ORIGINAL ARTICLE

Virological investigation on aerosol from waste depuration plants

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Key words

Aerosol • Virus • Depuration plants

Summary

Aerosol from activated mud decontamination plants used for the treatment of urban sewage can represent a vehicle for bacteria, virus and fungi. As a result, they become an infective hazard for plant personnel, the general population residing in the surrounding area and the occasional visitor. The present investigation focuses on the identification of enteric-type viruses in this kind of aerosol. The following methods were employed on 214 samples collected in the 1999-2000 period: cell culture (BGM, RD, Hep-2), electron microscopy, and polymerase chain reaction (PCR). Cytopathic effect was mild in 180 samples, and severe in 14, upon their first passage in culture. Virus identification was based on positivity to both electron microscopy (EM) and PCR. Thus, one positive sample was recognized to be of enteric-type virus and two positive samples were recognized as reovirus-type. All samples were negative for Norwalk-type virus or HAV. There was considerable discrepancy between electron microscopy and PCR concerning the number of enteric-type viruses recognized. A possible explanation is contamination with animal-type enterovirus.

Introduction

Active-mud plants used for urban waste depuration lead to public health concern associated with the environmental diffusion of contaminants. Aerobic treatment of the clarified waste produces aerosol, as a result of the aeration process. The aerosol particles can represent a vehicle for the bacteria, viruses, and fungi, commonly found in urban waste. The personnel at the depuration plant, the population in the surrounding area, and occasional visitors are all possible targets of infectious disease transmission. Virus identification in environmental sources, such as urban waste, is of utmost importance for the evaluation of viral hazard and epidemiological studies. Identification of viral particles in waste allows the circulation of distinct viral types in the environment to be monitored, and hence in the resident population, as well as the possibility to detect mutant or recombinant strains of vaccine virus of unpredictable behaviour. These procedures, however, are hampered by the high dilution of samples, small size of viral particles, species variability causing different biological characteristics, and interference by chemicals.
The human virus types mainly present in waste belong to the enteric transmission line. More than 140 such types have been identified in the faeces of patients or carriers, and include enterovirus, rotavirus, enteric adenovirus 40 and 41, hepatitis A and E virus, norovirus, calicivirus, and astrovirus [8]. Recognition is commonly based on the cytopathic effect on cell culture, a method that does not allow reliable identification of the isolate since several species can grow, often simultaneously, in cultures from environmental samples [1]. Confirmation using other methods then become essential for virus identification.
Aim of the present work was to identify virus types in aerosol particles from waste depuration plants by means of isolation in cell culture, electron microscopy (EM), and reverse transcriptase-polymerase chain reaction (RT-PCR).

Materials and methods

Aerosol samples were taken, in the period 1999-2000, from four activaded sluge decontamination plants serving different urban areas of Italy. A total of 214 samples were examined. The cytopathic effect was mild in 180 samples, and severe in 14, which were further investigated.
Samples were collected from the locations attended by the personnel and at a height of 160 cm from the ground, thus corresponding to the upper respiratory tract level. Impact samplers were used because they act as vehicles to known air volumes onto tryptcase soy agar (TSA) rodac plates.
Cell Culture: After chloroform decontamination, the samples were inoculated on Buffalo Green Monkey (BGM) cell monolayers in 25 cm^2 flasks. Cultures were checked daily for cytopathic effect. These same samples were inoculated in shell vials with three cell lines, BGM, Rhabdomyosarcoma (RD), Human Epidermoid Larynx (Hep-2) to allow amplification of the viral particle load.
Electron Microscopy: Cell culture were placed in test tubes and maintained at 4 °C for 3 hours to allow sedi-

mentation of the supernatant. Drops of the culture fluid, sampled at 2/3 from the bottom of the tube, were placed on 200-mesh grids covered with formwar coal; a drop of 2% phosphotungstic acid (pH 6.6) was added for 5 min. Grids were observed with a Philips M10 electron microscope [7].

RT-PCR: Viral nucleic acid was extracted from the cell lysate with proteinase K-phenol-chloroform. In the RT-PCR technique used to detect enterovirus gene expression specific primers were employed for amplification of a fragment of untranslated region at 5' [3]. Seminested-PCR for cDNA was adopted to enhance sensitivity and specificity of the amplification reaction. Reovirus and rotavirus gene expression was investigated with specific primers for amplification of a preserved 538 bp portion of the Sygma2 (S2) gene [2, 3].

Two different RT-PCR protocols were adopted to identify Norwalk-type virus genome sequences. One protocol employed primers differentiating genotypes 1 and 2. The other employed general primers detecting Norwalk-like virus with 85% confidence [11]. Primers detecting different viral subtypes were used to detect HAV virus since they link to highly preserved regions of the viral genome.

Results

Mild slow-developing cytopathic effects were seen. In general, these were detected at the first passage. However, in one sample, these were seen at the second passage, and in another at the third passage (N/99). Viral isolates with the BGM, Hep-2, and RD cell lines exhibited some differences. One sample (I/00) failed to produce a cytopathic effect on RD; another sample (N/99) showed effects only on BGM (Tab. I). Shell vial inoculation reduced the delay in the cytopathic effect from the 6-8 days, needed in flasks, to 3-4 days. Nonetheless, in one sample, effects were evident after only 3 days in flasks and after one day in shell vials.

Electron microscopy demonstrated enterovirus-like particles in 11/14 specimens, the morphological preservation of which, 28-30 nm diameter, and icosahedral shape, suggested Picornaviridae. Four samples (A/99, F/99, L/00, M/00) showed particles of shape and size (65-75 nm) compatible with Reoviridae (Fig. 1), while two samples (A/99, L/00) showed simultaneously enterovirus and reovirus (Fig. 2). All samples were negative for Norwalk-like and HAV virus.

PCR confirmed positivity for enterovirus in only one sample (A/99), and for reovirus in two other samples (L/00, M/00). One sample was negative both with the EM and RT-PCR techniques. All samples were negative for Norwalk-like and HAV virus.

Results are outlined in Table II.

Fig. 1. Reovirus-like particle.

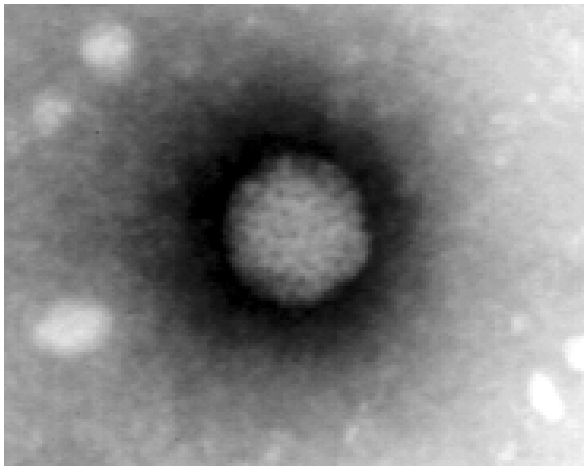
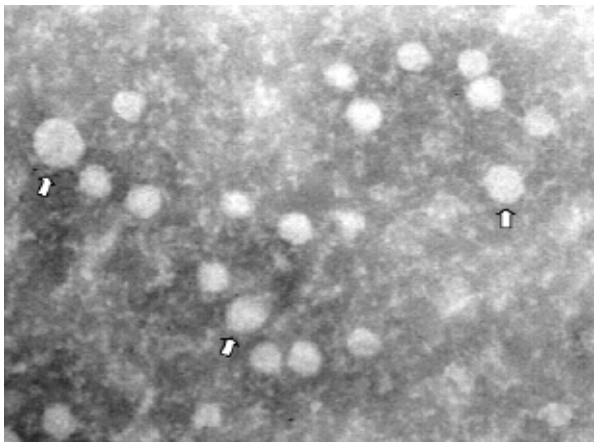


Fig. 2. Enterovirus-like particles and reovirus-like particles (arrow).



Tab. I. Viral isolation of samples in the tree cell lines.

Sample	Cell Lines		
	BGM	HEP-2	RD
A/99	+	+	+
B/99	+	+	+
C/99	+	+	+
D/99	+	+	+
E/99	+	+	+
F/99	+	+	+
G/99	+	+	+
H/99	+	+	+
I/00	+	+	-
L/00	+	+	+
M/00	+	+	+
N/99	+	-	-
I/00	+	+	-
L/00	+	+	+

Tab. II. Results obtained by the various methods.

Sample	Enterovirus		Reoviridae		HAV		Norwalk-like		Identification
	M.E	RT-PCR	M.E	RT-PCR	M.E	RT-PCR	M.E	RT-PCR	
A/99	+	+	+	-	-	-	-	-	Enterovirus, reoviridae
B/99	+	-	-	-	-	-	-	-	Enterovirus-like
C/99	-	-	-	-	-	-	-	-	Unidentified cytologic
D/99	+	-	-	-	-	-	-	-	Enterovirus-like
E/99	+	-	-	-	-	-	-	-	Enterovirus-like
F/99	-	-	+	-	-	-	-	-	Reoviridae
G/99	+	-	-	-	-	-	-	-	Enterovirus-like
H/99	+	-	-	-	-	-	-	-	Enterovirus-like
I/00	+	-	-	-	-	-	-	-	Enterovirus-like
L/00	+	-	+	+	-	-	-	-	Enterovirus, reoviridae
M/00	-	-	+	+	-	-	-	-	Reovirus
N/99	+	-	-	-	-	-	-	-	Enterovirus-like
O/99	+	-	-	-	-	-	-	-	Enterovirus-like
P/00	+	-	-	-	-	-	-	-	Enterovirus-like

Discussion and conclusions

Even if all the samples examined showed cytopathic effects on cell culture, this was not sufficient to characterize isolates or define the virus types in aerosol. As a result, identification was, therefore, based on the more stringent criteria of consensual of EM and RT-PCR findings, and was achieved in only three instances with enterovirus (A/99) and reovirus (L/00, M/00).

The frequent finding of enterovirus-like particles with EM, without RT-PCR confirmation, suggests the possibilty of an animal-type enterovirus and not human. In fact, the primers routinely used for environmental enterovirus detection with RT-PCR are often targeted at highly preserved sequences of the untranslated 5'-UTR region of the usual human enterovirus types. Even if universal primers, with group specificity, are employed, the highly specific nested PCR adopted, in this study, could miss animal-type enterovirus. Environmental samples comprise a very heterogeneous mixture of human- and animal-type virus, the isolation and identification of which, on a single cell culture, are laborious [4]. This is due to virus circulation in the population and to the steady shedding of vaccinal poliovirus in the faeces. Furthermore, animal-type enterovirus, although not representing a direct hazard for human health, could exhibit the capacity of host adaptation in the event of new viral species generation. This capacity, shown on cell culture, represents a risk of unknown potential for human health.

These results stress the importance of shell vial culture for environmental samples, a technique that capable of amplifying viral particle load. In our opinion, electron microscopy, a technique which reveals the morphology and all particles in a given sample [7], represents a useful tool in the identification of cell pathogens of difficult characterization. Albeit, findings must be supported by culture and molecular biology data.

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■ Received on February 22, 2005. Accepted on November 24, 2005.

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