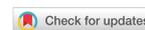


Commentary

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Environmental proteomics: a potential tool in wastewater-based epidemiology

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Abstract

Wastewater-based epidemiology (WBE) is a new complementary alternative to traditional epidemiological surveillance approaches for community health surveys. It provides objective, flexible, and comprehensive data on diseases, exposure to toxicants and pathogens, social habits, and lifestyle. Wastewater is regarded as an integrated pooled sample of the entire population served by a certain wastewater system, thus providing an average picture of its health status. WBE relies on the extraction, analysis, and subsequent interpretation of appropriate health status indicators, including both chemical and biological components, either exogenous or endogenous, altogether constituting the exposome. Instrumental development, especially mass spectrometry (MS) for the chemical analysis of small and large molecules, has been critical to the success achieved through WBE. Whereas all previous studies have been restricted to small molecules, the use of proteomics in WBE is an obvious extension of the intensive ongoing pursuit of biomarkers for community surveillance of diseases and health status. The relevance of human proteins present in wastewater as potential WBE indicators of population-prevalent diseases has been previously postulated, although no real examples to demonstrate their feasibility have been available until recently. These findings provide new insights into the characterization of wastewater proteomics useful for practical



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purposes related not only to WBE applications but also to environmental monitoring and wastewater treatment plant management.

Keywords: Environmental proteomics, wastewater-based epidemiology, sewage epidemiology, biomarkers, water fingerprinting, mass spectrometry

WASTEWATER-BASED EPIDEMIOLOGY: AN OVERVIEW

The term “Epidemiology” is a broad concept that has undergone significant changes in its definition over time, with the most widely accepted definition nowadays being “the study of the distribution and causes of health-related states or events in specified populations, and the application of this study to control health problems”^[1,2]. The recent worldwide outbreak of COVID-19 has highlighted the need for new epidemiological tools to assess population health. Factors like globalization - which is stimulated by trade and tourism- together with climate change, population growth, uncontrolled urbanization, and changes in land use contribute to the emergence or recurrence of infectious disease outbreaks, as well as to their rapid spread. Current approaches to epidemiological surveillance based on individual patient clinical monitoring data or statistical changes in rates (mortality/morbidity and drug prescribing rates), although very valuable, are insufficient as rapid alert systems. In this context, wastewater-based epidemiology (WBE)^[3], also known as sewage epidemiology^[4], has appeared as a new complementary alternative capable of providing comprehensive health and environmental information about communities. Under this approach, wastewater is considered an integrated aggregate sample of the entire population served by a certain sewage system; thus, its monitoring provides an average picture of its health status^[5,6]. Some of WBE’s well-established advantages are^[4]: (a) it provides objective, flexible, and comprehensive information (for example, surveillance of multiple aspects of health, such as disease, exposure to toxic substances and pathogens, social habits and lifestyle); (b) samples are available continuously and easily, allowing results to be obtained in (near) real-time; (c) it is scalable across the whole wastewater system and catchment area; (d) the samples are anonymous and, consequently, are not subject to specific personal and ethical consent requirements; and (e) it is cost-effective and thus economically feasible.

WBE is based on extracting, analyzing, and subsequently interpreting appropriate indicators of health status. These must be considered in their widest sense, encompassing both exogenous and endogenous chemical and biological components, making up, on the whole, the so-called exposome^[7,8]. The success of WBE can be linked to the development of analytical instrumental technologies, particularly mass spectrometry (MS) for the analysis of small and large molecules and, more recently, polymerase chain reaction (PCR) for genetic material analysis^[9]. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) with triple quadrupole (QqQ) or linear quadrupole ion trap (QTRAP) has risen as an effective analytical method for simultaneously detecting multiple biomarkers in wastewater. High-resolution mass spectrometry (HRMS), utilizing quadrupole time-of-flight (QqTOF) or Orbitrap, can often be a preferable choice for the direct identification of human biomarkers in sewage water, thus offering novel possibilities in WBE. Most studies utilizing LC-MS/MS do target analysis, where *a priori* selected analytes are monitored, thereby ensuring maximum sensitivity in the analytical determination. However, this approach may overlook the detection of other compounds present in the sample. Due to the complexity of wastewater samples potentially containing a large number of compounds, it is unlikely that the biomarkers of interest are the primary constituents. To address this analytical challenge, the so-called “suspected screening” approach has been developed. This requires constructing a database of reported compounds in the literature or other databases to assist in identifying relevant biomarkers^[10-12]. *In silico* tools, including the prediction of MS fragmentation, retention times, metabolism, biodegradation pathways, or transformation products, have proven effective in biomarker identification from raw MS data^[11,13]. Processing of wastewater

MS data profiles (fingerprinting) has been used as well for such purposes. However, this methodology demands the intensive application of bioinformatics and chemometric tools to support filtering and extraction of acquired MS data, cluster classification, which organizes in different groups ionic signals sharing common characteristics, estimates ionic signal intensity in space, and applies advanced multivariate statistical methods to develop predictive models.

Some successful applications of WBE deserving mention include the consumption of illegal drugs^[14,15], pharmaceuticals and personal care products^[16,17], tobacco^[18] and alcohol use^[19], the exposure to toxicants like pesticides^[20], and Bisphenol A^[21], and concerning biological response, oxidative stress^[22] or the monitoring of coronavirus prevalence during the recent COVID-19 outbreak using reverse transcription-PCR (RT-PCR) and other related methods^[23,24].

ENVIRONMENTAL PROTEOMICS IN WBE

While current WBE studies primarily focus on small molecules such as xenobiotics and their metabolites and transformation products, the potential for using proteomics in WBE is gaining interest as a means of identifying biomarkers for community biomonitoring of diseases and overall health status. Rice and Kasprzyk-Hordén^[6] previously suggested that human proteins found in sewage water could serve as indicators of prevailing diseases affecting a population. This approach emerged as a new concept in the assessment of community health, even though no experimental evidence was yet available to support its feasibility as a practical WBE tool, hence showing that the road from theory to reality is not straightforward. Although the use of large molecules in WBE has so far been scarce, recent studies^[25,26] have shown that analysis of influent and effluent from wastewater treatment plants (WWTPs) can provide valuable information about the large molecules present. As far as we know, these are the earliest non-target proteomics studies dealing with the analysis and identification of a range of peptides and proteins in the various steps of the WWTP process. Wastewater proteomics is thus a complex field that is still in its early stages of development, as it deals with the analysis of samples containing diverse molecules from various sources. Therefore, the amount of available information in this area is nowadays fairly limited, though more work is ongoing.

It is worth mentioning that the field of wastewater proteomics is in many cases capable of distinguishing human proteins from those of animal, plant, or microbial origin that are also present in these waters. Instead of being seen as a source of interference, these non-human proteomes provide valuable insights into the human environment^[25]. This initial discovery paved the way for the identification of human protein biomarkers in wastewater, which in conjunction with other biomarkers found in sewage water, may serve as indicators of the different anthropogenic activities occurring in the surroundings (i.e., domestic, agricultural, and industrial), pest control, as well as the population health status as assessed through WBE.

From the experimental point of view, protein analysis in wastewater involves certain preparative steps such as cleaning, concentration, and digestion. This is usually done by gel electrophoresis. Prior to the analysis by LC-HRMS, the bands with the concentrated proteins are excised and digested with trypsin^[27] to liberate the constituent peptides. MS methods based on LC-Orbitrap-MS and matrix-assisted laser desorption/ionization (MALDI)-TOF may allow for fast analysis and regular peptide monitoring, and related protein identification^[25,26]. Owing to the large amount of MS data acquired, as in the case of small molecules, the use of bioinformatics tools to process the complex omics pattern becomes crucial^[26].

Up to now, studies on sewage proteomics have predominantly focused on characterizing the bacterial microbiome in either sludge^[28] or wastewater^[29], which includes extracellular proteins^[30]. However, the

aforementioned recent studies^[25,26] have expanded upon this work. By utilizing passive sampling polymeric devices and LC-HRMS shotgun proteomic methods, these researchers have been able to profile the wastewater proteome beyond prokaryotes and include eukaryotic higher organisms, such as plants, animals, and human proteomes. In terms of higher organisms, these studies have identified not only major constituents, such as albumins and keratins, but also less abundant biomarkers of disease, including S100A8, uromodulin, and defensins, proposed as health markers associated with renal function and stress-related changes^[31]. This groundbreaking work represents the first attempt to identify the entire wastewater proteome, yet it also highlights the experimental and analytical challenges involved in its characterization, such as the need to overtake the predominance of bacterial proteins in comparison with those of other organisms.

Finally, it is worth noting that the feasibility of detecting disease-related viral structural proteins in wastewater, particularly in the case of SARS-CoV-2, has been discussed in the literature^[9]. However, it is important to point out that although MS has been utilized to identify viral proteins and peptides in clinical studies, its usage on WBE is not yet accomplished. In the case of SARS-CoV-2, the virion particle contains four structural proteins: spike, membrane, envelope, and nucleocapsid (referred to as S, M, E, and N, respectively). Some of these proteins have already been identified by MS in clinical samples and may also be present in wastewater. Therefore, the possibility of detecting them in wastewater should not be dismissed, so that they can be used as WBE biomarkers^[9]. At present, the foremost limitations to its practical application are the complexity of the sewage samples to be analyzed, together with the insufficient sensitivity of the currently available MS instruments.

FINAL REMARKS

In this commentary, we have outlined the recent progress of WBE application to large molecules. Notably, there are few studies currently available that refer to proteins and peptides as exposure biomarkers^[25,26]. However, their recent findings provide new insights into the characterization of wastewater proteomics that allow proposing specific bioindicators useful for practical purposes related not only to WBE surveys, but also to environmental monitoring, pest control, and wastewater treatment plant management.

Although significant progress has been made in studying the wastewater proteome, it should be noted that there is still much to discover and understand about the dynamics, fate, and emission rates of proteins in wastewater. Altogether, this highlights the need for continued research to fully grasp the potential of these biological molecules as WBE and environmental biomarkers.

Suggestions were made in the literature for future surveillance of viral proteins such as SARS-CoV-2 in WBE^[9], integrating previous experience gained in the use of LC-MS in biomedical studies to identify viral proteins and peptides. While PCR methodologies currently dominate in this area, MS detection may provide more detailed data concerning virus-specific peptides. In that sense, we believe that WBE MS could be in the future a convenient analytical methodology to complement PCR. However, for now, further improvements in MS sensitivity are needed to make it competitive with PCR tools.

DECLARATIONS

Authors' contributions

Made substantial contributions to the conception, writing, review, and editing: Ginebreda A, Barceló D, Picó Y, Carrascal M, Abián J

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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