*Bull. Org. mond. Santé*
*Bull. Wld Hlth Org.* } 1970, 43, 365-371

# Antibiotic-Resistant Strains of El Tor Vibrio in the Philippines and the Use of Furalazine for Chemotherapy

K. KOBARI,[1] I. TAKAKURA,[2] M. NAKATOMI,[3] S. SOGAME[4] & C. UYLANGCO[5]

*Among 1109 patients with bacteriologically confirmed El Tor cholera admitted to the San Lazaro Hospital, Manila, in 1969, 11 patients continued to excrete vibrios of the same biotype and serotype in stools for more than 1 week in spite of antibiotic treatment.*

*The strains isolated from these patients all belonged to the Ogawa serotype and were all highly resistant to streptomycin and chloramphenicol, and a few of them were resistant also to tetracycline. Other streptomycin-resistant strains of El Tor vibrio were detected, 5 in the Greater Manila area and 1 in Bacolod.*

*The antibiotic-resistant strains showed a high sensitivity to 3 kinds of antimicrobial chemicals, particularly dihydroxymethyl furalazine.*

*Furalazine was given to 33 adults and 15 children with bacteriologically confirmed cholera, and its effect in reducing the duration of diarrhoea and excretion of vibrios was investigated in comparison with the same number of cases treated with chloramphenicol. Furalazine was more effective in reducing the duration of positive stool culture than chloramphenicol, and the two antimicrobial agents were equally effective in decreasing intravenous fluid requirements.*

*Since furalazine was satisfactory in reducing the duration of diarrhoea and excretion of vibrios in stools, and since no resistant strains were found, the drug could be recommended as an alternative to chloramphenicol and tetracycline in the treatment of cholera.*

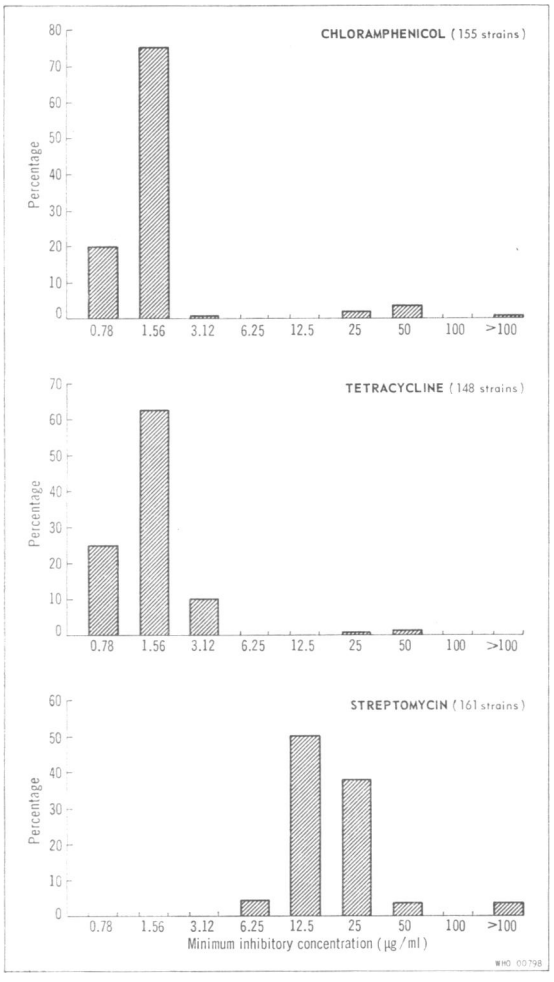
In the last 15 years, antibiotic-resistant strains of the pathogenic organisms *Shigella*, *Staphylococcus*, etc., have increased year by year, provoking serious problems in the treatment of infectious diseases. As far as *Vibrio cholerae* is concerned, it was found a long time ago that *V. cholerae* easily acquired a high resistance to streptomycin after use of the drug (Gohar, 1952). So far, tetracycline and chloramphenicol have remained effective against *V. cholerae*, in the sensitivity test *in vitro*, and have also remained effective for the treatment of cholera cases, although a few resistant strains of El Tor vibrio were found among the strains isolated in the Philippines during 1964 and 1965 (Pesigan et al., 1967; Kuwahara et al., 1967).

Since September 1961, the Philippines has never been free from El Tor cholera, the epidemic being repeated every year during the rainy season. Even in Manila, about 1500 cases of El Tor cholera have been confined in the isolation hospital each year. However, until 1968 it had not been demonstrated that drug-resistance had ever prolonged the duration of diarrhoea or the excretion of vibrios.

Between July and September 1969, it was recognized, at the San Lazaro Hospital, Manila, that in some patients with El Tor cholera, the results of stool culture continued to be positive for El Tor vibrio for more than 1 week in spite of the antibiotic treatment. The strains of El Tor vibrio isolated from these cases proved to be highly resistant to chloramphenicol, the drug used for treatment.

[1] WHO Cholera Team; present address: Institute for Tropical Medicine, Nagasaki University, Japan.
[2] WHO Cholera Team.
[3] Institute for Tropical Medicine, Nagasaki University, Japan.
[4] Institute for Tropical Medicine, Nagasaki University, Japan.
[5] San Lazaro Hospital, Manila, Philippines.

PERCENTAGE DISTRIBUTION OF SENSITIVITY TO ANTIBIOTICS OF EL TOR VIBRIO STRAINS




The sensitivity pattern of these strains to several antimicrobial agents and the clinical results of the cases from which the resistant strains were isolated are reported in this paper, and some results of chemotherapy using a nitrofuran derivative will also be given.

## ANTIBIOTIC-RESISTANT STRAINS OF EL TOR VIBRIOS

The distribution of sensitivity to chloramphenicol, tetracycline and streptomycin of the strains of El Tor vibrio isolated from patients admitted to the San Lazaro Hospital up to August 1969 is shown in the accompanying figure. Altogether 94% of 155 strains showed a high sensitivity to chloramphenicol (CP) at 0.78 μg/ml and 1.56 μg/ml MIC, but 6% of the strains were resistant to levels of 25 μg/ml MIC or higher. The majority of strains showed a high sensitivity to tetracycline; the growth of almost 88% of 148 strains was inhibited by 0.78 μg/ml and 1.56 μg/ml MIC. Only 2% of the strains were proved to be resistant to the drug at clinically obtainable levels.

The sensitivity to *streptomycin* (SM) was distributed mainly in the range of 12.5 μm/gl and 25 μg/ml, and there were resistant strains whose growth was not inhibited in an MIC of 100 μg/ml.

The chloramphenicol-resistant strains from the San Lazaro Hospital were examined for sensitivity, by the dilution method, to some other antibiotics including kanamycin (KM), erythromycin (EM), furadiomycin (FM), aminobenzyl-penicillin (ABPC) and the antimicrobial chemicals, furalazine (FL) and furazolidone (FZ), both nitrofuran-derivatives, and nalidixic acid (NA). The results are shown in Table 1.

None of the CP-resistant strains was inhibited by 100 μg/ml of SM. The MIC of CP with these strains was 50 μg/ml in most cases but with 2 strains it was 100 μg/ml and with another strain it was 25 μg/ml. Most of the strains were sensitive to tetracycline, except for 3 strains which showed resistance, the MICs being 25 μg/ml and 50 μg/ml.

As far as other antibiotics are concerned, the MICs were distributed between 3.12 μg/ml and 12.5 μg/ml. In comparison with these antibiotics, 3 chemotherapeutic agents showed much stronger antibacterial activity per unit weight against *V. cholerae*. In particular, all strains that were resistant to CP and SM were extremely sensitive to FL; they were not able to grow in an MIC 0.047 μg/ml. The MIC of FZ was distributed between 0.39 μg/ml and 1.56 μg/ml and that of NA was 0.39 μg/ml for all strains.

The total number of strains investigated for sensitivity to various kinds of antibiotic and antimicrobial chemical agent comprised 423 strains from patients admitted at the San Lazaro Hospital, 143 strains isolated from cases with diarrhoea detected in the cholera eradication project for the Greater Manila area and 185 strains recently isolated in Bacolod, Negros Island.

Among the strains from the San Lazaro Hospital, those isolated from 11 patients were resistant to CP and SM, and 3 of them were resistant to tetracycline as shown in Table 1. Strains isolated from the same

TABLE 1
SENSITIVITY PATTERN OF ANTIBIOTIC-RESISTANT STRAINS

| Strain no. | Minimum inhibitory concentration (μg/ml) [a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CP | SM | TC | EM | KM | FM | ABPC | FL | FZ | NA |
| A-27 | 50 | >100 | 0.78 | 3.12 | 12.5 | 6.25 | 6.25 | <0.047 | 0.78 | 0.39 |
| A-29 | 50 | >100 | 0.78 | 3.12 | 6.25 | 6.25 | 6.25 | <0.047 | 1.56 | 0.39 |
| B-27 | 25 | >100 | 0.78 | 3.12 | 6.25 | 6.25 | 6.25 | <0.047 | 1.56 | 0.39 |
| B-28 | 50 | >100 | 0.78 | 1.56 | 12.5 | 6.25 | 6.25 | <0.047 | 0.78 | 0.39 |
| C-21 | 50 | >100 | 50 | 1.56 | 12.5 | 6.25 | 3.12 | <0.047 | 1.56 | 0.39 |
| C-22 | 50 | >100 | 0.78 | 1.56 | 12.5 | 6.25 | 6.25 | <0.047 | 0.39 | 0.39 |
| C-24 | 100 | >100 | 50 | 3.12 | 12.5 | 6.25 | 6.25 | <0.047 | 1.56 | 0.39 |
| D-6 | 100 | >100 | 25 | 1.56 | 6.25 | 6.25 | 3.12 | <0.047 | 0.39 | 0.39 |
| D-11 [b] | 50 | >100 | 0.78 | 3.12 | 6.25 | 6.25 | 6.25 | <0.047 | 0.78 | 0.39 |
| E-4 [b] | 50 | >100 | 0.78 | 1.56 | 6.25 | 6.25 | 12.5 | <0.047 | 1.56 | 0.39 |
| H-1 | 50 | >100 | 0.78 | 3.12 | 12.5 | 6.25 | 3.12 | <0.047 | 0.39 | 0.39 |
| J-9 | 50 | >100 | 0.78 | 1.56 | 6.25 | 12.5 | 3.12 | <0.047 | 1.56 | 0.39 |

[a] CP = chloramphenicol; SM = streptomycin; TC = tetracycline; EM = erythromycin; KM = kanamycin; FM = furadiomycin; ABPC = aminobenzyl penicillin; FL = furalazine; FZ = furazolidone; NA = nalidixic acid.
[b] D-11 and E-4 isolated from the same patient on different days.

patient showed the same sensitivity pattern, except for one case in which two strains presented a slightly different pattern (D-11 and E-4). Five SM-resistant strains were found out of 143 strains isolated from cases with diarrhoea, but these strains were sensitive to CP and TC at MICs between 1.56 μg/ml and 6.25 μg/ml (Table 2). There was only one strain among those from Bacolod that was resistant to SM but not to CP and TC.

TABLE 2
SENSITIVITY PATTERN OF ANTIBIOTIC-RESISTANT STRAINS IN GREATER MANILA

| Strain no. | Minimum inhibitory concentration (μg/ml) | | |
|---|---|---|---|
| | Chloramphenicol | Tetracycline | Streptomycin |
| 1 | 6.25 | 1.56 | 100 |
| 2 | 6.25 | 3.12 | 100 |
| 3 | 6.25 | 3.12 | 100 |
| 4 | 3.12 | 3.12 | 100 |
| 5 | 6.25 | 3.12 | 100 |

All of the resistant strains belonged to the Ogawa serotype, which has been predominant in the Philippines since 1961. The biochemical behaviour of these strains is listed in Table 3; the only differences between strains were seen in the Voges-Proskauer reaction.

## CASES

The rainy season usually brings an outbreak of El Tor cholera to the city and surrounding area of Manila. The majority of cholera patients admitted at the San Lazaro Hospital, Manila, are seen during the period from July to September, when the streets are sometimes flooded after heavy rain.

The treatment normally given to cholera patients at the San Lazaro Hospital is as follows. Fluid treatment with the Ringer lactate solution is begun immediately after the hospitalization of the patient, in order to compensate for severe dehydration. At the same time, adults are given 250 mg of chloramphenicol orally 4 times daily for 3 days to shorten the duration of diarrhoea and the excretion of vibrios. The daily stool cultures usually become vibrio-negative within 2 or 3 days after the commencement of the antibiotic treatment.

During the period from the end of July to the beginning of September 1969, however, it was noticed that some patients continued to excrete El Tor vibrios in stools for more than one week, as shown in Table 4, in spite of the treatment with chloramphenicol. When stool cultures continued to be positive for *V. cholerae*, treatment with chloramphenicol was repeated for a further 3 days. However, in these cases the repeated use of chloramphenicol was not successful in stopping the excretion of vibrios, whereas tetracycline was effective in the two such cases in which it was employed.

On the other hand, prolonged excretion of CP-resistant strains has not been accompanied by prolonged diarrhoea, except in one case in which diarrhoea continued for 48 hours.

### FURALAZINE AS A CHEMOTHERAPEUTIC TREATMENT FOR CHOLERA

It seemed necessary to investigate alternative chemotherapy, in place of chloramphenicol and tetracycline in view of the possibility that antibiotic-resistant strains might spread in the Philippines. As a result of sensitivity tests, dihydroxymethyl furalazine was chosen as one of the alternative drugs for the treatment of El Tor cases.

TABLE 3

BIOCHEMICAL REACTIONS OF THE RESISTANT STRAINS

| Reaction | Result |
|---|---|
| Arginine | − |
| Lysine | + |
| Ornithine | + |
| Maltose | + |
| Mannose | + |
| Sucrose | + |
| Arabinose | − |
| Malonate | − |
| Urea | − |
| $H_2S$ | − |
| Methyl red | − |
| Voges-Proskauer | + or − |
| Indole | + |
| Simmon's citrate | + |
| Motility | + |
| Haemolysis | − |
| Haemagglutination | + |
| Polymyxin-B resistance | + |

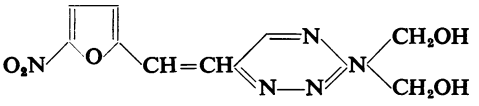
TABLE 4

RESULTS OF STOOL CULTURES FROM SOME PATIENTS RECEIVING CHLORAMPHENICOL

| Patient | Strain no. | Age | Sex | Date of admission | Days of hospitalization | Duration of diarrhoea (h) | Daily stool culture [a] |
|---|---|---|---|---|---|---|---|
| S. G. | A-27 | 5 | male | 24 July | 15 | ? | + + + + + − + + − − − − − |
| E. G. | A-29 | 10 | male | 24 July | 12 | ? | + + + + + + + + + + + − − − |
| B. R. | C-21 | 29 | male | 14 Aug. | 13 | 48 | + + + + + + + − + + absconded |
| R. C. | B-28 | 30 | female | 16 Aug. | 11 | ? | + + + − + + + + − − |
| G. G. | C-22 | 24 | male | 17 Aug. | 9 | 29 | + − + + + + − − + |
| M. G. | C-24 | 16 | female | 17 Aug. | 12 | 17 | + + + + + + + + − − |
| F. L. | D-11 | 29 | male | 18 Aug. | 9 | 14 | + + + + + + − − |
| | E-4 | | | | | | |
| J. S. | B-27 | 20 | female | 26 Aug. | 10 | 27 | + + + + + + + + − − |
| E. F. | J-9 | 26 | female | 5 Sept. | 10 | 30 | + + + + + + + + − − |

[a] ——— Administration of chloramphenicol
═══ Administration of tetracycline

Dihydroxymethyl furalazine is a nitrofuran derivative developed by the Toyama Chemical Co. in Japan. Its chemical structure is:



3-di(hydroxymethyl)amino-6-(5nitro-2-furylethenyl)-1,2,4-triazine. This drug shows a wide spectrum of antibacterial activity against various pathogenic organisms, both Gram-positive and Gram-negative. Gram-negative rods such as *Shigella* and *Salmonella*, in particular, are extremely sensitive to the drug.

The drug has been used frequently in Japan for the treatment of *Shigella* infections caused by strains highly resistant to chloramphenicol, tetracycline and streptomycin. The effect of the drug has been generally satisfactory, although chloramphenicol and tetracycline were more effective in patients infected with non-resistant strains.

As dihydroxymethyl furalazine became available for the treatment of El Tor cases in September 1969, it was given orally, in form of granules, to patients admitted to the San Lazaro Hospital, and its effect was compared with that of chloramphenicol. A sachet containing 250 mg of the drug was given every 6 hours 4 times daily for 3 days. The granules were dissolved in cold or warm water; the solution tasted sweet, and was easy to drink even for children. Children 4–12 years old were given half the adult dose and those under 3 years of age were given one quarter of the adult dose.

The results for the duration of diarrhoea, the duration of culture positivity and for the required amounts of intravenous fluid are compared for FL and CP in Table 5.

The treatment with FL was tested on 33 adults and 15 children who revealed typical cholera symptoms on admission; the same number of patients were treated with CP. The intravenous fluid requirements of patients receiving FL were very similar to those of patients receiving CP. The mean duration of diarrhoea was observed to be somewhat less in the patients receiving CP, both among adults and children. These differences could well have been due to chance but in any case the magnitude of the observed differences is not of much clinical importance.

On the other hand, the mean duration of positive stool culture was almost twice as long in the CP group as in the FL group. In the majority of cases treated with FL, the stool cultures became negative for vibrios within 2 days from the start of therapy, and only a few cases showed a positive result for vibrios on the third and fourth days. FL, however, appears to have a less rapid effect than tetracycline in eradicating vibrios from the stool (Pierce et al., 1968; Karchmer et al., 1970).

TABLE 5

COMPARISON OF THE EFFECTIVENESS OF FURALAZINE (FL) AND CHLORAMPHENICOL (CP) IN REDUCING THE DURATION OF DIARRHOEA AND POSITIVE STOOL CULTURE

| Drug | No. of cases [a] | Duration of diarrhoea (h) | Duration of culture positivity (days) | Amount of intravenous fluid (litres) |
|---|---|---|---|---|
| | | Adults | | |
| CP | 33 | 25 | 3.4 | 15.1 |
| FL | 33 | 32 | 1.8 | 14.5 |
| | | Children | | |
| CP | 15 (6.6) | 7 | 3.1 | 3.8 |
| FL | 15 (4.5) | 16 | 1.6 | 2.8 |

[a] Average age of children (years) in parentheses.

## DISCUSSION

Since antibiotic resistant strains of most pathogenic bacteria have developed following the widespread use of antibiotics, it is not surprising that we have now discovered antibiotic resistant strains of *Vibrio cholerae*. SM-resistant strains of *V. cholerae* have been found not infrequently in the past few years; in the Philippines it was reported that highly SM-resistant strains were relatively often isolated from El Tor cases treated with antibiotics, and one patient yielded a strain resistant to TC, CP, SM and sulfafurazole. During the epidemic in 1969, we found that the strains isolated from 11 cholera cases were highly resistant to CP and SM, and a few of them were resistant to TC as well. In these cases stool cultures continued to be positive for *V. cholerae* for more than one week. In 1969, only 11 out of 1109 bacteriologically confirmed cases at the San Lazaro Hospital yielded CP- and SM-resistant strains, but it is possible that the resistant strains could spread rapidly, not only in Manila but also over the whole country. The strains are being carefully investigated by the bacteriologists who are especially interested in genetics, to determine whether the resistance of these strains is due to a "resistance factor" or not.

In the last two years, CP has been widely used in the city and the surrounding area of Manila for the treatment of patients with diarrhoea and for contacts with stool cultures positive for El Tor vibrio. It can be assumed that the widespread use of CP may have played an important role in the appearance of the resistant strains of El Tor vibrios in this district. In addition, the fact that most patients treated with CP were discharged from hospital after less than one week may have helped to promote the spread of any resistant strains that survived in the intestines of convalescents.

In our studies the strains isolated from patients on the first day of hospitalization, before the administration of any antibiotic, showed resistance to CP and SM; in other words, these cases were infected with a resistant strain of El Tor vibrio. During the hospitalization, they continued to excrete resistant strains. Two of them absconded from the hospital, before excretion of resistant strains in stools had ceased. Thus it is probable that resistant strains will persist somewhere in this district, and may give rise to an outbreak in the coming epidemic season.

As far as the drug resistance is concerned, TC will not always be effective in the case of CP-resistant strains, because a cross-resistance was recognized between CP, SM and TC in 3 of the resistant strains. Among other antibiotics, EM can be recommended for the treatment of cholera on the basis of the results of the sensitivity tests against the El Tor vibrio and clinical experience of the authors, but the high cost of the drug makes it less readily available in cholera endemic areas.

Furalazine was selected for testing as a chemotherapeutic agent as an alternative to CP and TC for the treatment of cholera, in view of the extremely high sensitivity of the El Tor vibrio to the drug and previous experience with the drug in 1964 in Manila (Kobari, 1965; Kobari et al., 1967). Furazolidone, another nitrofuran-derivative, was reported by Chaudhuri et al. (1965) to be effective for shortening the duration of diarrhoea and the excretion of vibrios and to be effective against both the classical and El Tor biotypes of *V. cholerae*. Recently, the same authors (Chaudhuri et al., 1968) compared the effect of FZ with TC, and found that FZ and TC were equally effective in reducing both the duration of diarrhoea and the amount of intravenous fluid required, and that even a single daily dose of FZ was effective.

On the other hand, Pierce (1968) concluded that FZ was significantly less effective than TC in rapidly and consistently terminating vibrio excretion, and that TC remains the antibiotic of choice in cholera but that FZ would be a useful adjunct to cholera therapy when TC is unobtainable or if strains of *V. cholerae* with clinically significant resistance should be encountered. However, if the sensitivity of FZ reported by Pierce is compared with that of FL found in our present studies it will be noticed that the MIC of FL against El Tor vibrio is almost 200 times lower than that of FZ, the MIC of FZ being 8.0 μg/ml–16.0 μg/ml, while that of FL is less than 0.047 μg/ml. The duration of excretion of vibrios in stools seems to have been almost the same in both groups.

FL would be a useful alternative drug for the treatment of cholera instead of CP or TC. The drug may also prove to have an important advantage in that no resistant strains of pathogenic organisms, including *V. cholerae*, have yet been found.


## RÉSUMÉ

### SOUCHES DE VIBRIONS EL TOR RÉSISTANTES AUX ANTIBIOTIQUES AUX PHILIPPINES ET EMPLOI DE LA FURALAZINE DANS LA CHIMIOTHÉRAPIE DU CHOLÉRA

Sur 1109 malades atteints de choléra El Tor (sérotype Ogawa) admis en 1969 à l'Hôpital San Lazaro, à Manille, 11 ont continué à excréter des vibrions appartenant au même biotype et au même sérotype pendant plus d'une semaine en dépit d'un traitement par antibiotiques. Les souches isolées chez ces patients étaient très résistantes à la streptomycine et au chloramphénicol, et 3 d'entre elles l'étaient également à la tétracycline. Six autres souches de vibrions El Tor résistantes à la streptomycine ont été identifiées, dont 5 dans le Grand Manille et 1 à Bacolod.

Les souches résistantes aux antibiotiques se révélant en revanche très sensibles à divers agents antimicrobiens, dont la furalazine, on a administré ce dernier produit à 33 adultes et 15 enfants atteints de choléra bactériologiquement confirmé. Une étude comparative a montré que la furalazine avait une action plus favorable que le chloramphénicol sur la durée de positivité des cultures de

selles; les deux médicaments permettaient, dans une mesure équivalente, de réduire la quantité de liquide à administrer par voie intraveineuse.

L'efficacité de la furalazine, qui abrège la durée de la diarrhée et de l'excrétion des vibrions, et le fait qu'aucune résistance à son égard n'a été signalée, amènent à la considérer comme un médicament susceptible de remplacer le chloramphénicol et la tétracycline dans le traitement du choléra.

## REFERENCES


Chaudhuri, R. N., Neogy, K. N., Sanyal, S. N., Gupta, R. U. & Manji, P. (1968) *Lancet*, **1**, 332

Chaudhuri, R. N., Sanyal, S. N., Neogy, K. N., Barua, D. & Manji, P. (1965) *Lancet*, **2**, 909

Gohar, M. A., El-Mofty, A., Eissa, A. A., Mousa, A. H., Sorour, A., Doss, H., Goma, T., Abou-El Wafa, M., El-Azhari, A., Mitri, S. & Ibrahm, M. (1952) *J. trop. Med. Hyg.*, **55**, 241

Karchmer, A. W., Curlin, S. T., Huq, M. I. & Hirschhorn, N. (1970) *Bull. Wld Hlth Org.* **43**, 373

Kobari, K. (1965) *Treatment of cholera El Tor with antimicrobial drugs.* In: *Proceedings of the Cholera Research Symposium*, Honolulu, Washington, D.C., US Government Printing Office, p. 199

Kobari, K., Uylangco, C., Vasco, J., Takahira, Y. & Shimizu, N. (1967) *Bull. Wld Hlth Org.*, **37**, 751

Kuwahara, S., Goto, S., Kimura, M. & Abe, H. (1967) *Bull. Wld Hlth Org.*, **37**, 763

Pesigan, T. P., Gomez, C. Z. & Barna, D. (1967) *Bull. Wld Hlth Org.*, **37**, 814

Pierce, N. F., Banwell, J. G., Mitra, R. C., Caranos, G. J., Keimowitz, R. I., Thomas, J. & Mondal, A. (1968) *Brit. med. J.*, **2**, 3277