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Infectious virus isolation in Pusan, 1998

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1. Introduction

Viruses cause a variety of infections including in men, animals and plants. Enteroviruses and respiratory viruses are the predominat agents in men. Enteroviruses multiply throughout the alimentary tract and stable in acid, they spread throught the body via the blood stream and most infections occur during childhood whereas respiratory viruses showing their adaptation to the nasopharynx and infect all ages.

Outbreak of viral disease is ensuing throughout the world. Control of diseases caused by these viruses has not yet been fully established. Isolation and identification of virus play an important role in diagnosis and surveillance of disease.

This report includes part of results to monitor the occurrence of viral infection in Pusan, 1998.

2. Materials and Methods

2.1. Collection of specimens

Specimens from patient were collected from hospitals in Pusan.

2.2 Treatment of specimens

10% stool samples in were obtained by suspending in 10㎖ complete PBS suspension. Vortexing and centrifugation (3,000 rpm, 20 min) is followed. Bring the supernatant into a new tube and add 1/10 volume of chloroform and mixed well. This mixture was centrifuged (3,000rpm, 20 min) and 6-7㎖ were harvested and used for further cell culture and the remainings were stored at -70℃. Sample dilution of 1X, 10X, 100X were made for inoculation with 1% FBS-DMEM. Throat swab cultures were taken in transport tubes and squeezed. Antibiotics(penicillin 5 units/㎖, streptomysin 5 ㎍/㎖, nystatin 1,000units/㎖) were added and left at 4℃ for 1 hr. Supernatant was collected after centrifugation. One tube of the supernatant was inoculated into a cell culture whereas the other was stored at -70℃.

2.3 Propagation and Assay in cell culture

The RD cell line derived from a human rhabdo sarcoma, HEp-2 from human epidermoid carcinoma, and Vero cells from monkey kidney, MDCK cells from canine kidney were cultured in 24 well plate with EMEM or DMEM medium and used for cytopathic effect(CPE). All cells were obtained from National Institute of Health.

Incubation at 34℃ in 5-7% CO_2 incubator were performed for at least 5-10 days and checked for CPE for daily.

2.4 Electron Microscopy

Viruses which showed positive reaction on CPE after serial culture were examined under electron microscope after negative staining with 4% uranyl acetate.

2.5. Identification

Isolated enteroviruses serotypes were identified by the neutralization antibody test. Influenza viruses were identified by Indirect fluorescent antibody test using FITC-conjugated anti-mouse immunogloblin. All these were done by National instituite of Health.

3. Results and Discussions

Viruses were isolated from January to December in 1998 in Pusan. Enteroviruses were found in adult as well as sick children. Influenza viruses were isolated from both children and adults.

4 cases of echovirus 6 serotype, 2 cases of echovirus 25 serotype, 1 case of echovirus 30 serotype, 3 cases of coxsackievirus B2, 8 cases of coxsackievirus B3, 2 cases of coxsackievirus B4, 1 case of coxsackievirus B6, and 8 cases of calicivirus were isolated.

Two types of viruses influenza A/Sydney/05/97-like(H3N2) and Influenza A/Beijing/262/95-like(H1N1) were identified. Adenovirus was also confirmed.

References

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