu/cm^2 after 1 day of contact, respectively. After 8 days of contact, the counts of *E. faecalis* increased by about 3 log cycles, while *E. faecium* counts increased by about 2 log cycles for both temperatures. Therefore, the presence of the two species must be controlled by ricotta processing industries, since they were able to form biofilms under conditions similar to those encountered during processing.

Keywords: *E. faecalis*, *E. faecium*, biofilm.

Efficiency of sanitation procedures on removing biofilms of *Enterococcus faecium* and *Enterococcus faecalis* on stainless steel surface

Fernandes, M. S.[1*]; Kabuki, D. Y. [1]; Kuaye, A. Y. [1]
[1]University of Campinas (UNICAMP) – Department of Food Technology.
Rua Monteiro Lobato, 80 – Cidade Universitária Zeferino Vaz – Campinas, SP, Brazil – CEP 13083-862. P.O. Box 6121.
*Presenting author. Email: meg@fea.unicamp.br. Phone numbers: +55 19 35214011.

Abstract

E. faecium and *E. faecalis* are capable of forming biofilms on food contact surfaces and its removal depends on the sanitation procedures employed. In this work, the efficiency of cleaning and sanitization procedures for the control of biofilms of *E. faecalis* and *E. faecium* on stainless steel coupons was evaluated. The coupons were subjected to different sanitation procedures: 1- alkaline cleaning; 2- acid-alkaline cleaning; 3- sanitation; 4- alkaline cleaning + sanitation, and 5- acid-alkaline cleaning + sanitation. The sanitizers used were: peracetic acid (0.2%), sodium hypochlorite (0.2%), quaternary ammonium (3%), and biguanide (1%). For biofilms formed after 1 day, all sanitation procedures were effective, with a reduction of the counts to less than 1 cfu/cm^2, except for the use of biguanide, that reduced 3.65 and 2.32 log cfu/cm^2 for *E. faecalis* and *E. faecium*, respectively. For biofilms formed after 8 days, the sanitation procedures 2, 4, and 5 reduced the counts to less than 1 cfu/cm^2. In procedure 3, the peracetic acid was the most efficient sanitizer (less than 1 cfu/cm^2 counts). The biguanide was the least effective, with a reduction of only 0.03 and 0.05 log cycles cfu/cm^2 for *E. faecalis*, and 0.49 and 1.08 log cycles cfu/cm^2 for *E. faecium* at 25 and 39 °C, respectively. Furthermore, procedure 1 reduced 3.93 and 4.28 log cycles cfu/cm^2 for *E. faecalis* and *E. faecium*, respectively. The results demonstrated the importance of the cleaning step and the type of sanitizer in the effective removal of the biofilms.

Keywords: *E. faecalis*, *E. faecium*, sanitation.

"Microbiological Evaluation of Air and Surfaces of Eye Surgery Rooms from a Lisbon Hospital"

S.Alexandra[1], M.Paula[1], F.Pedro[2], P. José Moniz[1]

[1]Laboratório de Controlo Microbiológico – ADEIM, College of Pharmacy, University of Lisbon

[2] Ordem Terceira de S. Francisco da Cidade Hospital, IPSS

Assessment of microbiological quality of the interior of operating rooms is crucial in controlling and maintaining aseptic environment. Ordem Terceira Hospital in collaboration with a University Laboratory in Lisbon, held annually, microbiological control of the air and surfaces (equipment and utensils) of eye surgery room (Lasik room ±15m^2) and post surgical recovery room (±8m^2). The impaction method by Merck MAS 100 equipment is used for the collection of indoor air and the surfaces and utensils were evaluated using contact plates and Swab Test. In all samples the Count of Total Aerobic Bacteria (TAMC) and Count of Total yeast and fungi (TYMC) was performed. During three years, were analyzed in both room's, thirty air samples and hundred and fives samples from surfaces and utensils. In surgery room (Lasik), 80% to 100% air samples showed TYMC <3 cfu/m^3 and TAMC <11cfu/m^3 whereas 90-99% of the samples from the surfaces of this room had TYMC <2cfu/m^2 and TAMC <30cfu/m^2. In the recovery room, 80% to 100% air samples showed TYMC <25cfu/m^3 and TAMC <50cfu/m^3 whereas 76-99% of the surfaces samples TYMC <3cfu/m^2 and TAMC <50cfu/m^2.

These results are consistent with the framework of indoor air quality (D.L 78/2006), which recommends TAMC and TYMC < 500 cfu/ m^3, and illustrate that a periodic review of the microbiological quality of air and surfaces of surgical rooms, associated with cleaning and disinfection plans, contribute to quality control of these rooms, detection of critical points of contamination and to prevent post surgical infections.

Gamma radiation effects on microbial inactivation of two medicinal plants

Authors: Márcia Meneses[1,2], Amilcar L. Antonio[1,3], Sandra Cabo Verde[1]

[1] *Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Portugal*

[2] *Environmental Health RG - Lisbon School of Health Technology - Polytechnique Institute of Lisbon, Portugal*

[3] *Centro de Investigação de Montanha (CIMO), ESA, Instituto Politécnico de Bragança, Portugal.*

Presenting Author: Márcia Meneses E-mail: marcia17meneses@gmail.com Tel. no.: +351 911880273

The consumption of natural products has become a public health problem, since these medicinal teas are prepared using natural plants without an effective hygienic and sanitary control. The aim of this study was to assess the effects of gamma radiation, on the microbial burden of two medicinal plants: *Melissa officinalis* and *Lippia citriodora*.

Dried samples of the two plants were irradiated at a Co-60 experimental equipment. The applied gamma radiation doses were 1, 3, and 5 kGy at a dose rate of 1.34 kGy/h. Non-irradiated samples followed all the experiments. Bacterial and fungal counts were assessed before and after irradiation by membrane filtration method. Challenging tests with *Escherichia coli* were performed in order to evaluate the disinfection efficiency of gamma radiation treatment.

Characterization of *M. officinalis* and *L. citriadora* microbiota indicated an average bioburden value of 10^2 CFU/g. The inactivation studies of the bacterial mesophilic population of both dried plants pointed out to a one log reduction of microbial load after irradiation at 5 kGy. Regarding the fungal population, the initial load of 30 CFU/g was only reduced by 0.5 log by an irradiation dose of 5 kGy. The dynamics with radiation doses of plants microbial population's phenotypes indicated the prevalence of gram-positive rods for *M. officinalis* before and after irradiation, and the increase of the frequency of gram-negative rods with irradiation for *L. citriadora*. Among fungal population of both plants, *Mucor, Neoscytalidium, Aspergillus* and *Alternaria* were the most isolated genera. The results obtained in the challenging tests with *E. coli* on plants pointed out to an inactivation efficiency of 99.5% and 99.9% to a dose of 2 kGy, for *M.officinalis* and *L. citriadora*, respectively.

The gamma radiation treatment can be a significant tool for the microbial control in medicinal plants.

ACKNOWLEDGEMENTS: The authors are grateful to project PRODER nº 53515, AROMAP, for financial support of the work. The authors thank to "MaisErvas - Aromáticas e Medicinais" company for supplying the samples. The authors are grateful to FCT (RECI/AAG-TEC/0400/2012) for financial support.

Abstract

Identification and sequencing of acetylase expressed from the pMdT1 recombinant plasmid in *Escherichia coli*

P. Magalhães[1,2], A. Gonçalves[1,2], L. Pinto[1,2], E. Araújo[3], H. Santos[3], J. Capelo-Martinez[3], C. Torres[4], P. Poeta[1], G. Igrejas[1,2]

[1]*University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*

[2]*Institute for Biotechnology and Bioengineering, Vila Real, Portugal*

[3]*Bioscope Group, University of Nova de Lisboa, Caparica, Portugal*

[4]*University of La Rioja, Logroño, Spain*

Introduction and objectives. *Escherichia coli* is a commensal microorganism of the gastrointestinal tract of animals and humans. Already a mainstay of molecular biology, the *E. coli* model is increasingly used to study the biology and genetics of antibiotic resistance, an emerging public health problem. The presence of the pMdT1 plasmid in *E. coli* is important in the study of antibiotic resistance as it contains a gene that encodes a variant of the AAC (6')-lb-cr protein which confers resistance to kanamycin and tobramycin, and decreases susceptibility to ciprofloxacin and norfloxacin.

Methods. Protein separation and quantification were carried out by two-dimensional electrophoresis and subsequent analysis by matrix-assisted laser desorption/ionization-time of flight/mass spectrometry. High performance liquid chromatography and mass spectrometry coupled with searching of bioinformatics databases was used to identify which proteins and peptides were expressed differently.

Results and discussion. Two *E. coli* samples were compared. Electromax DH10B is a transformation-ready strain and TF-SE20 is a strain that contains the pMdT1 plasmid expressing the acetylase gene. Proteins identified were related to biological processes such as glycolysis and oxidation-reduction processes. The protein of interest, aminoglycoside N(6')-acetyltransferase type 1, was sequenced and identified.

Conclusions. Using proteomics methods made it possible to detect the protein expressed by the pMdT1 plasmid in a strain of *E. coli*. Proteomics was used to obtain more information on resistance mechanisms and how they function.

Abstract

Application of two-dimensional gel electrophoresis to the study of antibiotic resistance in clinical *Enterococcus* spp.

L. Pinto[1,2], P. Poeta[1], C. Torres[3], C. García[4], J. Capelo-Martinez[5], G. Igrejas[1,2]

[1]*University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*

[2]*Institute for Biotechnology and Bioengineering, Vila Real, Portugal*

[3]*University of La Rioja, Logroño, Spain*

[4]*Complutense University of Madrid, Madrid, Spain*

[5]*Bioscope Group, University of Nova de Lisboa, Caparica, Portugal*

Introduction and objectives. Humans are colonized by an enormous variety of bacteria which are normally commensal but are increasingly developing antibiotic resistance. The purpose of this work was to extract and separate sub-proteomes of *Enterococcus* spp. sampled from humans to obtain more information about these strains.

Methods. The genotypes of *Enterococcus* spp. from clinical sources were analyzed and antibiotic resistance genes were detected. Through rigorous protein extraction protocols supported by microbiological methodologies, fractionated proteomes were obtained by using two-dimensional gel electrophoresis (2-DE) followed by matrix-assisted light desorption/ionization-time of flight/mass spectrometry.

Results and Discussion. Four different protocols were used to extract different fractions, which were then separated by 2-DE. In total 1022 protein spots were obtained in gels of the extracellular, periplasmic, cytoplasmic and external membrane sub-proteomes. New insights provided by this analysis were related to genetic characterization of these strains. Dividing one bacterial sample into different sub-proteomes may add valuable information on localization of the expressed proteins to the functional predictions from bioinformatics analysis or confirm experimental observations.

Conclusions. Clinical bacteria can be accurately analyzed by proteomics rapidly bringing information on molecular function to the study of the public health issue of antibiotic resistance.

Abstract

A contribution for an alternative method in *Salmonella* spp. and *Listeria monocytogenes* detection

Martins, C. and Santos, V.

Laboratório Regional de Veterinária, Angra do Heroísmo, Açores-Portugal

carla.ap.fernandes@azores.gov.pt, +351 295404245, +351 295216492

Salmonella spp. and *Listeria monocytogenes* are pathogenic microorganisms of great importance for food safety. Detection and identification of microorganisms by standard methods are laborious and time-consuming.

This study assessed the performance of alternative method real-time PCR for *Salmonella spp.* and *L. monocytogenes* detection, compared with the respective ISO methods ISO 6579: 2002 and ISO 11290-1: 1996 Amendment 1: 2004.

In the present work, 20 samples of meat and derivatives, with negative result for *Salmonella* spp. were used which were subsequently contaminated with *Salmonella tiphymurium*-ATCC14028. For the assessment of *L. monocytogenes*, 15 samples of cured cheese were contaminated experimentally with *L. monocytogenes* -ATCC 7644. Moreover, twenty samples of cheese were analyzed as blind samples.

The sensitivity, specificity and relative agreement were 100% comparing the results obtained by real-time PCR with those obtained by ISO methods.

The real-time PCR allowed detecting *Salmonella* spp. in samples of meat products with a contamination level of 3 UFC/25g, and *L. monocytogenes* in samples with a contamination level of 6 UFC/25 g for cheese. Comparing the relative detection limit for real-time PCR with the respective standard methods there is no statistics differences. It can be concluded that the use of real-time PCR technique represents an added value in routine analysis because it allows obtaining reliable results within a short period. The real-time PCR is an excellent tool for the screening of food free of *Salmonella spp*. and *L. monocytogenes* obtaining results in 2 days.

Key-words: *Salmonella* spp.*, Listeria monocytogenes,* Real-time PCR

Inactivation of phage T4 by High Pressure Processing

Reis S.[1,2,*], Almeida A.[1], Saraiva J.[2]

[1]Department of Biology, University of Aveiro, Portugal; [2] Department of Chemistry, University of Aveiro, Portugal

[*]Corresponding author: Email: silviareis@ua.pt; Tel.: +351234370350, Fax: +351234372587.

Abstract: High Pressure Processing (HPP) has been considered a promising nonthermal technology to inactivate food viral waterborne pathogens. Because human pathogens viruses are difficult to cultivate in a laboratory setting, use of surrogates like bacteriophages for this kind of viruses is frequent.

Te aim of this work was to investigate the baroresistance of bacteriophage T4, a dsDNA virus. Samples were treated by high pressure at 150-500 MPa at 25 °C in different times. The results show that viral inactivation depends on applied pressure and time of pressurization. T4 phage was almost totally inactivated by HPP applying a pressure of 500 MPa for 5 minutes (reduction of approximately 7.5 log), which is important, because for food industry reductions to low safe levels are sufficient. When low pressures (150-250 MPa) were applied during short times (until 5 minutes of pressurization), reductions of 2 log were obtained. D and z values were calculated from T4 pressure inactivation kinetics profiles.

Higher pressures combined with low times of treatment are more efficient in the inactivation of DNA virus than high times combined with low pressures.

Potential of phage cocktails in the inactivation of *Enterobacter cloacae*

Pereira S.[1]*, Reis S.[1], Pereira C.[1], Silva Y.J.[1], Costa L[1] and Almeida A.[1]

Department of Biology and CESAM, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

*Corresponding author: Email: sarasouto@ua.pt; Tel.: +351234370350, Fax: +351234372587.

Enterobacter cloacae is part of the normal flora of the gastrointestinal tract of 40-80% of people. This opportunistic microorganism is capable of causing infection in debilitated and hospitalized patients. *E. cloacae* is resistant to a broad number of antibiotics therefore infections caused by this bacterium are difficult to control. Phage therapy may be a useful tool to control infections caused by resistant bacteria. Three previously isolated phages E-2, E-3 and E-4 produced on *Enterobacter cloacae* were used to examine survival and host-phage dynamics. The survival was determined in PBS through quantification by soft agar overlay technique. The host-phage dynamics was characterized in tryptic soy broth, through quantification of phages by soft agar overlay technique and host quantification in TSA medium. The concentration of E-2 decreased by two orders of magnitude in the first 105 days. The concentration of E-3 decreased by one order of magnitude in the first 20 days and reached a plateau until 77 days. Afterwards, the phage titer decreased by three orders of magnitude until 156 days. E-4 concentration only decreased by one order of magnitude after 255 days. The results show that the growth of the *E. cloacae* was inhibited by the three phages, resulting in a decrease of ≈ 3 log after 4-10 h of incubation. The use of cocktails with two or three phages was significantly more effective, with reductions of ≈ 4 log after 2 hours. Phages E-2, E-3 and E-4 showed and an efficient inactivation of *E. cloacae*, being potential candidates as agents for the control of nosocomial infections caused by *Enterobacter cloacae*.

Detection of bacteria of the *M. tuberculosis*-complex in different food matrices on production and retail level

U. Messelhäusser, S. Hörmansdorfer, P. Kämpf, C. Höller, A. Rampp

Bavarian Health and Food Safety Authority, Veterinärstr. 2, 85764 Oberschleißheim, Germany

Corresponding author:

e-mail: ute.messelhaeusser@lgl.bayern.de,

phone:+49-(0)9131-6808-5314

fax:+49-(0)9131-6808-5110

Tuberculosis in cattle, the most important known source of a foodborne infection of humans, is predominantly associated with *M. bovis* and *M. caprae*, a species, which was numbered among *M. bovis* the last years. Since 1997 Germany has been recognised officially free of bovine tuberculosis (OTF), but this does not mean that all herds are free of bovine tuberculosis (EFSA, 2003). Therefore in regions with tuberculosis positive herds a contamination of food of animal but also of plant origin by use of dung or liquid manure cannot be totally excluded.

In 2011 the Bavarian Health and Food Safety Authority developed a combined cultural and molecular method for the detection of bacteria in milk and milk products (Messelhaeusser, 2011). During the last three years this method was used to screen more than 230 food samples of animal and plant origin for the presence of bacteria of the *M. tuberculosis* complex. Samples were taken on production level (cheese) and on retail level (other food matrices like meat, vegetables and milk products). Two samples of raw milk cheese, which were taken on production level gave a positive result using molecular methods, but the cultural detection did not succeed. The cheese came from a lot which was processed with milk of cows tested positive for tuberculosis. All other food samples were tested negative with cultural and molecular methods.

Official controls of sprout production in Bavaria –

lessons learnt from 2011

U. Messelhäußer, J. Krams, S. Eckert, P. Kaiser, S. Reitenauer, M. Butzenlechner, A. Jüngling

Bavarian Health and Food Safety Authority, Veterinärstr. 2, 85764 Oberschleißheim, Germany

Corresponding author:

e-mail: judith.krams@lgl.bayern.de,

phone:+49-(0)9131-6808-5877

fax:+49-(0)9131-6808-5349

Since the German foodborne EHEC O104:H4 outbreak in 2011, which caused 2,987 human cases of illness and 53 cases of death, food hygiene in primary production and especially in sprout production is an important item of official food controls.

During the last two years the European Union also tightened the legal requirements for the microbiological quality of seeds and sprouts intended for human consumption. Furthermore there are new regulations with regard to the approval of establishments producing sprouts and the hygiene in sprout production.

However, the new hygienic requirements for sprout production laid down in the Regulation (EU) 210/2013 are very general and the microbiological criteria in the Regulation (EC) 2073/2005 only cover pathogenic agents and not the classical hygienic parameters like *Escherichia coli*, yeast and molds. Therefore the Bavarian Health and Food Safety Authority created comprehensive checklists for standardized official controls of sprout companies which concretize the general legal requirements. In parallel to the official food hygiene controls of sprout companies in Bavaria product samples were taken and not only analyzed for the parameters of Regulation (EC)

2073/2005 but also for different hygienic parameters. Results of microbiological investigations in combination with hygienic parameters of the sprout companies will be presented.

New perspectives of Juglans regia L. phytochemicals against Candida species

Natália Martins,[1,2] Lillian Barros,[1] Ana Maria Carvalho[1], Sónia Silva,[2] Mariana Henriques,[2] Isabel C.F.R. Ferreira[1]

[1]Mountain Research Centre (CIMO), ESA, Polytechnic Institute of Bragança, Portugal.
[2]IBB - Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Braga, Portugal.

Opportunistic fungal infections have deserved special relevance in the last decades, presenting itself, a serious problem in terms of public health. Despite Candida albicans was considered the main agent responsible for those infections, other non-albicans Candida species have also been described in the last years [1-3]. Most of the species are susceptible to antimicrobial drugs, but recently it has been observed a growing number of microorganisms with drug resistance. Therefore, the discover/use of alternative therapies is crucial [4].

Juglans regia L. (walnut) leaves are commonly used in traditional medicine as antiseptic, antimicrobial and anti-inflammatory [5]; those benefits could be related with its richness in phenolic compounds [6]. In the present work, the antifungal potential of the hydroalcoholic extract prepared from walnut leaves was evaluated against a total of nineteen Candida strains (from the species: C.albicans, C.glabrata, C.parapsilosis and C.tropicalis), using the disc diffusion halo assay.

All the tested strains were sensible to the plant extract. The obtained values of the inhibitory zones ranged between 0.9-1.4 cm, being the halo maintained after 48h. The observed antifungal activity is certainly related to the phenolic compounds previously determined in the extract [6]: five phenolic acid derivatives- caffeoylquinic and p-coumaroylquinic acid derivatives, two dimers and one trimer of procyanidins, twelve flavonols- quercetin, myricetin and kaempferol derivatives, and five taxifolin O-pentoside isomers; 3-O-caffeoylquinic acids and quercetin O-pentoside were the main phenolic compounds. Further studies are necessaries in order to elucidate the most active compounds and the specific role of each one.

Acknowledgments: The authors are grateful to FCT (Portugal) for financial support to CIMO (PEst-OE/AGUI0690/2011), N. Martins (SFRH/BD/87658/2012), L. Barros ("Compromisso para a Ciência 2008) and S. Silva (NORTE-07-0124-FEDER-000027; QREN) contracts.

References:

[1] Kim J, Sudbery P. (2011) J Microbiol. 49, 171-177.
[2] Tsai P-W, Chen Y-T, Hsu P-C, Lan C-Y. (2013) BioMedicine. 3, 51–64.
[3] Vázquez-González D, Perusquía-Ortiz AM, Hundeiker M, Bonifaz A. (2013) J Ger Soc Dermatology. 11, 381–94.
[4] Kanafani ZA, Perfect JR. (2008) Clin Infect Dis - Antimicrob Resist. 46, 120–128.
[5] Murray MT. (2004) 2nd ed. New York, NY. Random House.
[6] Santos A, Barros L, Calhelha RC, Dueñas M, Carvalho AM, Santos-Buelga C, Ferreira ICFR. (2013) Ind Crops Prod. 51, 430-436.

In vitro study of the antifungal potential of Apiaceae hydroalcoholic extracts against Candida species

Natália Martins,[1,2] Isabel C.F.R. Ferreira[1] Lillian Barros,[1] Mariana Henriques[2], Sónia Silva[2]
[1]IBB - Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Braga, Portugal.
[2]Mountain Research Centre (CIMO), ESA, Polytechnic Institute of Bragança, Portugal.

The use of medicinal plants is an ancient practice, but recently there is an increasing interest towards the evaluation of their bioactive properties. Opportunistic fungal infections, linked with higher rates of fungal resistance to the current antifungal drugs, have deserved special relevance in the last decades. Candida albicans was identified as the main responsible agent for those infections, but other non-Candida albicans Candida (NCAC) species have been also found [1]. Thus, it is urgent to discover new alternatives against those pathogens with high resistance.

In the present work, the antifungal potential of hydroalcoholic extracts obtained from two Apiaceae plants (Coriandrum sativum L. and Pimpinella anisum L.), commonly used in folk medicine, were evaluated against a total of 19 Candida strains (from the species: C. albicans, C. glabrata, C. parapsilosis and C. tropicalis).

The fruits of Coriandrum sativum L. (coriander) and Pimpinella anisum L. (anise) showed similar antifungal potential considering the studied strains, being effective against three of the nineteen strains. However, regarding the tested Candida species, the extracts presented considerable variations. Whereas coriander was effective against C. parapsilosis (ATCC22019 and 513143) and C. tropicalis (ATCC750), anise was effective against C. parapsilosis (513143 and 491861) and C. albicans (558234). Furthermore, the inhibitory zones were different at 24 and 48h. Further studies are being carried out in order to characterize the mechanism of action and the compounds responsible for the bioactivity, but the use of these extracts seems to have potential in antifungal therapy.

Acknowledgments: The authors are grateful to FCT (Portugal) for financial support to CIMO (PEst-OE/AGUI0690/2011), N. Martins (SFRH/BD/87658/2012), L. Barros (“Compromisso para a Ciência 2008) and S. Silva (NORTE-07-0124-FEDER-000027; QREN) contracts.

References:

[1] Martins N, Ferreira ICFR, Barros L, Silva S, Henriques M. (2014). Mycopathol. 177, 223–240.

THANKS FOR YOUR PARTICIPATION!

TEMPH 2014

TRENDS IN ENVIRONMENTAL MICROBIOLOGY FOR PUBLIC HEALTH