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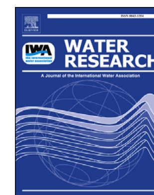

Detection of oncogenic viruses in water environments by a Luminex-based multiplex platform for high throughput screening of infectious agents

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A B S T R A C T

Recent studies documented the detection of viruses strongly associated with human cancer in urban sewages and other water environments worldwide. The aim of this study was to estimate the occurrence of human oncogenic viruses in environmental samples (sewage, river, marine, and pool/spa water) using highly sensitive and specific multiplex bead-based assays (Luminex technology). A total of 33 samples were analysed for 140 oncogenic viral agents, including mucosal and cutaneous human papillomaviruses (HPVs), human polyomaviruses (HPyV), human herpesviruses (HHV) and mouse mammary tumour virus (MMTV).

Eighty-eight percent of the samples tested positive for at least one viral pathogen and the simultaneous presence of more than one virus was frequent (mean number of positivities/sample = 3.03). A total of 30 different Alpha, Beta and Gamma HPVs were detected, including mucosal and cutaneous types. The high-risk type HPV16 was the most frequently detected virus, identified in 73% of the samples. Of the 12 HPyVs tested, only two (BKPyV and MCPyV) were detected. At least one of these two was present in 48% of the samples. MMTV was detected in 21% of the samples, while herpesviruses - HHV-6 and HHV-1 - were detected in two samples (6%).

The present study is the first to provide a comprehensive picture of the occurrence of oncogenic viruses belonging to different families and species in diverse water environments, and the first to successfully use, in environmental samples, a Luminex-based multiplex platform for high throughput screening of infectious agents. Our findings, showing that oncogenic viruses are ubiquitous in water environments, pave the way for future studies on the fate of these pathogens in water environments as well as on their potential for transmission via the waterborne route.

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1. Introduction

The International Agency for Research on Cancer (IARC) estimates that 15–20% of cancers are associated with infectious agents (Stewart and Wild, 2014; Torre et al., 2016). Several DNA and RNA viruses belonging to various viral families, are associated with cancer in humans (McLaughlin-Drubin and Munger 2008; De Flora and Bonanni, 2011). To date, seven viruses have been classified by IARC as "carcinogenic to humans" (group 1): hepatitis B virus (HBV), hepatitis C virus (HCV), Epstein-Barr virus (EBV), Kaposi's sarcoma herpes virus (KSHV), also known as human herpesvirus 8 (HHV-8), human immunodeficiency virus type-1 (HIV-1), human T cell lymphotrophic virus type-1 (HTLV-1), and human papillomavirus (several genotypes).

In the present study, we investigated the occurrence of a panel of cancer-related viral infectious agents in water samples, including mucosal and cutaneous human papillomaviruses (HPVs), human polyomaviruses (HPyVs), herpesviruses (HHVs) and mouse mammary tumour virus (MMTV).

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Human papillomaviruses represent a large group of epitheliotropic viruses, associated with various genital, oral, and cutaneous conditions, both benign and malignant (Bernard et al., 2010). They are divided into 5 genera: Alphapapillomavirus (species Alpha 1–14), Betapapillomavirus (Beta 1–6), Gammapapillomavirus (Gamma 1–20), Mupapillomavirus (species 1 and 2) and Nupapillomavirus (species 1) (http://ictvonline.org/index.asp). The IARC Working Group on the Evaluation of Carcinogenic Risks to Humans has classified some Alpha, Beta, and Gamma HPVs into carcinogenicity Groups 1, 2A, 2B and 3 (De Flora and Bonanni, 2011; IARC, 2013).

Polyomaviruses are small, non-enveloped viruses known to infect different tissues and organs, usually causing subclinical infections in immunocompetent individuals, and serious diseases in immunocompromised hosts (Dalianis and Hirsch, 2013). Thirteen species have been reported to infect humans: BK virus (BKPyV), JC virus (JCPyV), KI virus (KIPyV), WU virus (WUPyV), Merkel cell polyomavirus (MCPyV), human polyomavirus-6 and 7 (HPyV6 and HPyV7), trichodysplasia spinulosa-associated PyV, HPyV9, HPyV10, Saint Louis polyomavirus (STLPyV), and HPyV12. In addition, a non-human, primate PyV — simian virus 40 (SV40) — seems to circulate in human populations as well (Moens et al., 2014). Among Polyomaviruses, MCPyV is classified by IARC as "probably carcinogenic to humans" (Group 2A) (Bouvard et al., 2012); other potentially carcinogenic polyomaviruses are JCPyV and BKPyV, both classified as Group 2B ("possibly carcinogenic to humans"). BKPyVs have been detected in many tumour specimens, while neurotropic JCPyV, the aetiologic agent of progressive multifocal leukoencephalopathy (PML), has been associated with brain tumours (Berger et al., 1998). The aetiological role of both viruses in human cancer remains controversial, however (Dalianis and Hirsch, 2013).

Human herpesviruses have the ability to establish lifelong latency in the host, and to cause a wide spectrum of diseases leading to severe complications in immunocompromised patients. The herpes family of viruses includes eight different viruses that affect human beings: Herpes simplex virus 1 (HSV-1), Herpes simplex virus 2 (HSV-2), Varicella zoster virus (VZV), Cytomegalovirus (CMV), Human herpes virus 6 (HHV-6), Human herpes virus 7 (HHV-7), Epstein-Barr virus (EBV) and Human herpes virus 8 (HHV-8). Some Herpesviruses cause human cancer: HHV-8 is linked to Kaposi's sarcoma and EBV, to Burkitt's lymphoma (De Paoli and Carbone, 2016). Human herpes virus 6 has been detected in lymphomas, leukemias, cervical cancers and brain tumours, but evidence on its involvement in cancer induction is still elusive, because viral DNA has also been identified in many other non-pathological tissues (Kofman et al., 2011).

Mouse mammary tumour virus is the aetiological agent of mammary cancer in mice. Yet, over the past 15 years, a number of studies have suggested it may play a role in human cancer as well (Wang et al., 1995). The papers in question reported that DNA sequences displaying 90–95% identity with MMTV have been found in almost 40% of human breast cancers (Joshi and Buehring, 2012).

In recent years, HPVs and HPyVs have been detected in urban sewages and other water environments worldwide, and have been proposed as potentially emerging waterborne pathogens (Bofill-Mas, 2016; Di Bonito et al., 2015; Fratini et al., 2013; Iaconelli et al., 2015a; La Rosa et al., 2013; La Rosa, 2016; Reynolds, 2012). Herpesviruses (along with HPVs) have also been identified in sewage sludge as well as in river waters by metagenome analysis (Bibby and Peccia, 2013; Dann et al., 2016).

The aim of this study was to estimate the prevalence of human oncogenic viruses - HPVs, HPyVs, HHVs, and MMTV - in water samples (raw sewage, river, marine, and pool/spa waters), using a Luminex-based multiplex platform for high throughput screening of infectious agents.

2. Materials and methods

2.1. Water samples

Water samples belong to a repository of environmental samples collected in Italy during 2011–2015, and previously analysed for enteric viruses in the framework of different projects. A total of 33 samples were tested: 10 raw sewage samples, 11 river samples, five pool/spa water samples, and seven seawater samples. Sewage samples were collected, handled and analysed as previously described (La Rosa et al., 2014). Briefly, 20 ml of untreated wastewater samples was treated with two ml of 2.5 M glycine pH 9.5 and incubated in ice for 30 min. The solution was then treated with 2.2 ml chloroform and centrifuged at 5000 rpm for 10 min. The supernatant was used for extraction.

River and spa water samples (10 L each) were filtered by adsorption to and elution from 1MDS electropositive filters (Cuno Meriden CT, USA), as previously described (Iaconelli et al., 2015a, b; La Rosa et al., 2015). Viruses were eluted with 70 ml of 1.5% beef extract with 0.05 M glycine (pH 9.5), recirculated through the filters for 20 min. Eluates were then neutralised to pH 7 ± 0.1 and stored at −20 °C for future use. Marine waters were concentrated by the adsorption/elution method, with the use of negatively-charged microporous filters (Cellulose Nitrate Membrane Filters, Sartorius), rather than electropositive filters.

2.2. DNA extraction

Viral nucleic acids were extracted from 10 ml of samples, using the NucliSENS easyMAG (BioMerieux, Marcy l'Etoile, France) semi-automated extraction system with magnetic silica, according to the manufacturer's instructions. Eluates (100 µl each) were divided into small aliquots and subsequently frozen at −80 °C.

2.3. Viral DNA detection

The identification of 140 infectious agents (see Table 1) was performed using type-specific multiplex genotyping assays, which combine multiplex polymerase chain reaction (PCR) and bead-based Luminex technology (Luminex Corp., Austin, TX, USA). The Luminex assays used here for HPVs, HPyVs, and HHVs have been previously described (Corbex et al., 2014; Schmitt et al., 2006, 2010). The Luminex assay for the detection of MMTV-like sequences in human specimens is based on a multiplex PCR that targets the proviral DNA intermediate. Specific primers that amplify two distinct sequences (132 bp and 160 bp) of the MMTV–like envelope protein gene have been designed in regions that are not homologous to known human endogenous retrovirus (Nartey et al., 2017).

The PCR products were generated, denatured, and hybridised to the bead-coupled probes in 96-well plates. After transfer of the products into wash plates with filter bottoms, the unhybridised DNA was removed. Subsequently, the biotinylated PCR products were stained with a streptavidin-R-phycoerythrin conjugate. After further washing steps, the beads were analysed in the Luminex reader, which contains two lasers to identify the bead set by the internal bead colour and to quantify the reporter fluorescence on the bead. The results are expressed as the median fluorescence intensity (MFI) of at least 100 beads per bead set. MFI values reflect a semiquantitative measure of the number of copies of target DNA in the sample. For each probe, the cutoff for positivity was computed as described previously (Schmitt et al., 2010).

Negative controls were systematically included in all the assays: (i) several tubes containing water were blindly processed along with the other samples for the extraction of DNA and the Luminex

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