Laboratory Test Findings of the First Indigenous *Vibrio cholerae* O139 Case in Taiwan

Abstract

From the human specimens of the patient, *Vibrio cholerae* O139 was isolated. This was the first new O139 type ever isolated in Taiwan in the last 30 years since the O1 outbreak in 1962 and the control of cholera in 1965. Drug resistance of the pathogens was also tested at the same time. These testing procedures should help in the early identification of infection sources and also treatment of patients. The fact that O139 was promptly and correctly isolated indicates the improvement in the disease reporting system, the disease control system and the laboratory technology in preventing the spread of the infection. It also suggests the importance of developing SOP for laboratory procedures and also the advancement in technology and facilities. Furthermore, the public should be educated about the prevention and control of the high-risk notifiable diseases such as cholera.

Introduction

By serotypes, *Vibrio cholerae* can be classified into some 150 types. They are named O1, 02 and so on by the 0 surface antigens. In the past, it was believed that only the toxigenic O1 types (including Ogawa, Inaba and Hikojima subtypes) could produce serious clinical symptoms and lead to pandemics. Therefore, only the toxigenic O1 type cholera was made notifiable. However, in the period between October 1992 and January 1994 in India and Bangladesh, several outbreaks of non-O 1 cholera were reported. The clinical symptoms of this infection were as serious as those of the O1 types, and the number of individuals infected had exceeded 150,000. As the clinical symptoms of the infection were similar to those of the O1 types and yet the *Vibrio cholerae* isolated from the infection was not one of the 138 already identified types, it was named O139. Since the first strain was

isolated from the coastal areas of Bengal, the new pathogen was then named *Vibrio cholerae* O139 Bengal. WHO subsequently in 1993 informed its member states that this toxigenic non-O1 *Vibrio cholerae* (O139) was as dangerous as the notifiable O1 cholera and therefore should be handled accordingly with care. The new strain soon spread to other countries. Even in USA, Japan and Hong Kong, imported cases of O139 had been reported. *Vibrio cholerae* O139 is a new toxigenic non-O1 type (very few non-toxigenic *Vibrio cholerae* O139 have been isolated thus far), it is little known to the public and more education of the public as to its danger and measures of prevention should be conducted [1-4].

At 11 am of 27 August 1997, the Southern Branch Laboratory of the National Institute of Preventive Medicine, DOH, received two specimens of ¡§suspected enteritis¡¨ sent by the Kaohsiung Veterans¡¦ General Hospital through the Kaohsiung City Health Department. The two specimens were collected from the patient and his wife, the contact without symptoms. It was learned later that the case was a 71-year male of poor physical conditions, with two-thirds of stomach gastretomized earlier. The patient though did not suffer from abdominal pain, had watery diarrhea for more than 30 times a day. Baktar (trimethoprim-sulfamethoxazole, T/S) had been given to the patient before specimen collection.

Materials and Method

1. Materials
   1) selective agars: TCBS (thiosulfate-citrate-bile-sucrose agar)
      PMT (polymyxin mannose tellurate agar)
      DHL (desoxycholate-hydrogen sulfide-lactoseagar)
      SS (salmonella-shigella agar)
      ANA (alkaline nutrient agar)
   2) antisera: O1; poly, Ogawa, Inaba, Hikojima, and O139 antisera
   3) biochemical kits: API 20E, Bio-test No.1
   4) toxigenicity testings:
      (1) phenotype: RPLA (reverse passive latex agglutination)
      (2) genotype: DNA probe and PCR (polymerase chain reaction)
   5) strains: suspected strains collected after the selective cultures; standard O139 strains and standard O1 non-toxigenic strains were used as controls.
   6) drug sensitivity tests: drugs used for testings are listed in Table 1.

2. Method: the SOP for the Testing of Specimens for Disease Control of the National Institute of Preventive Medicine was modified to shorten testing time; the procedures are shown in the flow chart of Figure 1

Results and Discussion

1. That the results could come out in such a short period of time, preliminary identification of O139 in 15 hours, confirmation in 24 hours and finalization in 30 hours, was due to: 1) the earlier administration of drugs could have simplified the colonies of the specimens; 2) adequate planning and use of the SOP and work items. The time table of the process is shown in Figure 2. Testings of all specimens were completed in one week (Table 2). When the environmental specimens collected from the patient's house were found positive, more specimens were collected and the environment disinfected. It was not until the contamination of the drainage was proved negative and the contacts and environments were tested negative that the investigation was called off.

2. Seedswab specimens were smeared for culture directly on selective agars (Figure 1). The colonies were very simple and grew rather slowly. It was suspected that drug resistance had resulted from the administration of drug prior to the specimen collection. The colonies were therefore simple as protoplast, and the growth was slow. Drug sensitivity was tested at the same time (Table 1). Baktar (trimethoprim-sulfamethoxazole) administered to the patient is drug- resistant. It could have inhibited the growth of other bacteria to result in a "pure culture" environment for the O139 strains. Multiplication by peptone water gave similar findings as the seedswab specimens, all indicating to Baktar-resistant *Vibrio cholerae* O139.

3. It is generally believed [4-6] that though *Vibrio cholerae* does not agglutinate with antisera of either single or multiple strains of O1 group, its biochemical specifications are similar to those of the El Tor type. This O139 strain was proved through PCR in the laboratory to be the El Tor type. The finding corresponds to the general reports that all *Vibrio cholerae* since 1960 are of the El Tor type. Their fatality factors such as CT (cholera enterotoxin), CF (colonization factor), TCP (toxin-coregulated pilus) and TOXR (regulatory element) are similar to those of the O1 types but not to the non-O1 types. The CT factor directly associated with enterotoxins is composed of A and B subunits. Subunit A is related to toxicity intensity; subunit B to the mechanism of watery diarrhea by connecting to the Gml receptors of the small intestine mucosal cells. DNA hybridization and PCR [7] were performed on the toxigenic genes to prove the existence of toxigenic genes. RPLA was conducted further to confirm the expression of toxigenic genes. Detection of genes is useful to promptly find out the existence of genes. RPLA though time-consuming, can prevent incidents of false-positive reaction.

4. Many reports have mentioned that most *Vibrio cholerae* O139 is resistant to T/S (Baktar) [4, 8]. The strains in the present case showed the same resistance Some

reports suggested that this drug-resistance was associated with the transposon of the *stx* series. The preliminary isolation of plasmid in the present case did not indicate the existence of plasmid in the strains. Further studies are needed to understand the mechanism of drug-resistance. It is likely that the transposon containing stx series is already in (insertion?) the chromosomes of the strains.

5. Selenite samples were also used for multiplication. They were cultured on DHL and SS agars for observation. No suspicious colonies were detected. The possibility of cross-infection by drug-resistant strains such as Salmonella and Shigella was removed.

Conclusion

In the present case, *Vibrio cholerae* O139 was identified from the specimens only after the patient was given some medicine. This practice, though not advisable, in a way helped simplify the testing procedures. Health authorities, however, are urged to collect specimens before any medication to avoid any incorrect findings of the testings. This case for instance, if some effective medicines had been administered in advance, the *Vibrio cholerae* in question could have not been detected, and the chances of detecting cross infection by some gastro-intestinal pathogens would have been very small.

Acknowledgement

Thanks are due to all agencies concerned for their cooperation and support in this investigation. Particular thanks are due to the Central and Eastern Branch Laboratories of the National Institute of Preventive Medicine for their laboratory testings of contacts; to Prof KH Lin of the Kaohsiung Medical College for the use of their facilities; to Prof HL Wu and the Kaohsiung Quarantine Station I for their reagents; and to Dr YC Chuang, Director of the Infection Department, National Chengkung University Hospital, for his consultation on the clinical symptoms and treatment of *Vibrio cholerae* O139 infection.

Prepared by: SY Hsu, LL Kuo, MM Hsu, CC Chen, SC Shih, KJ Wang, CB Horng
(National Institute of Preventive Medicine, DOH)

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Table 1. Drug Sensitivity Tests

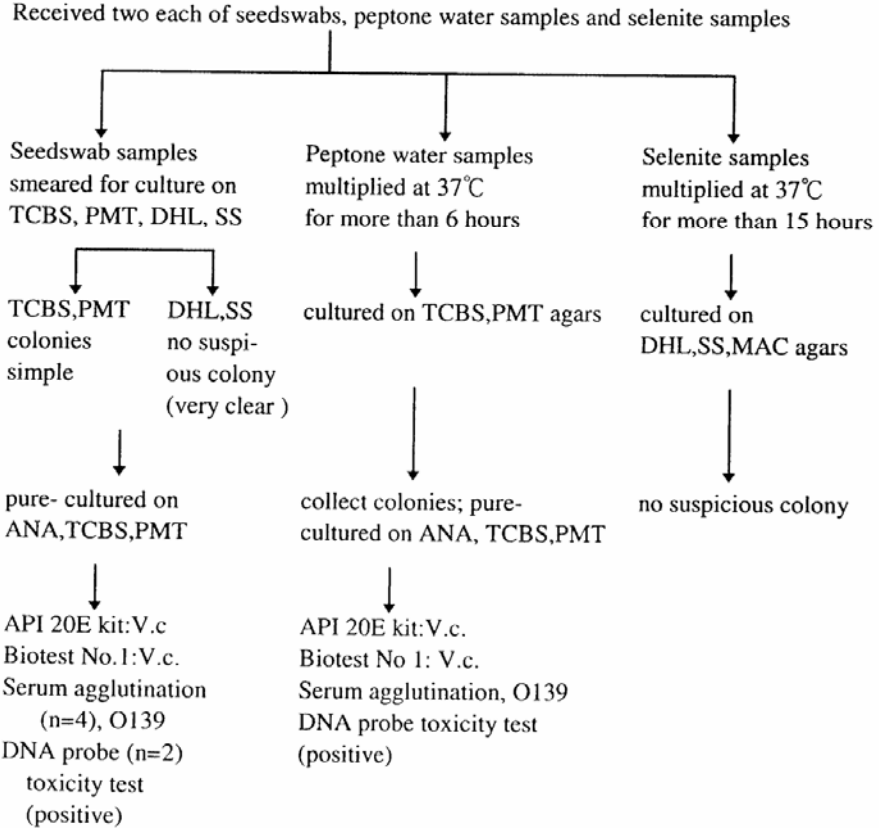
Antibiotics (μg)	Results
Ampicillin 10 (AMP 10)	S
Tetracycline 30 (TE 30)	S
Chloramphenicol 30 (C 30)	S
Gentamicin 10 (CN 10)	S
Nalidixic acid 30 (NA 30)	S
Ciprofloxacin 5 (CIP 5)	S
Cephalexin 30 (CL 30)	S
Cefaclor 30 (CFC 30)	S
Cefotaxime 30 (CTX 30)	S
Trimethoprim-Sulfamethoxazole 0.0002-32(T/S; Baktar)	R

Table 2. Findings of Laboratory Testings

	No.	Negative	Positive	Notes
Person-times	159	158	1	Patient tested for
No. of persons	156	155	1	three times; all
Environment	55	54	1	negative

Note: Case tested for the fourth time on 8 September, negative; follow-up removed.

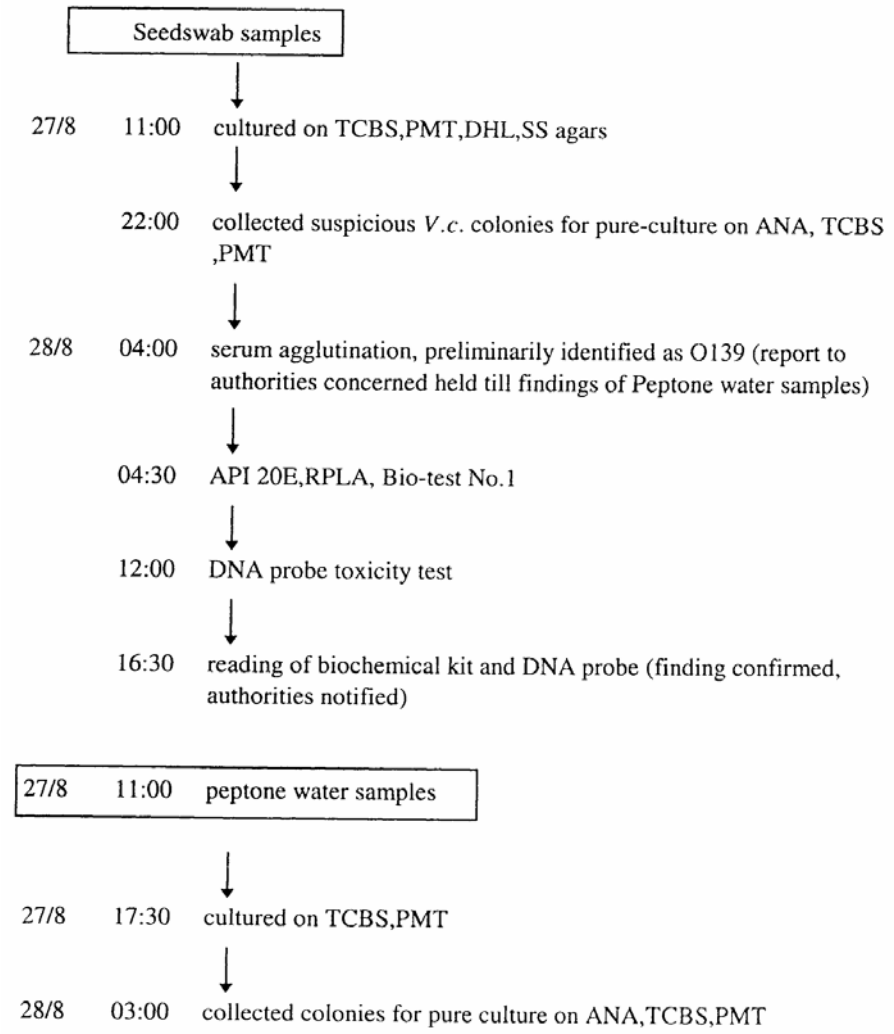
Figure 1. Flow of Testings and Findings

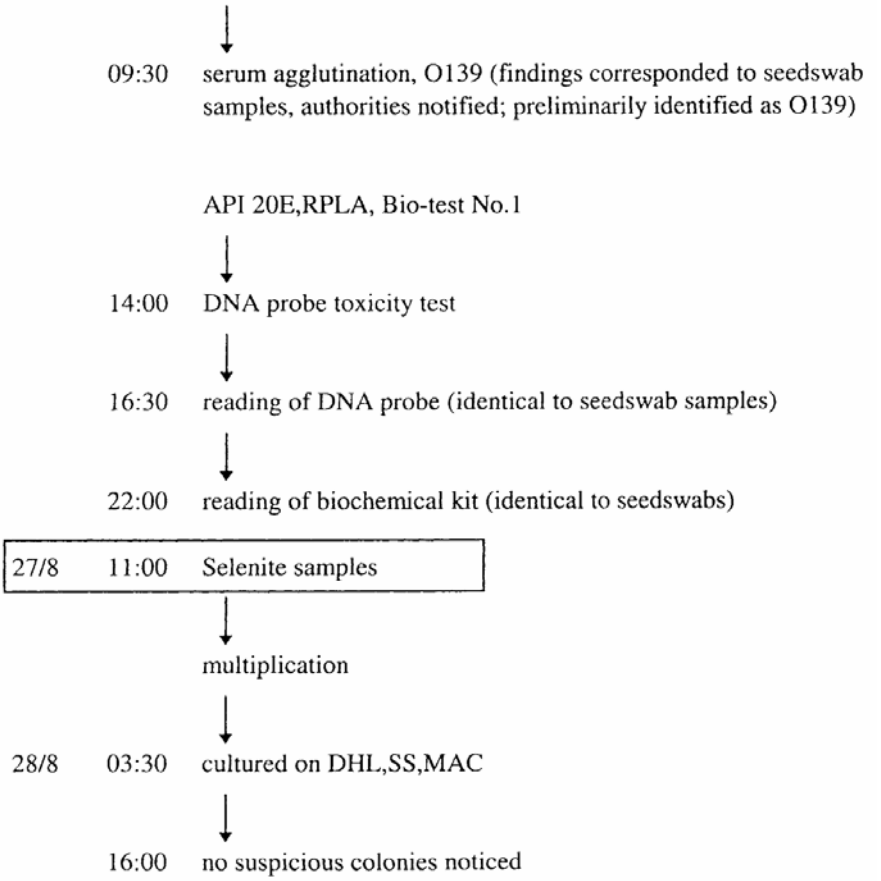


RPLA (n=2) (positive)

Note: Only specimens of case showed positive; n for the number of tests performed under different test groups, all findings corresponded.

Figure 2. Time Table of Testings





Erratum (Vol.13 No.10)

Page	Error	Correction
182	Acute Flaccid Fever	Acute Flaccid Paralysis
182	*Rabies	Tetanus
182	*Yellow Fever	*Neonatal Tetanus