Determination of Phytocannabinoids in Cannabis Samples by Ultrasound-Assisted Solid-Liquid Extraction and HPLC-DAD Analysis

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ORIGINAL ARTICLE

ABSTRACT

Introduction: The cannabis plant has been used by humankind for several millennia. Cannabis has over 120 phytocannabinoids[1], from which Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD) are present at higher amounts[2]. While Δ^9-THC is primarily responsible for the psychoactive activity[3], CBD is associated with anti-inflammatory, anxiolytic and analgesic properties, attenuating the psychoactive effects caused by Δ^9-THC consumption[4]. The characterisation of cannabis plant has gained enormous importance in the last decade, mainly due to changes in cannabis control. Thus, it is particularly relevant for the forensic, medical or recreative industry to have a rapid, inexpensive, and reliable methodology to identify and quantify phytocannabinoids.

Methodology: To optimise the extraction process, the solvent type, extraction volume, the steps number and the sonication time were evaluated using different design of experiment (DoE). The herbal samples were extracted by ultrasonication solid-liquid extraction (US-SLE) using only 50mg of sample and the plant oils were diluted in ethanol. The samples were analysed by high-performance liquid chromatography with diode array detection (HPLC-DAD) to identify and quantify Δ^9-THC, Δ^8-THC, cannabidiol, cannabinol, Δ^9-tetrahydrocannabinolic acid and cannabidiolic acid in cannabis products.

Results: Extraction optimisation used an ethanol:acetonitrile mixture, with only one cycle and one minute in the US step. The optimised US-SLE/HPLC-DAD procedure allowed ≈100% extraction efficiency of the target cannabinoids. The method was successfully applied to cannabis samples, demonstrating its suitability for routine analyses (Figure1).

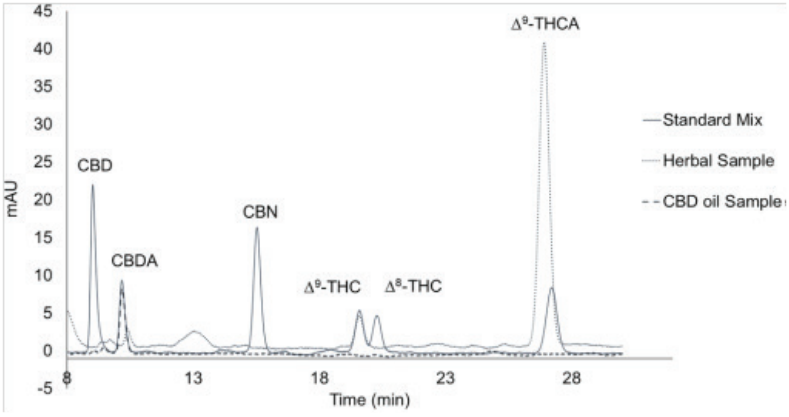

Figure 1. Overlayed chromatograms of standard mix containing the 6 target cannabinoids (solid line), the herbal cannabis sample (dotted) and a "CBD" oil sample (dashed).

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Discussion: The validation assays showed that the method is linear (R^2>0.997), selective, precise and accurate, with suitable limits of detection (0.125-0.250 μg mL-1) and quantification (0.500 μg mL^{-1}). The present study compares very favourably against other works, particularly in regards to the extraction efficiency, speed of the overall procedure, method sensitivity, and ability to monitor Δ8-THC spiked samples using a novel solvent mixture.

Keywords: HPLC-DAD, cannabis, THC.

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Dissemination of Colistin Resistance from One Health Perspective

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ORIGINAL ARTICLE

ABSTRACT

Introduction: The emergence of plasmid-mediated colistin resistance genes (mcr) in bacteria from animals raised the concern of resistance dissemination to human isolates, where colistin have been used to treat multidrug resistant infections. A better understanding of the biological cost imposed by mcr-plasmids may facilitate a decision on the ban of colistin in livestock. The main goal of this study was to assess the prevalence of mcr-borne plasmids in Escherichia coli isolated from food-animal producers and the impact on bacterial fitness of mcr-1 acquisition.

Methods: Ninety-eight Escherichia coli were isolated from Iberian pigs, rabbits and poultry samples. Plasmid identification, mcr-1 to -10 and ESBL genes were screened by PCR and identified by sequencing. The potential of plasmid spread was evaluated by conjugation assays. Growth kinetics were conducted in LB-broth without and with colistin (4mg/L). Plasmid loss was evaluated in six transconjugants and donors by serial transfer during 10 days. Statistics were done with paired, two-tailed t-test and ANOVA test.

Results: The mcr-1 gene was found in 15,3% of the isolates, mostly from pigs; genes blaTEM-1; blaCTX-M-1, and blaCTX-M-15 co-existed with mcr-1 in four strains. The plasmids had the potential to spread. Trasconjugants showed a biological cost in the presence of colistin (growth rate 1,32 vs 1,66). Significant differences in growth rates ($p<0.05$) were observed in transconjugants with different plasmids. After several cell generations plasmid retention was diverse.

Discussion: Overall, mcr-1 in zoonotic strains have the potential to disseminate to others that might enter in contact with humans. Despite an observed fitness cost to the cell, colistin withdrawn from animal production will not revert completely the resistance; other factors beyond colistin pressure (like plasmid type) regulate the stability of the plasmid maintenance in the bacterial population with consequent spread to other niches.

Keywords: plasmid-mediated colistin resistance genes, horizontal gene transfer, one health.

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Disclosing the Health-to-Disease Transition by Untargeted Metabolomics Based on HRMS and Chemometric Tools

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Untargeted analysis is a valuable approach for sample classification and identification of unknown compounds, including potential health status indicators. Comparing metabolomic profiles of different groups such as control and disease samples can unveil biomarkers and shed light on disease mechanisms[1]. Presently, untargeted metabolomics relies on highly sensitive techniques like liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). The post-experimental data processing is challenging due to the vast amount of complex information generated and chemometric tools may help in this endeavour. This study aimed to profile human plasma samples using LC-HRMS and the chemometrics method Regions of Interest-Multivariate Curve Resolution (ROIMCR)[2] to search for potential biomarkers of the health-to-disease transition, focusing on chronic kidney disease (CKD). While several biomarkers have been identified for this worldwide health problem[3], finding indicators of disease progression remains an open challenge. To our knowledge, this study is the first application of ROIMCR to CKD research.

Methodology: LC-HRMS analysis with an Orbitrap mass detector operated in full scan mode was performed for the plasma of three groups: healthy controls, intermediate-stage CKD patients, and final-stage CKD patients. Positive and negative ionization modes were analyzed simultaneously. Data processing was implemented in MATLAB environment.

Results and discussion: In a first stage of data processing, the ROI procedure compressed and filtered the data, followed by MCR-Alternating Least Squares (MCR-ALS) to resolve the elution profiles and spectra of sample constituents. Statistical tools such as PCA, PLS-DA, and ASCA were used to analyze the peak heights of the elution profiles, revealing a distinct separation and clustering of samples according to health status. The metabolites exhibiting significantly different peak heights, potentially serving as biomarkers of disease onset and/or progression, were tentatively identified using the Human Metabolome Database and CFM-ID online tool.

Keywords: untargeted analysis, data processing, biomarkers.

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NMR Metabolomic Analysis of Plasma from Long-Term Care Facilities People to Detect Blood Biomarkers for Stroke

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Worldwide, 12.2 million people suffer a new stroke each year, one every 3 seconds, and half of them die from it[1]. In Portugal, stroke remains the first cause of death and 82% of the total number of deaths due to cerebrovascular diseases occur in people aged over 74 years[2]. It can have different causes and is frequently the result of complex interactions of a variety of risk factors, often related with age progression. Some of them are related to lifestyle, such as unhealthy diet, alcohol abuse, smoking, physical inactivity, and stress. Other, such as hypertension, arrhythmias, heart failure, angina pectoris, heart attack (cardiovascular diseases) and metabolic disorders (diabetes, high cholesterol, and obesity), are the traditional major risk factors for stroke. Despite all the well-known risk factors, it is not possible to fully prevent the disease onset or clearly differentiate its risk stages. Thus, the objective of this study was to use Nuclear Magnetic Resonance (NMR) spectroscopy to identify small molecule variations between different stroke risk stages.

Methodology: To predict the probability of suffering stroke in 197 elders from EBIcohort (cohort of Elderly from 20 long-term care facilities of Beira Interior), we used the Framingham Stroke Risk Score[3]. It combines modifiable and non-modifiable risk factors, namely, systolic blood pressure, use of antihypertensive drugs, presence of cardiovascular diseases, diabetes, and current smoking status. Within a time window of 5 years probability of suffering a stroke, we constitute three study groups: low risk [0%-5%[(control), moderate risk [5%-20%[and high risk [20-100%]. NMR spectroscopy was used to analyse the metabolites present in each participant plasma sample. All the samples were acquired on an AVANCE III 600 MHz NMR spectrometer equipped with a quadruple resonance cryoprobe. Were acquired 1D (NOESY and CPMG) and 2D (1H-1H J-res) pulse sequences to identify and quantify the metabolites.

Results: Trough univariate statistical analysis several metabolites were identified from which asparagine, creatinine, dimethyl sulfone, urea, formic acid and two unknown metabolites were found in significantly higher relative concentrations.

Discussion: The differences found in the metabolic profiles seem to be a promising tool in the clinical diagnosis of stroke risk, also to assess its severity and progression.

Keywords: stroke, metabolomics, elders.

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Pharmacogenomic Characterization of SLC Transporters in Iberoamerican Countries

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Pharmacogenomic variation in genes coding for drug metabolizing enzymes or transmembrane transporters is now well established as the most important determinant of variable risk for pathologies or variable drug response, both in terms of efficacy and safety. Among the transmembrane transporters, SLC (solute carriers) co-transporters have recently been unveiled as central determinants of drug bioavailability, intra-and extracellular concentrations, drug metabolism and pharmacological activity. These genes are polymorphic, with some variants leading to significant phenotypic changes, with established or potential clinical impact. The identification of polymorphic variants in these genes and their frequencies in different world populations is of paramount importance to predict drug action, probability of adverse effects or risk of non-responsiveness to treatment. However, the pharmacogenomic characterization in most countries is still scarce, particularly in the Iberoamerican region, and more so in native or admixed populations.

Methodology: We have characterized selected single nucleotide polymorphisms (SNPs) in SLC transporters (namely SLCO1B1 T217C, A388G, C463A, T521C and SLCO1B3 T334G, G699A, G1564T) and determined their frequencies in population samples from Portugal, Brazil and Colombia.

Results: The results show, for some SNPs, a variable genetic profile, while other polymorphic variations present with similar frequencies in these three populations.

Discussion: These results contribute to the growing effort in characterization of pharmacogenomic variability worldwide, which is expected to provide genetic information and testing tools that may be useful in the definition and optimization of therapeutic strategies.

Keywords: pharmacogenomics, SLC transporters.

Acknowledgements: This study received Portuguese national funds from FCT - Foundation for Science and Technology through projects UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020.

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Biopelículas de Nocardia Brasiliensis

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ORIGINAL ARTICLE

RESUMEN

Introducción: Las biopelículas (BP) son organizaciones microbianas que ofrecen ventajas a los microoganismos que las producen, tales como la resistencia a los agresores ambientales y a los tratamientos antisépticos o antibióticos. Crecen embebidas en una matriz de exopolisacárido autoproducido y se adhieren a una superficie inerte o a un tejido vivo Se han descrito estas BP para diversas bacterias y hongos lo que permite comprender su participación en la incidencia de enfermedades nosocomiales, resistencia a los antibióticos o la recurrencia de diversas patologías entre otras. De aquí la importancia de conocer si las BP participan como mecanismos de virulencia en bacterias saprófitas como lo son los actinomicetos que son responsables de enfermedades crónicas y de difícil tratamiento como son la tuberculosis o el micetoma. Nocardia brasiliensis y Actinomadura madurae son bacterias de suelo responsables del actinomicetoma que por estudios de histoquímica se ha demostrado la producción de un "cemento" de naturaleza polisacárida que es característico del microoganismo que lo produce, sin embargo, a este cemento no se le asociado como componente de una biopelícula.

Metodología: Se evaluaron diferentes tipos de soporte inerte que permitieron la adherencia sobre acetatos, vidrio o catéteres como fase inicial en la generación de las biopelículas por Nocardia brasiliensis, así como su cinética de formación para corroborar su capacidad de crear filamentos.

Resultados: Se encontraron diferencias en la capacidad adherente y en la homogeneidad de crecimiento entre los distintos materiales. Se determinó la cinética de adherencia al acetato de esta bacteria, y se observó un aumento en las UFC/placa y la formación de filamento de las colonias en estas condiciones.

Discusión: Se logró la descripción de la formación de biopelículas por Nocardia brasiliensis

Palabras clave: nocardia brasiliensis, biopelículas, actinomicetos.

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Calidad Fisicoquímica y Microbiológica del Agua Mineral de Manantial Embotellada Comercializada en Quito. Ecuador

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ORIGINAL ARTICLE

RESUMEN

Introducción: De acuerdo con el informe realizado en marzo del 2023 por la United Nations University Institute for Water, Environment and Health[1], cada minuto se venden más de un millón de botellas de agua en todo el mundo, de manera que este sector económico mueve aproximadamente 270.000 millones de dólares cada año, con un crecimiento del 73 % entre 2010 y 2020 en 109 países[1]. El consumo de agua envasada puede causar riesgos para la salud cuando el producto presenta contaminación microbiológica y/o química. Por ello el objetivo del presente trabajo fue evaluar la calidad fisicoquímica y microbiológica del agua mineral de manantial envasada durante su comercialización en los anaqueles de establecimientos comerciales de la ciudad de Quito. Las muestras de agua envasada fueron obtenidas en establecimientos comerciales, siguiendo un muestreo aleatorio. Se evaluaron cinco marcas de agua de manantial envasada sin gas expendida en la ciudad de Quito. De cada marca se recolectaron durante dos campañas de muestreos un total de ocho botellas de plástico, de un volumen de 1,50 litros. Para analizar los parámetros fisicoquímicos se empleó la metodología indicada por la American Public Health Asociation[2], utilizando un medidor multiparámetro marca Hanan para pH, temperatura, oxígeno disuelto, turbidez, conductividad y solidos disueltos totales y para los análisis microbiológicos, las metodologías de filtración por membrana indicadas por Andueza[3] y Araque[4], así como siembra en superficie en placas Compact Dry EC. Los resultados fisicoquímicos indican valores promedios de pH 6,46 (±0.02) temperatura 19,55 °C (±0.15 °C), oxígeno disuelto 6,76 mg/l (± 1.5% de la lectura o ± 1.0% de saturación) turbidez 0,31 NTU (± 2% de la lectura o ± 0,01 PSU), conductividad 324,51 μS/cm (± 1% de la lectura o ± 1 μS/cm) y sólidos disueltos totales 214,03 mg/l (±2% F.S.). Los análisis microbiológicos señalan la presencia de bacterias heterótrofas en un rango de 10 UFC/100 mililitros a más de 1600 UFC/mililitros y de bacterias del género Pseudomonas en un rango de 10 a más de 520 UFC/100 mililitros. En ninguna de las muestras estudiadas se observó presencia de coliformes totales y de la bacteria Escherichia coli. Los parámetros fisicoquímicos de todas las marcas de agua estudiadas se encuentran dentro de los rangos establecidos por la normativa nacional e internacional. Los resultados del análisis microbiológicos evidencian la presencia de un número moderado de bacterias heterótrofas, superior a lo establecido en la normativa de la Comunidad Económica Europea. Se requiere evaluaciones microbiológicas periódicas para evitar brotes de infecciones de origen hídrico.

Palabras clave: agua envasada, agua mineral, calidad fisicoquímica, calidad microbiológica.

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The Potential of Dried Saliva Spots for Antidepressant Drugs Monitoring

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Portugal has one of the highest rates of mental illness in Europe, and the consumption of antidepressants amongst its population has doubled between the years 2013 and 2016. Approximately 30 million packs of medication are prescribed and dispensed annually in this country to treat major depressive disorders[1]. Therapeutic Drug Monitoring (TDM) is a practice established for a small number of drugs, for which there is a direct relationship between concentration and pharmacological effect at the site of action. Commonly, TDM involves measuring drug concentrations in serum, plasma or blood, being complemented with clinical observation measures to assess patient's condition, support the individualization of therapy, detect changes in the patient´s pathophysiological status or the modification of the drug pharmacokinetics, but also to assess adherence to therapy.

Methodology: The present work describes a methodology with great potential for TDM purposes, allowing the determination of antidepressants (fluoxetine, venlafaxine, citalopram, sertraline, paroxetine and metabolites) using 100 μL of oral fluid with dried saliva spots (DSS) and analysis by gas chromatography-tandem mass spectrometry (GC-MS/MS).

Results: For both sampling and extraction with DSS, several parameters were optimized using the statistical tool Design of Experiments (DoE), namely: drying and extraction times, extraction solvent and volume. The final optimized conditions were: 1 hour of drying, 1 mL of methanol and 5 minutes of extraction. The method was validated showing linearity within the adopted therapeutic ranges, with limits of detection and quantification between 10 and 100 ng/mL. All validation parameters were assayed according to the SWGTOX guidelines.

Discussion: This is the first work that uses DSS to extract antidepressants from oral fluid samples, proving to be a sensitive, simple and fast alternative to conventional techniques and samples used in TDM (solid phase extraction based procedures and plasma samples). It also reveals potential to be routinely applied in TDM and forensic toxicology purposes, as it has been successfully applied to the analysis of authentic patient samples[2].

Keywords: antidepressants, DSS, drug monitoring.

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Evaluation of hair hydrolysis and MEPS columns for the detection of amphetamines derivatives in hair

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Different extraction and cleanup procedures have been used to identify amphetamines in hair samples. The microextraction by packed sorbent (MEPS), a miniaturized approach of the classic solid-phase extraction (SPE), has only been used so far to determine the presence of amphetamine (AMP) and methamphetamine (MAMP) in hair. The aim of this work was to evaluate different hair sample extraction conditions, namely hydrolysis, to maximize the detection of AMP, MAMP, MDA, MDMA, MDE, and MBDB in combination with MEPS and GC-MS.

Methodology: Pulverized authentic hair (50mg) was incubated with 500μL of NaOH 1M at 80ºC for 1H, 60ºC overnight and 45ºC overnight[1]. After incubation, the extracts were neutralized with 50μL of 10M HCl and subsequently centrifuged. The supernatant was tested with several conditions to access the most suitable when paired with MEPS clean-up. The studied conditions were as follows: the addition of 500μL of H_2O; supernatant filtration followed by addition of H2O; and precipitation with cold ACN followed by centrifugation. After clean-up, 30μL of MBTFA were added for evaporation under nitrogen gas. After being fully dried, 50μL of MBTFA was added and derivatization was done with a microwave oven. Finally, the extract was injected into a GC-MS and analysed.

Results: Results obtained from these tests indicated that the best hydrolysis for this purpose was obtained with 500μL NaOH 1M at 45ºC overnight, with the addition of H_2O before MEPS clean-up. The clean-up procedure was previously optimized by design of experiments (not being the scope of this study). Furthermore, M1 and C18 MEPS sorbents were tested to check which one would provide cleaner extracts and greater analyte signals. A concentration of 2.00 ng/mg of AMP, MAMP, MDA, MDMA, MDE, and MBDB was used for this test.

Discussion: Considering the results, both columns presented similar recoveries for each amphetamine, when using equal hydrolysis and MEPS conditions.

Keywords: hair analysis, amphetamines, hydrolysis.

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An analysis of the Association Between MMP-8 +17 C/G Polymorphism and Periodontitis in a Portuguese Population

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Matrix Mettaloproteinases (MMPs) are structurally related but genetically distinct proteolytic enzymes involved in the breakdown of extracellular matrix and basement membrane (ECM) proteins. This action during the inflammatory process of periodontal disease leads to the destruction of the periodontium[1]. Several studies have been conducted to understand its severity and course, concluding that the individual genetic susceptibility of MMPs is an important factor. Specifically related to this disease, MMP-8 also known as collagenase-2 participates in the breakdown of various proteins from the connective tissue, including collagen type IV, V, and XI, proteoglycans and elastin[2]. Single nucleotide polymorphisms (SNPs) in the region of MMP-8 gene (chromosome 11), at position +17 C/G; a cytosine to a guanine replacement occurs at the first exon in the coding region, has been described to have putative functional significance[3]. To date, no study has analyzed if MMP- 8 +17 C/G polymorphism might modify the relative risk for the development periodontitis in a Portuguese population. As such, this pilot study aimed to determine the allele and genotype frequencies and the relationship between the existence of this polymorphism and the presence of periodontitis in a Portuguese population of users of the Egas Moniz Dental Clinic.

Methodology: A sample of 43 individuals was used, 21 of which were periodontal patients, and the remaining 22 were periodontally healthy (approved by the IUEM Ethics). The samples from the jugal mucosa were collected with an OmniSwab, for later DNA extraction. Genetic analysis for the MMP-8 +17C/G polymorphism was performed through PCR (Polymerase Chain Reaction) and RFLP (Restriction Fragment Length Polymorphism) with Dde I , followed by electrophoretic analysis. Statistical analysis was carried out using the R Statistical 4.0 Software (R package based on $\chi 2$ test, Statistical Computing and Graphics, Inc.).

Results/Discussion: Our results showed that all of our polymorphisms were in Hardy-Weinberg equilibrium ($p=0.13$). The allelic and genotypic distributions for this polymorphism were the same in periodontal patients and controls ($p=0,48$ and $p= 0,49$, respectively). In this sense, the present pilot study provides no evidence of an association between MMP-8 polymorphism and periodontitis susceptibility.

Keywords: metalloproteinase, mmp 8, periodontitis, molecular genetics, SNP.

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Molecular Characterization of Carbapenem-Resistant *Klebsiella* spp. and *Acinetobacter* spp. Collected from a Portuguese Tertiary Hospital

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Antimicrobial resistance has been rising dramatically. The World Health Organization classified as critical carbapenem-resistant *Enterobacteria* and *Acinetobacter* spp., bacteria that show resistance to all beta-lactam antibiotics due to carbapenemases production. The phenotypic and genotypic study of antimicrobial susceptibility is crucial to track resistance, implement novel antibiotic policies, and improving infection prevention/control responses in clinical setting. The main goal of this study was to evaluate the antimicrobial susceptibility profiles and the molecular mechanism underlying the resistance to carbapenems in *Klebsiella* spp. and *Acinetobacter* spp., isolated from patients of the Centro Hospitalar Universitário de Coimbra (CHUC) between December 2022 and March 2023, and to assess their potential spread.

Methods: Antibiotic susceptibility evaluation was performed by VITEK-2 (BioMérieux) and the disc diffusion method. Breakpoint interpretation was based in EUCAST guidelines. The most frequently found carbapenemase genes ($bla_{IMP,}$ bla_{VIM}, bla_{KPC}, bla_{OXA-48}) were screened by PCR, and further identified by amplicon sequencing. Bacterial genotype was assessed by the MultiLocus Sequence Typing technique. The potential for carbapenemase-gene dissemination was assessed by conjugation assays.

Results: A total of 26 carbapenem-resistant isolates were collected. *Klebsiella* spp. isolates (n=24) were more prevalent in urine samples (58.3%; n=14) and from the Emergency ward. KPC gene was detected in 79.2% (n=19) and bla_{OXA-48} in 20.8% (n=5). The KPC variants identified were KPC-3 and KPC-19. The bla_{OXA-48} gene was successfully transferred to E. coli J53 by conjugation. The MLST revealed the Sequence Types ST15, ST231 and ST13. Only two isolates of *Acinetobacter* spp. were recovered: *A.baumannii* carried the bla_{VIM} gene.

Discussion: To our knowledge, this is the first study reporting the bla_{VIM} gene in Acinetobacter baumannii in Portugal and the co-existence of the bla_{IMP} and bla_{OXA-48} genes in *Klebsiella pneumoniae*. Our results highlight the spreading potential of bla_{OXA-48} borne plasmids to other species. Previous studies showed that KPC-3 was prevalent in this hospital, but a novel variant, KPC-19, only detected in a Lisbon hospital, was identified. Overall, this study shows that carbapenem resistance is associated with carbapenemase production and the gateway to novel strains/genes is the Emergency ward, highlighting the screening of patients transferred from other health facilities to improve antibiotic stewardship and infection prevention.

Keywords: *Klebsiella* spp., *Acinetobacter* spp., carbapenem-resistance.

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Amperometric Biosensors for High Spatial and Temporal Resolution Monitoring of Lactate *in vivo* in Rat Brain

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ORIGINAL ARTICLE

ABSTRACT

Introduction: The role of lactate has been re-evaluated from a metabolic dead-end product to a key metabolic intermediate produced under fully aerobic conditions, used as a metabolic fuel by a variety of cells[1]. In the brain, lactate plays a pivotal role in coupling neuronal activity to both energy metabolism and signalling processes. Recent evidence has linked changes in lactate levels in brain extracellular space to neurodegenerative diseases and brain disorders. Moreover, oxygen is presented as one of the major substrates for aerobic energy metabolism, which is altered in a wide range of perturbations that can occur in the brain. It is therefore essential to develop tools to monitor extracellular concentrations of lactate and oxygen to elucidate their roles in brain function and understand their involvement in certain medical conditions such as TBI and epilepsy.

Methodology: Microelectrodes coupled with fast electrochemical techniques are widely used as an attractive analytical tool for monitoring the concentration dynamics of non-electroactive neurotransmitters and metabolic substrates in the brain extracellular space. Thus, microelectrodes can be used to detect and monitor lactate and oxygen levels in the brain extracellular space with high spatial and temporal resolution, sensitivity, selectivity, and minimal tissue damage.

Results and Discussion: In this study, microelectrode-based biosensors have been designed and developed using platinum modified carbon fiber microelectrodes (CFM/Pt) and ceramic-based Pt microelectrode arrays (MEA) as sensing platforms for lactate measurements, with the immobilization of lactate oxidase (LOx). The *in vitro* evaluation of the lactate microbiosensors was performed by amperometry at +0.7 V vs Ag/AgCl. Analytical performance and enzyme kinetic parameters were evaluated in a wide range of lactate concentrations (0.05-40 mM). Oxygen sensors were developed by using carbon fiber microelectrodes modified with multiwall carbon nanotubes. The *in vitro* evaluation of the oxygen sensors was performed by amperometry at -0.6 V vs Ag/AgCl. Oxygen sensor sensitivity was determined by adding saturated O2 solution to a previously N2 purged solution in a range of oxygen concentration (5.02-41.6 μM). The lactate microbiosensors have been successfully used to monitor tonic changes and basal lactate levels in the brain extracellular space with high spatial and temporal resolution under various experimental paradigms.

Keywords: lactate biosensor, *in vivo* electrochemistry, epilepsy.

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Recreational Athletes' Perception of Contaminants in Food Supplements and their Risks for Health

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ORIGINAL ARTICLE

ABSTRACT

Introduction: The use of food supplements (FS) by athletes has significantly increased in recent years[1]. Through some inherent toxicity as well as the possibility of contamination or adulteration, FS may cause health issues[2]. Heavy metals, illicit compounds including doping substances, undeclared drugs, and other contaminants and adulterants have all been detected in FS2. Thus, the study aims to investigate how recreational athletes perceive the main concepts of FS as well as its risks of adulteration and contamination and their effects on human health.

Methodology: In the current cross-sectional analysis participated 303 gym-goers from Lisbon, Portugal, who were 133 women and 170 males (30.8 ± 12.9 years old). Face-to-face interviews were used by qualified researchers to gather data.

Results: Men were the predominant consumers of FS, accounting for 71.95% of the population. The accurate definition of FS (Directive 2002/46/EC, 10 June) was unknown to about 95% of the participants. Those with hypertrophy training objectives did use more FS, compared to those who trained for well-being ($p<0.01$). There were notable discrepancies between the beliefs of FS consumers compared to no consumers: People who do not use FS were more aware of possible drug interactions, believed that FS can be used as a replacement for a balanced diet, and expressed worries about the likelihood of FS overdose death. The participants' knowledge of market control (46.54%) and regulation (40.92%) was low. While customers feel that FS may contain prohibited additives that alter nutritional value, those who do not use FS are more concerned about the possibility of their contamination with industrial pollutants.

Discussion: In general, athletes have consistently reported a lack of knowledge about the main concept of FS, but also about its possible contamination and adulteration and their potential health impacts and risks. The results obtained seem to show the need for more educational programs to guide consumers and increase their knowledge about FS.

Keywords: food supplements, athletes, contaminants.

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Effects of Antihypertensive Therapies in Serum Metabolites and Sociodemo-Graphic and Clinical Data in Elders from Nursing Homes

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ORIGINAL ARTICLE

ABSTRACT

Introduction: Hypertension (HT) increases the risk of suffering from other cardiovascular diseases, which are responsible for up to 30% of mortality worldwide. People suffering from HT can be treated with antihypertensive therapies from different classes.

Methods: From a cohort (EBIcohort) of ~450 users of nursing homes/long-term care facilities, we collected biological samples (serum) and clinical and sociodemographic data (age, Body Mass Index, systolic blood pressure, diastolic blood pressure, current diagnosis and medications, number of cardiovascular diseases, and scores of Global Deterioration Scale, Mini Mental State Examination, Addenbrooke's Cognitive Examination-Revised, Frontal Assessment Battery, and Quality of Life). We analyzed the effects of different antihypertensive treatments (AHT) in the serum metabolomic profiles using NMR metabolomics, and also measured several serum biochemical parameters (such as glucose, triglycerides, cholesterol, HDL, and LDL). We constituted a control group (AHT0) with participants without hypertension and four groups with participants with hypertension treated with one (AHT1), two (AHT2), three (AHT3) or four (AHT4) antihypertensive drugs.

Results and Discussion: We observed significant differences between the groups (AHT0, AHT1, AHT2, AHT3, and AHT4) when analyzing sociodemographic and clinical parameters. Namely, we found higher BMI levels in participants that were being treated with two or more AHT in comparison with participants from the control group or AHT1. We also found that the number of cardiovascular diseases increased when increasing the number of AHT, and also differed among AHT1 groups. Regarding metabolite levels, we also found significant differences between groups. Creatinine serum levels significantly increased from AHT0 to AHT4. Then, when analyzing metabolite levels among each group, we found differences in aminobutyrate (AHT1), dimethylsulfone (AHT2), hydroxybutyrate (AHT3), and choline and methylglycine (AHT4) levels. The observed differences might help to understand the impact of the antihypertensive therapies on the metabolism and other disease-related parameters.

Keywords: hypertension therapy, NMR, metabolomics.

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The Potential of Waters Analysis in Toxicology: A Review of Publications Between 2019-2023

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ORIGINAL ARTICLE

ASTRACT

Introduction: Water quality-threats have been emerging over the years, presenting a devastating public health issue[1]. The analysis of water from the standpoint of toxicology is essential for assessing the risks posed by contaminants, safeguarding human health and ecosystems, complying with regulations, and making informed decisions to manage and protect water resources. It enables a comprehensive understanding of the complex interactions between pollutants, organisms, and the environment. Thus, the present study aimed to explore descriptively which are the current most common extraction and detection techniques, as well as the tested analytes and types of waters.

Methodology: To achieve this purpose, the Web of Science database was used to retrieve papers published from 2019 to 2023 (10th august 2023), with the search string "determination in water" for terms included in the title, abstract or keywords. Given the generality of the search's thematic, only articles written in English and performing analyte extraction and detection in water samples were included.

Results: Taking these criteria into consideration, 2962 articles were found from which 333 were considered. Articles were classified according to the type of paper, analysed water(s), detected analyte(s), and extraction and detection approaches. From the 306 original articles and the 27 reviews analysed, the majority assessed surface waters (195), followed by wastewaters (152), tap (60), drinking (29), rain (7) and hospital waters (5). Regarding analytes, pharmaceuticals (39%) and pollutants (34%) emerged as the main concerns, whereas heavy metals (14%), biomarkers (5%), substances from cosmetics (5%), illicit drugs (2%) and caffeine (2%) were less studied. Correlation between contaminants and type of water was also described (e.g., surface water and pharmaceuticals were detected 45%). The great majority of studies used solid-phase extraction (71%), while others chose miniaturized techniques (21%), liquid-liquid extraction (5%) or protein precipitation and direct injection (3%). Liquid chromatography (63%) was the most used instrumentation method, followed by gas chromatography and biosensors (both 12%), spectrophotometric methods and atomic absorption spectrometry (both 5%) and fluorometric methods (2%).

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Discussion and conclusions: In conclusion, considering the crucial role of water quality assessment in public health and safety, further investigation is needed in order to improve and optimize the existing approaches, taking into account the type of analyte and sample.

Keywords: toxicology, water analysis, public health.

Acknowledgements: FCT: UIDB/00709/2020; UIDP/00709/2020; SFRH BD/148753/2019.

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Presencia de Células CD161+ productoras de 17A (TH17) Actinomicetomas por *Nocardia Brasiliensis* y *Actinomadura Madurae*

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ORIGINAL ARTICLE

RESUMEN

Introducción: La hiperreactividad inflamatoria del actinomicetoma requiere de la búsqueda de las células y mediadores involucrados en esta patología, por tanto, el objetivo de este trabajo fue la identificación de las poblaciones de linfocitos CD161+ productores de IL-17 A (TH17) y de esta citocina en actinomicetomas por *Nocardia brasiliensis* y *Actinomadura madurae*.

Metodología: Se realizó el estudio de las células CD161+ y de la presencia de IL-17A en la que se utilizaron cortes histológicos de muestras de 8 pacientes con actinomicetoma, 7 por *Nocardia brasiliensis* y una muestra por *Actinomadura madurae*. Para la identificación de IL-17A se obtuvieron 9 muestras (7 de Nocardia y 2 de Actinomadura a las cuales se les efectuaron 2 cortes, uno para la tinción H-E y el otro para el marcaje con anticuerpos.

Resultados: Solo el 29% de los actinomicetomas por N. brasiliensis mostró abundante número de células productoras de IL-17A en la zona II de la lesión. Para Actinomadura se presentó un número muy abundante en ambas muestras.

Discusión: se encontró la presencia de células TH17 en los actinomicetomas, sin embargo, puede apreciarse IL-17 A en alta concentración en la zona II como respuesta inflamatoria de la lesión por lo que su presencia puede provenir de otras células.

Palabras clave: tinomicetoma, *nocardia brasiliensis*, célulars TH17, *actinomadura madurae*.

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Actividad Antiinflamatoria en Modelo Murino y Antimicrobiana en Bacterias Periodontopatógenas de Aceite Árbol del Té

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ORIGINAL ARTICLE

RESUMEN

Introducción: Las Enfermedades Periodontales (EP) pueden iniciar por disbiosis en la biopelícula de la placa dental por bacterias periodontopatógenas, provocando una respuesta inmune con proceso inflamatorio que puede destruir el periodonto[1]. Por la resistencia a los antimicrobianos, la OMS incentiva la obtención de alternativas; resurgiendo el estudio de productos naturales utilizados por la Medicina Tradicional (MT)[2]. Así, conociendo el uso antimicrobiano y antiinflamatorio que la MT y algunas investigaciones[3] atribuyen a *Melaleuca alternifolia* o Árbol del Té, cuyos datos de citotoxicidad indican un uso tópico seguro; se desarrolló un gel tópico bucal conteniendo su aceite (AAT), ya que los enjuagues comerciales por su sabor tienen poca aceptación. Este trabajo evaluó la acción antiinflamatoria in vivo del ATT en modelo murino, y comparó la actividad antimicrobiana *in vitro* del AAT y del gel desarrollado con un gel comercial de clorhexidina. Los periodontopatógenos evaluados fueron: *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Parvimonas micra* y *Prevotella intermedia*. Estas dos últimas no evaluadas antes con ATT.

Método: El uso del modelo animal cumplió con las reglas del Comité Interno para el Cuidado y Uso de Animales UAMX, la NOM-062-ZOO-1999-SAGARPA (manejo animales) y la NOM-087-SEMARNAT-SSA1-2002 (control de residuos biológicos); ambas de México. Los periodontopatógenos se obtuvieron de pacientes con consentimiento informado, cumpliendo con lineamientos bioéticos. ATT, fármacos y excipientes, reactivos y medios de cultivo, fueron de calidad farmacéutica o analítica. Para el ensayo antiinflamatorio se utilizó el modelo de Edema Auricular inducido por aceite de Croton (ricino),usando ratones proporcionado por el bioterio de la UAMX distribuyendo los grupos en 5 y (n=6) en control positivo con indometacina y diclofenaco sódico, control negativo (acetona) y dos grupos experimentales administrados con gel elaborado en UAMX, uno conteniendo 2.0% y el otro, 2.5% de ATT. Para el ensayo antimicrobiano. se realizaron tres diluciones con PBS a partir de la muestra 1:0 posteriormente se inoculo con las muestras obtenidas de las diluciones, se cultivaron por siete días en atmosfera anaerobia a 37°C , se hizo la identificación macroscópica y microscópica siendo estas Gram negativas, así como una batería de

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pruebas bioquímicas. Una vez identificadas las bacterias se procedió a la prueba de susceptibilidad antibiótica por el método de Kirby-Bauer y sensibilidad con la escala de Duraffout con dos concentraciones diferentes, 2.0% y 2.5% de ATT, se midieron los halos de inhibición (mm) y se comparó contra Clorhexidina al 2% como positivo en las cuatro bacteria.

Resultados: La prueba de edema auricular en concentración al 2% mostró un 51.21% de inhibición, de la actividad antiinflamatoria mientras que para la concentración del 2.5%, fue de 54.74%. Los estudios preclínicos en la fase de ensayos *in vivo* con animales no humanos demostró poseer una actividad antiinflamatoria. En la sensibilidad antimicobiana con la escala de *Duraffourt* para *Porphyromonas gingivalis* 93.3% *Fusobacterium nucleatum*, 97.8% *Parvimonas micra* 80% y *Prevotella intermedia* 100%.

Discusión: Acerca de la actividad antimicrobiana del gel con AAT fue muy similar al ser comparada con la clorhexidina, obteniendo resultados equivalentes a otras investigaciones, como Hans y col. en el año 2016. Se han realizado diferentes pruebas para verificar su actividad, encontrándose que el AAT es efectivo contra microorganismos patógenos en la cavidad oral. Referente a la actividad antiinflamatoria, se han realizado diferentes estudios, sin embargo, no existe similitud de los estudios ya que cada uno es diferente en cuanto a metodología y técnicas utilizadas, no obstante, concluyen que el AAT, presenta una acción antiinflamatoria al inhibir la actividad antiinflamatoria coincidiendo en los resultados de este estudio.

Conclusiones: Los resultados de este estudio *in vitro* e *in vivo*, demuestran que el Aceite de Árbol de Té, tienen una actividad antimicrobiana y antiinflamatoria, lo cual puede considerarse como una alternativa del tratamiento de la enfermedad, aunque se requiere completar con estudios toxicológicos y clínicos para comprobar su sensibilidad y posibles eventos adversos.

Palabras claves: aceite de arbol de té, antimicrobiano, antiinflamatorio.

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