Antimicrobial susceptibility of *E. coli* isolates from meat industry wastewater

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Abstract

Wastewater originating from the meat processing industry is characterized by the high presence of organic waste load as well as potentialy pathogenic microorganisms. This study consisted of four sampling campaigns, during four seasons in 2013 and 2014. Wastewater was collected from three meat processing plants in the Province of Vojvodina, Republic of Serbia. The aim of this research was to determine the posible resistance of isolated *E. coli* strains to selected antibiotics.The evaluation of the antimicrobial susceptibility was performed on 37 strains of *E. coli* to 9 different antibiotics.Antibiotics, as emerging pharmaceuticals, used for susceptibility testing were: ampicillin, cefotaxime, ciprofloxacin, chloramphenicol, gentamicin, nalidixic acid, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole. Monitoring of antimicrobial resistance in commensal bacteria *E. coli* is of great significance because this bio - species is commonly present in animal faeces, in human organism, and can often acquire conjugative plasmids from other present enteric bacteria, thus serving as a valuable reservoir of resistance genes which can be transferred to other bacteria present in same environment. From a total of 37 strains of *E. coli*, a moderate degree of resistance was shown to tetracycline (37.83%); low degree of resistance to ampicillin (21.62%), streptomycin (24.32%), trimethoprim-sulfamethoxazol (18.92%) and nalidixic acid (16.22%); a very low to: chloramphenicol (13.51%), ciprofloxacin (2.7%), gentamicin and cefotaxime (0.0%). Ten of the tested *E. coli* strains (18.92%) were multidrug-resistant (MDR). Antibiotics used in this experiment are present on the NORMAN list of emerging substances of concern, since they are most frequently detected in aquatic environment. Due to their detectable presence in the environment, we have to be very careful because it increases the possibility for antimicrobial ressistance in microorganisms which have been in contact with antibiotic residues. High public pressure considering the consequences of mass appearance of multi-resistant bacterial strains that have spread from the animals to humans, managed to significantly reduce the use of antibiotics, especially as growth promoters.Serbia also adopted this EU legislation but more stringent control of law implementation is needed.

Key words: antimicrobial susceptibility,*E. coli*, meat industry, wastewater

Acknowledgement

This work has been done within the NATO project (Ref. 984087), and it has also been financially supported by Ministry of Education and Science, Republic of Serbia (Project No. 46009).

most common cyanobacteria species in Portuguese freshwater reservoirs, often exhibition long residence time in those reservoirs. This work aimed to evaluate the antibiotic susceptibility patterns and resistance mechanisms in *P. agardhii* in order to assess their putative contribution to the global pool of resistance determinants in freshwater. **Methods:** We investigated 8 strains of *P. agardhii*, previously isolated from different freshwater reservoirs. The antibiotic susceptibility was evaluated by a microdilution method previously adapted for cyanobacteria, against beta-lactams, aminoglycosides, quinolones, trimethoprim and tetracycline. Minimum inhibitory concentrations (MIC) were determined according to cell density (DO, 450nm) and microscopic examination of cultures integrity. All strains were searched for antibiotic resistance genes and class 1, 2 and 3 integrons by PCR/sequencing. **Results:** The results showed that *P. agardhii* is not susceptible to trimethoprim and quinolones within the tested concentrations (0.0015-1.6 mg/L). However, the cell growth is strongly inhibited by amoxicillin (median MIC value of 0.1mg/L). The other antibiotics presented the following median MICs: ceftazidime, 1.6 mg/L; ceftriaxone, 0.8 mg/L; tetracycline, 0.4 mg/L; kanamycine, 0.2 mg/L and gentamicine, 0.1mg/L. None of the *P. agardhii* strains exhibited genes associated with trimethoprim and quinolones resistance, which suggests that these cyanobacteria are intrinsically non susceptible to this antibiotics. Conversely, genes associated with streptomycin (*str*A-*str*B) and sulfonamide (*sul*1) resistance, as well as a 1-type integron, were detected in three of the strains. These strains were isolated from the freshwater reservoirs where *Planktothrix* blooms are frequent and. This suggests that the presence of a common antibiotic resistant determinant in *P. agardhii* might be a result from a similar selective pressure within those reservoirs. **Conclusions:** The presence of antibiotic resistance genes and integrons, as well as the reduced susceptibility to antibiotics, suggest that cyanobacteria may play a role on freshwater resistome and eventually contribute to the dissemination of antibiotic resistance in freshwater environments.

WE089

Antimicrobial susceptibility of E. coli isolates from meat industry wastewater

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WE090

Presence of multiple antibiotic resistant Enterococcus spp. harbouring virulence genes in WWTP effluent and receiving water systems in the North West Province, South Africa

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Faecal contaminants from poor operating waste water treatment plants pose a health risk to humans and animals. The latter is heightened when multiple antibiotic resistant bacteria harbouring virulence genes are present. The aim of the current study was to determine the antimicrobial resistance patterns and presence of virulence genes in *Enterococcus* spp. isolated from the final effluent of three municipal wastewater treatment plants and receiving water systems in the North West Province, South Africa. Sixty three *Enterococcus* isolates were isolated and antimicrobial susceptibility test performed on all isolates. Antibiotic inhibition zone diameter data was subject to cluster analysis. The cluster composed of *Enterococcus* spp. from all WWTPs final effluent was predominated by *E. faecalis* spp. followed by *E. faecium* and *E. hirae* spp. Whereas the cluster composed of *Enterococcus spp*. from downstream sites of receiving water systems was predominated by *E. gallinarum, E. casseliflavus* and *E. mundtii*. All 63 *Enterococcus* spp. were screened for the presence of five virulence determinants (*asa1, cylA, esp, gelE* and *hyl*). All five virulence genes were detected and six multi-virulence profiles observed. Analysis of the antimicrobial susceptibility of the 63 *Enterococcus* isolates revealed that resistances to Ampicillin (67%), Vancomycin (62%), Tetracycline (58%), Penicillin (52%) and Erythromycin (51%) were most frequent. Sixty eight percent of the screened *Enterococcus* spp. were resistant to three or more antibiotics. Seventy six of the screened *Enterococcus* isolates resistant to multiple antibiotics had plasmids. Differences in MAR phenotypes were observed for all profiles pre- and post-plasmid curing with lower resistance profiles post plasmid curing. This study has demonstrated that *Enterococcus* spp. harbouring virulence factors and plasmids that mediate multiple antibiotic resistance are present in WWTPs final effluent and receiving water systems that support a variety of social needs in South Africa. Thus, it is recommended that *Enterococcus* spp. be used as an additional faecal indicator in conjunction to *E. coli*. **Keywords:** *Enterococcus* spp.; WWTP final effluent; multiple antibiotic resistance; virulence genes

WE091

Antibiotic-resistant bacteria from air samples collected in nursing homes

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Introduction Antibiotic resistance is currently a worldwide public health problem that can include diverse mechanisms and bacterial species. The understanding and monitoring of such phenomena is essential to avoid further dissemination. The main aim of this study was the characterization of antibiotic resistance mechanisms in bacterial strains collected in air samples from nursing homes and compare them with clinical samples isolated from the same geographic area. **Materials and methods** Air samples were collected from bedrooms, living rooms and outdoor of 4 nursing homes located in Lisbon, Portugal. Screening of antimicrobial susceptibility of 18 Gram-negative (5 *Acinetobacter* spp., 1 *Klebsiella oxytoca*, 4 *Pantoea* spp., 7 *Pseudomonas* spp., 1 *Sphingomonas paucimobilis*) and 12 Gram-positive (3 *Micrococcus luteus*, 3 *Staphylococcus* spp.) isolates was performed by disk diffusion method. Different antibiotic resistant genes were searched by PCR. PFGE was used to evaluate clonality between *K. oxytoca* isolated from the air environment and other strains from clinical origin. **Results and discussion** The majority of strains were susceptible to all antibiotics tested. Among beta-lactam antibiotics, reduced susceptibility to cefoxitin was detected in *Staphylococcus capitis*, through expression of the *mecA* gene, and to ampicillin, piperacillin and piperacillin-tazobactam in 1 *K. oxytoca* expressing a $bla_{OXY\text{-}5}$-type beta-lactamase. Nonsusceptibility to meropenem and piperacillin-tazobactam was observed in 2 *Pseudomonas putida* however no antibiotic resistance gene was detected. Regarding quinolones, non-susceptibility was found in 1 *S S. capitis*, 2 *Staphylococcus haemolyticus*, and 1 *K. oxytoca*. The molecular characterization of the *mecA*-positive *S. capitis* from the nursing homes and from a hospital within the same region suggests a potential dissemination of strains between these two environments. The genetic relatedness of *K. oxytoca* from nursing homes (n=1) and clinical isolates (n=9) recovered within the same region, allowed to conclude that they were not genetically related. **Conclusions** Globally, nursing homes environments may act as complementary reservoirs of antibiotic resistant bacteria and antibiotic resistance genes. Thus, a better understanding of the antibiotic resistance mechanisms and dissemination pathways in other reservoirs than human is essential to control its emergence and spread.

WE092

Antimicrobial susceptibility and integron diversity in organic and conventionally grown fruits and vegetables

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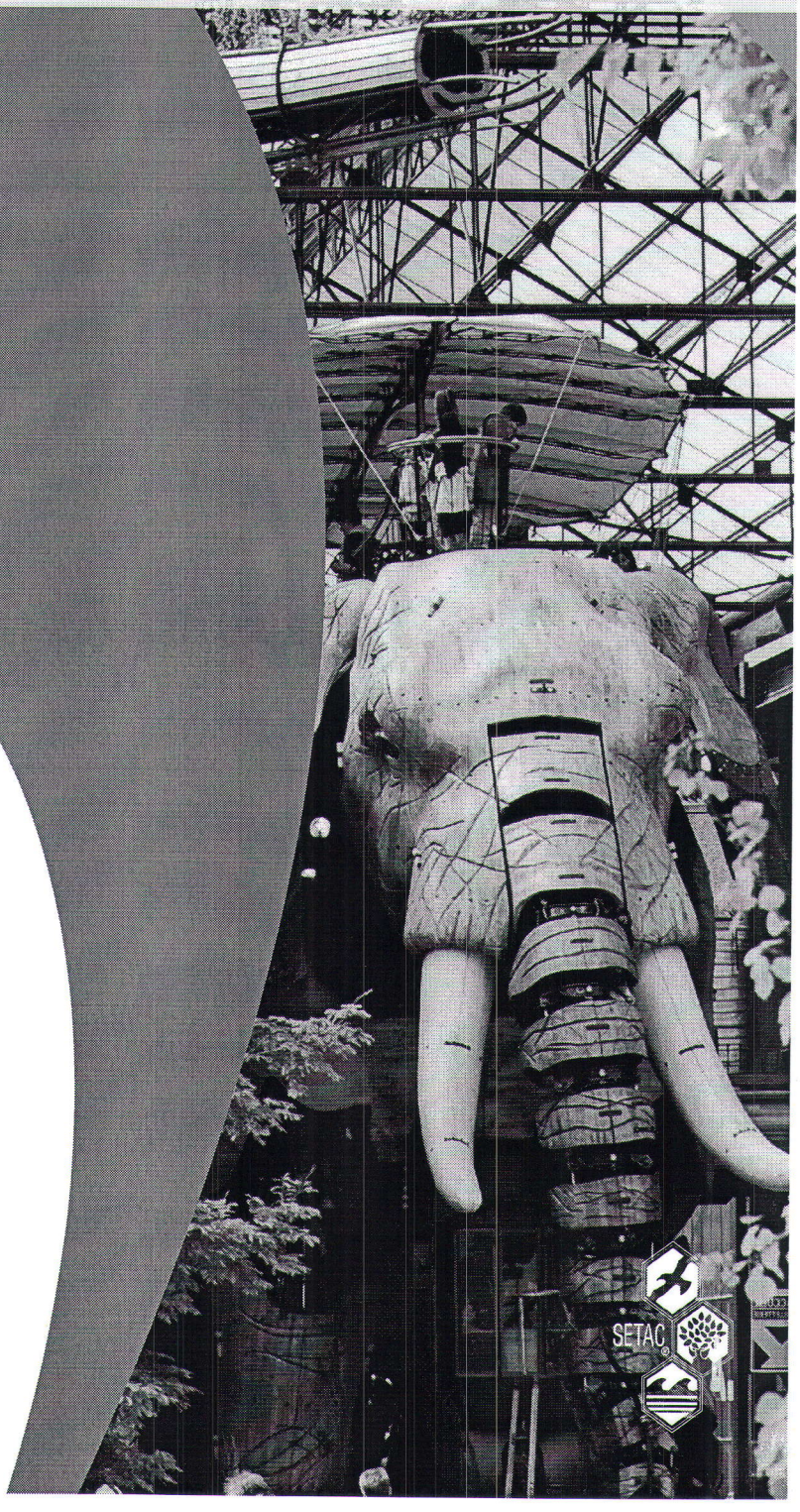
ABSTRACT BOOK

SETAC Europe 26th Annual Meeting
22-26 May 2016, Nantes, France

Environmental contaminants from land to sea: continuities and interface in environmental toxicology and chemistry



Nantes 2016
SETAC Europe

ABSTRACT BOOK
SETAC Europe 26th Annual Meeting

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This book composes the abstracts of the presentations for the platform and poster sessions of the 26th Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), conducted at La Cité Nantes Congress Center in Nantes, France, from 22 - 26 May 2016. The abstracts are reproduced as accepted by the Scientific Committee of the meeting and appear in order of abstract code, in alphabetical order per presentation type. The poster spotlight abstracts are included in the list of poster abstracts. The presenting author of each abstract is underlined.

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SOCIETY OF ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY

In the 1970s, no forum existed for interdisciplinary communication among environmental scientists, biologists, chemists, toxicologists as well as managers and engineers others interested in environmental issues. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in North America in 1979 to fill the void. Based on the dynamic growth in the Society's membership, meeting attendance and publications, the forum was clearly needed. SETAC has two administrative offices, in Pensacola, Florida, USA, established in 1990, and in Brussels, Belgium, established in 2003.

A unique strength of SETAC is its commitment to balance the scientific interests of government, academia and business. The Society by-laws mandate equal representation from these three sectors for officers, World Council, Geographic Unit Boards of Directors and Councils, and Committee members and governance of activities. The proportion of members from each of the three sectors has remained nearly equal over the years.

SETAC publishes two globally esteemed scientific journals and convenes annual meetings around the world, showcasing cutting-edge science in poster and platform presentations. Because of its multidisciplinary approach, the scope of the science of SETAC is broader in concept and application than that of many other societies.

The Society is concerned about global environmental issues. Its members are committed to Environmental Quality through Science®, to timely and effective communication of research, and to interactions among professionals so that enhanced knowledge and increased personal exchanges occur. SETAC's growth has been marked the establishment of geographic units around the world: SETAC Europe in 1989, SETAC Asia/Pacific in 1997, SETAC Latin America in 1999 and SETAC Africa in 2012. As evidence of international acceptance of the SETAC model and of the great interest at the local level, regional chapters of the geographic units are being considered for a number of countries.

Publications

Environmental Toxicology and Chemistry, an internationally acclaimed scientific journal, has grown from a quarterly publication of fewer than 400 pages annually in 1980 to a monthly publication of nearly 3,000 pages annually.

Integrated Environmental Assessment and Management, launched in 2005 to bridge the gap between scientific research and its application in environmental decision-making, regulation and management, has become a well-respected quarterly publication of 700 pages annually.

SETAC Books total more than 100, encompassing workshop results and other scientific studies.

The Society of Environmental Toxicology and Chemistry (SETAC) is a not-for-profit, global professional organisation comprised of some 5500 individual members and institutions dedicated to the study, analysis and solution of environmental problems, the management and regulation of natural resources, research and development, and environmental education. SETAC Europe is one of the five Geographic Units of the global Society, established to promote and undertake activities of SETAC in Europe, and to support activities of SETAC in the Middle East and Africa. The Society is dedicated to the use of multidisciplinary approaches to examine the impacts of stressors, chemicals and technology on the environment. We also provide an open forum for scientists and institutions engaged in the study of environmental problems, management and regulation of natural resources, education, research and development, and manufacturing. SETAC Europe is incorporated in Belgium as a not-for-profit organisation. The Society is governed according to its articles of association and by-laws. SETAC Europe maintains its administrative office in Brussels, Belgium.

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