

## Drug Sensitivity of El Tor Vibrio

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**Abstract:** One hundred and forty-nine strains of El Tor vibrio were examined for their susceptibilities against 12 antimicrobials. Ninety-seven strains out of 149 were isolated in Kenya in 1975, and 52 strains were isolated in the Philippines from 1973 to 1978. The organisms were most sensitive to minocycline which inhibited the growth at the concentration of 0.39 mcg/ml or less. Naridixic acid was the second effective antibiotics to inhibit the organisms. Minimum inhibitory concentration (MIC) of chloramphenicol was 0.78 mcg/ml in 85% of the strains. Rifampicin showed almost same MICs with chloramphenicol. Aminoglycosides and beta-lactam antibiotics were not excellent with the MICs of 6.25 mcg/ml or so in majority. But newly developed antibiotics of cephalosporin, i.e., SCE-963, was valued about same as chloramphenicol as far as susceptibility in vitro is concerned. There was no significant discrepancy of drug sensitivity pattern in the strains from Kenya and from the Philippines. Highly resistant strains were not found.

*Vibrio cholerae* is noteworthy as a pathogen of intestinal infection, as well as *Shigella* and *Salmonella*. Although the drug sensitivities of Enterobacteriaceae including *Shigella* and *Salmonella* has been well studied so far, few studies on the activities of antibiotics against *Vibrio cholerae* have been reported<sup>1-8),11)</sup>; because the therapy of cholera almost completely depends on fluid infusion, and little on antibiotics.

The seventh cholera pandemic at present does not decline yet. It is still invading the virgin area in the world. And for the treatment of cholera, antibiotics such as tetracycline are routinely used with the fluid infusion. Antibiotics, actually, are beneficial for shortening the period of the disease<sup>9)</sup>. In these situation, it is necessary to survey the annual changes and geographical feature of the drug sensitivity pattern of the pathogen. The present report deals with the drug sensitivity pattern of El Tor vibrio isolated in Kenya and the Philippines in the recent years.

### MATERIALS AND METHODS

**Strains:** Ninety-seven El Tor vibrio strains isolated in Kenya in 1975 and 52 El Tor vibrio strain isolated in the Philippines from 1973 to 1978 were tested. Kenya strains had been stocked in butt of soft agar media consisted of 0.5% NaCl, 1% peptone (Difco), and 0.3% agar. The Philippines strains had been stocked in butt of meat extract agar.

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Contribution No.817 from the Institute for Tropical Medicine, Nagasaki University.  
Received for publication, February 10, 1979

Drugs: Twelve antimicrobials were used; chloramphenicol (CP), minocycline (MNC), erythromycin (EM), rifampicin (RFP), naridixic acid (NA), gentamicin (GM), kanamycin (KM), amoxycillin (AM-PC), sulbenicillin (SB-PC), cefalexin (CEX), cefazolin (CEZ), and newly developed cephalosporin (SCE-963). Solution of each drugs were arranged at 1,000 mcg/ml with distilled water, and then serially diluted with normal saline solution. Naridixic acid was poorly soluble in water, so, small amount of 1N NaOH was used as a solvent. With the same reason, small amount of ethanol was used for erythromycin and rifampicin.

Determination of sensitivity: Minimum inhibitory concentration (MIC) of the drugs were determined by agar plate dilution method. Two milliliters of drug solutions with 1,000 mcg/ml to 2 mcg/ml in two-fold dilution series were mixed with 18 ml of melted (56°C) heart infusion agar (Eiken). Then, 10 agar plates containing 100 mcg/ml to 0.2 mcg/ml of each drug were prepared. Inoculums were made by 10-fold dilution of 20 hours culture of each strain in heart infusion broth (Eiken) at 37°C.

Normal saline solution was used as the diluent. The inoculums were streaked on the agar plates in about 1 cm in length, and incubated at 37°C for 20 hours.

## RESULTS

### *Drug sensitivity pattern in general aspect*

As far as the drugs which can be used as tablets are concerned, the range of MICs were separated into 2 groups as shown in Fig. 1. MNC, NA, CP and RFP showed low MICs less than 1.56 mcg/ml against all 149 strains. On the contrary, the other group of drugs, CEX, AM-PC, KM and EM showed higher MICs of 6.25 mcg/ml against about 90% of the strains. Regarding the parenteral antibiotics, GM, CEZ, SB-PC, and SCE-963, variable MICs were seen (Table 3). CEZ was almost the same as AM-PC in the activity pattern, and SCE-963 was almost the same as CP. GM and SB-PC were between the 2

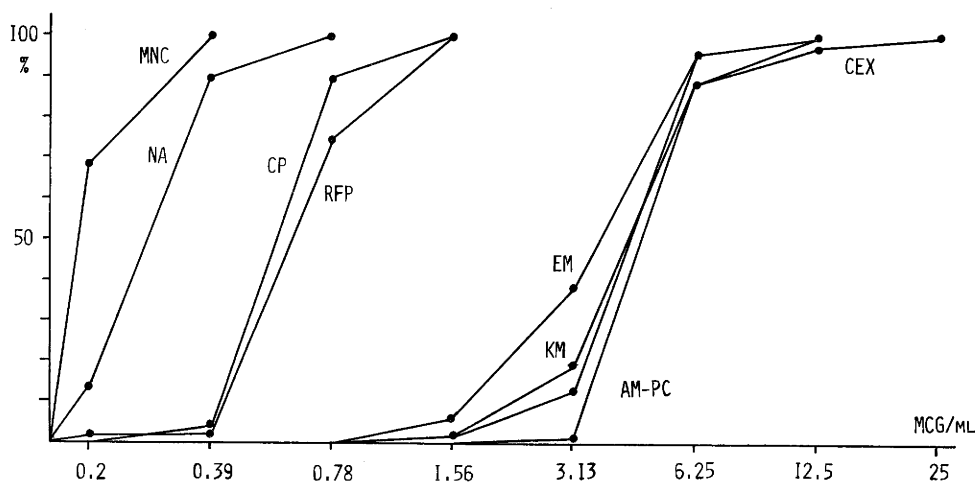


Fig. 1. Cumulative distribution of MICs of 8 antibiotics against 149 El Tor vibrio strains.

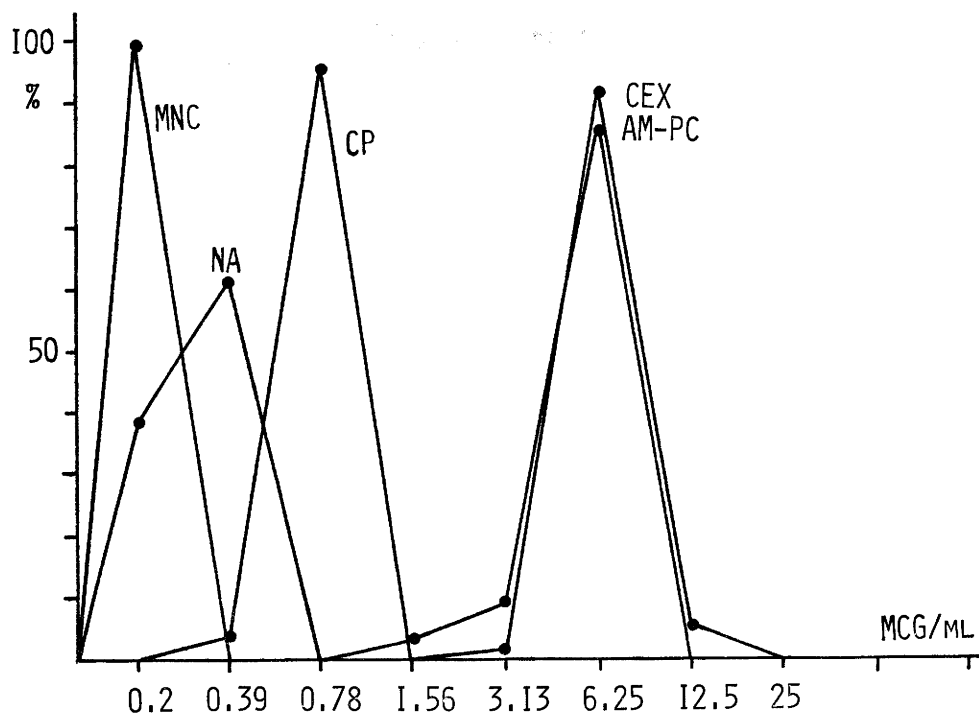
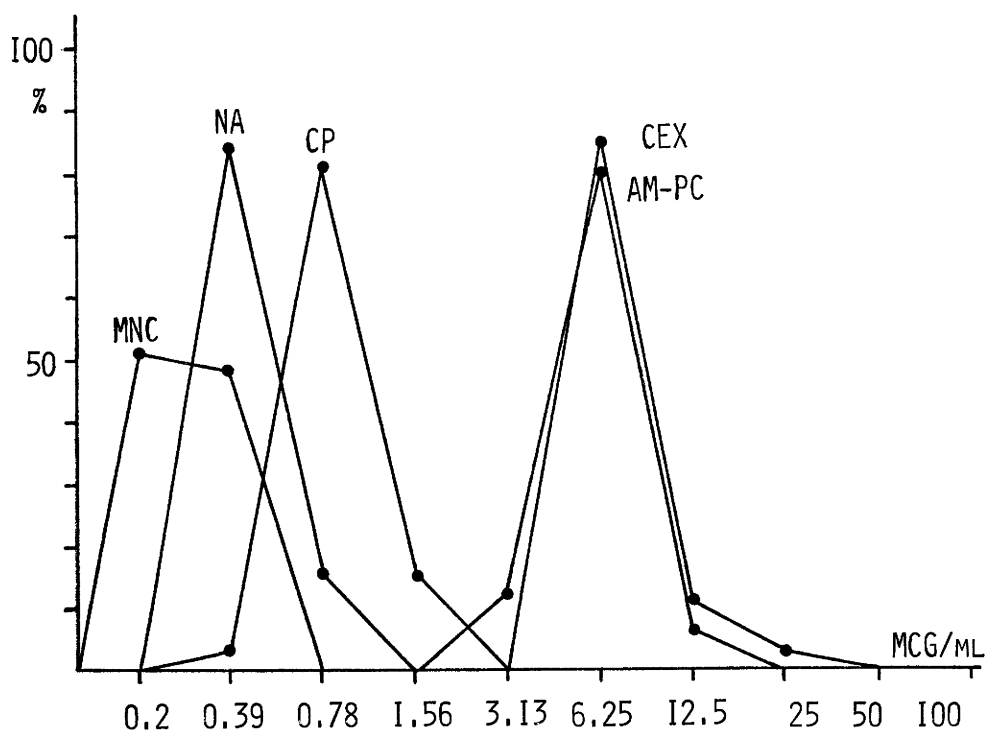


Fig. 2. Susceptibility of El Tor vibrio against tablet antibiotics, 97 strains from Kenya (upper) and 52 strains from the Philippines (lower).

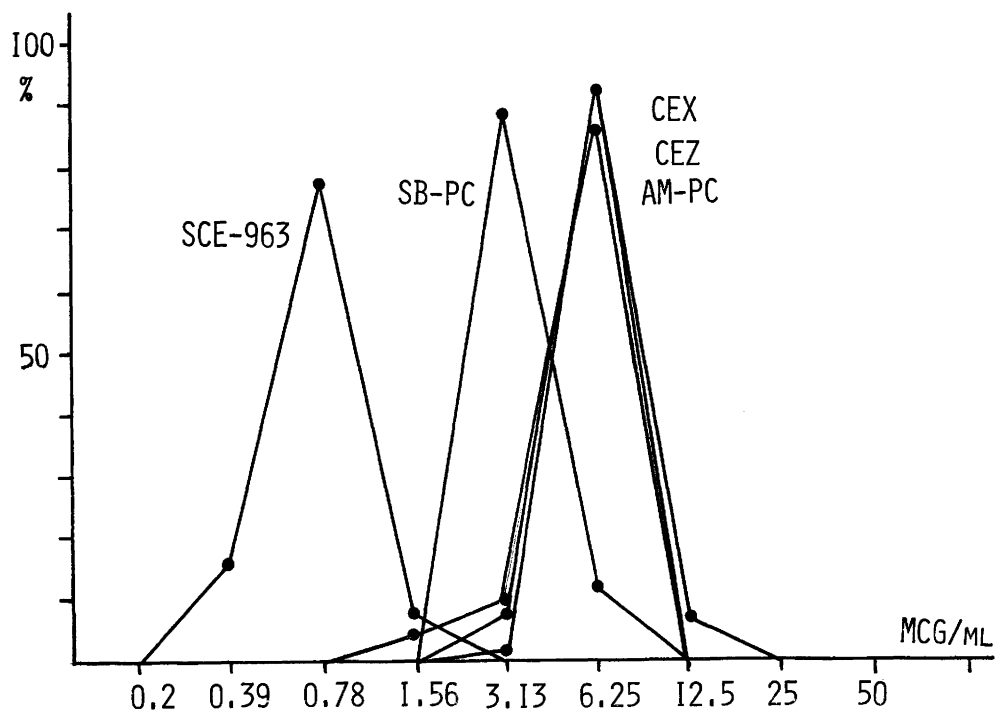
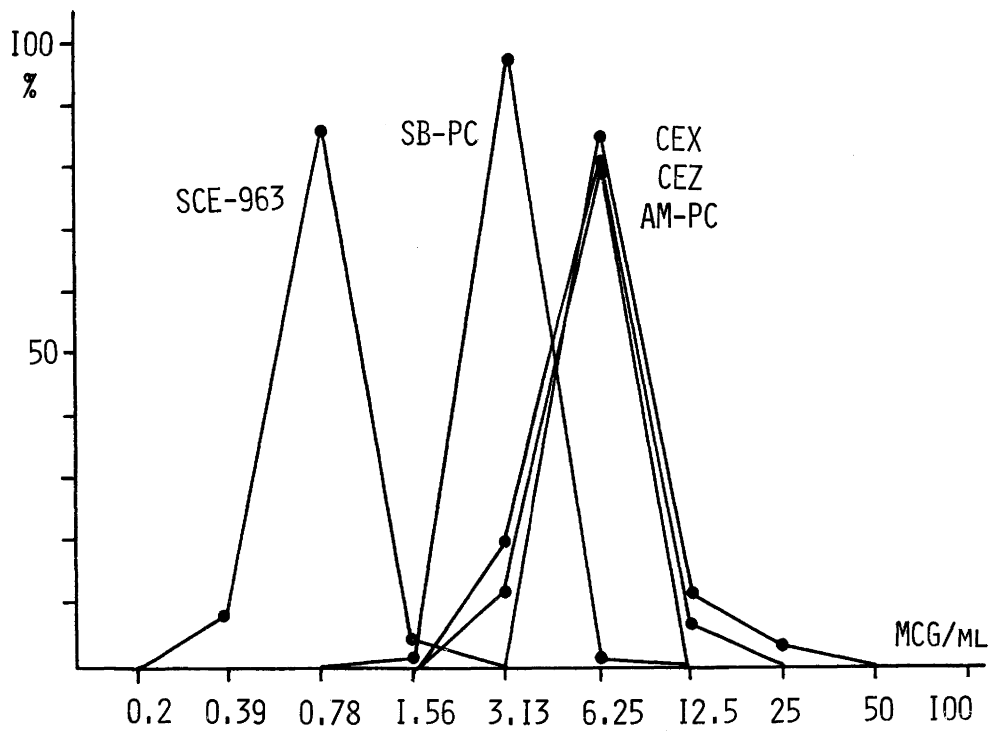


Fig. 3. Susceptibility of *El Tor* vibrio against 5 beta-lactam antibiotics, 97 strains from the Kenya (upper) and 52 strains from the Philippines (lower).

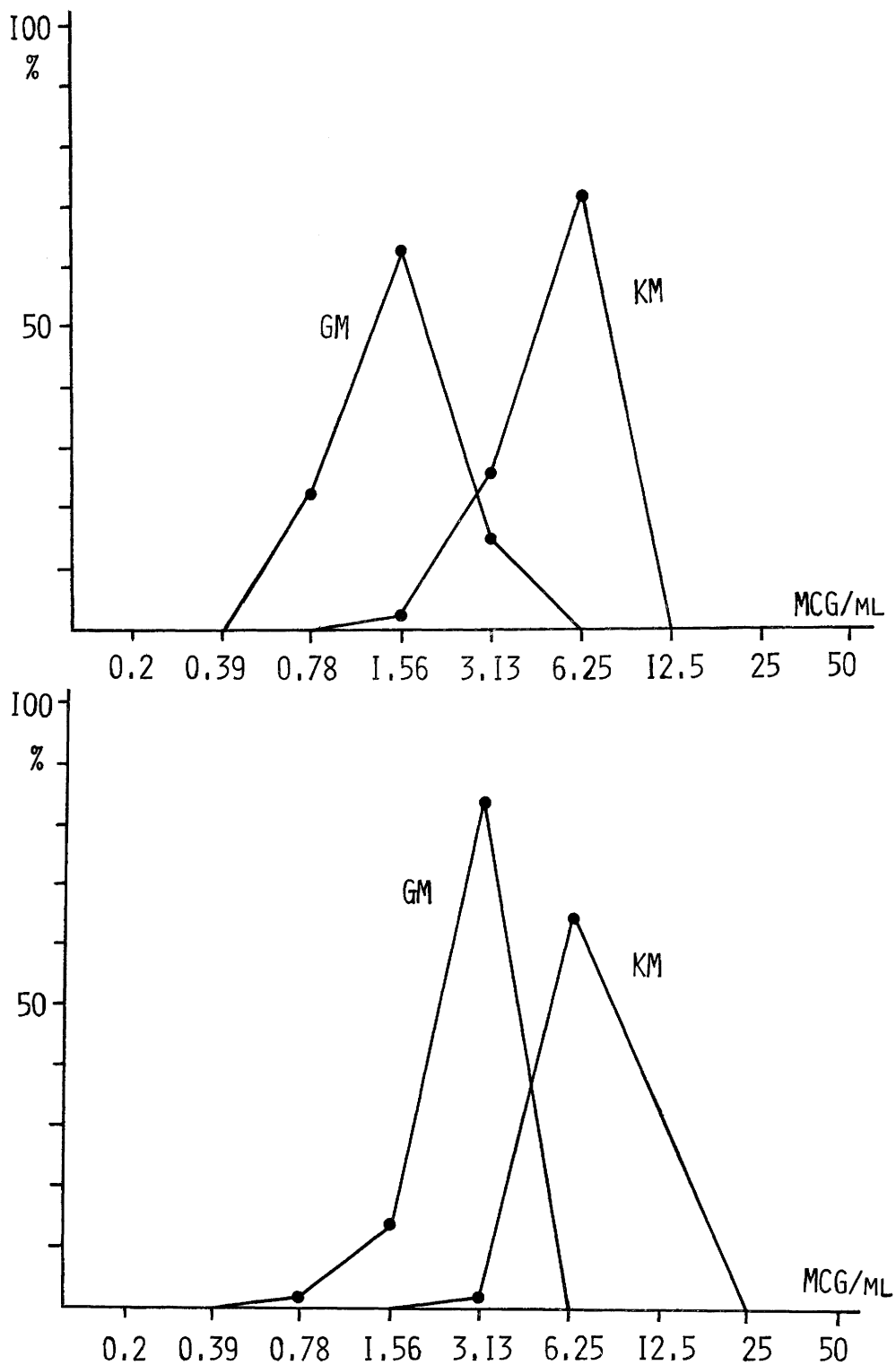


Fig. 4. Susceptibility of El Tor vibrio against 2 aminoglycosides, 97 strains from Kenya (upper) and 52 from the Philippines (lower).



Table 2. MICs of antimicrobial agents against El Tor vibrio from Kenya

mcg/ml drug	0.2 ↓	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	100 ↑
CP		3	79	15							
MNC	50	47									
CEX						83	11	3			
AM-PC					12	79	6				
RFP	2	1	75	19							
EM	1			6	28	56	6				
CEZ					19	78					
SCE-963		8	84	4		1					
GM			22	61	14						
SB-PC				1	95	1					
KM				2	25	70					
NA		82	15								

Table 3. MICs of antimicrobial agents against 149 strains of El Tor vibrio

mcg/ml drug	0.2 ↓	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	100 ↑
CP		5	129	15							
MNC	102	47									
CEX					1	131	14	3			
AM-PC				2	17	124	6				
RFP	2	1	108	38							
EM	1			8	48	86	6				
CEZ					23	126					
SCE-963		16	124	8		1					
GM			23	68	58						
SB-PC				1	141	7					
KM				2	26	104	17				
NA	20	114	15								

*Geographical feature*

Susceptibility of El Tor vibrio isolated in Kenya and in the Philippines were compared in geographical point of view (Figs. 2,3,4). As shown in the figures, sensitivity pattern of El Tor vibrio seemed to be quite similar, although slight discrepancies were seen in MNC, NA, GM and KM. Strains from the Philippines were slightly more sensitive to NA and MNC, and those from Kenya were slightly more sensitive to GM and KM.

Summary of the results is shown in table 1,2, and 3.

## DISCUSSION

The effect of sulfonamide drugs on *Vibrio cholerae* was reported by Griffiths in 1942<sup>5)</sup>. That was the first report on antimicrobial activity against *Vibrio cholerae*, as far as our knowledge is concerned. Thereafter, several studies have been reported, but the number of these reports is so small as compared with the same kind of studies on the other pathogens such as *Salmonella* and *Shigella*.

The seventh cholera pandemy due to El Tor vibrio has not declined yet. Moreover, it is still invading into the virgin area of the world. In this pandemic situation in which antibiotics such as tetracycline has been routinely used for the treatment, the information on geographical feature and on annual changes of drug sensitivity pattern of the organism should be required.

Present study revealed that minocycline (as a substitute of tetracycline) and chloramphenicol were excellent in anti-vibrio activity as before. Naridixic acid was also excellent, and it might be able to substitute chloramphenicol. Anti-vibrio activity of rifampicin was about the same with chloramphenicol. This drug is absorbed in the intestine and is mainly excreted in bile. Because of this hepato-intestinal circulation, the drug concentration level in blood is significant even at 20 hours after medication. Therefore, it is expected that one dose of this drug may eliminate all vibrio in the intestine.

Regarding the geographical feature of sensitivity pattern, classical strains isolated in four areas (Egypt, Bombay, Bengal, and Indochina) were examined by Felsenfeld *et al.* (1951)<sup>2)</sup>. They examined 10 antibiotics, and concluded that the result showed a wide variation of susceptibility to these drugs, according to the geographical origin of the vibrios. Looking at the results on chloramphenicol in their report, mean inhibitory concentration against the strains from Egypt was 5.3 mcg/ml, and that from Bengal was 0.9 mcg/ml including a few strains which were resistant to 100 mcg/ml.

The drug sensitivity pattern of El Tor vibrio was reported first by Kao in 1964, who examined four drugs against 300 strains of El Tor vibrio isolated in Taiwan<sup>6)</sup>. Kuwahara *et al.* reported the geographical feature in the Philippines (1967), on the strains isolated in 1964 and 1965, and noted some variation of susceptibility<sup>8)</sup>. But present study revealed that the sensitivity patterns of El Tor vibrios originated in Kenya and in the Philippines were quite similar each other. Moreover, the sensitivity patterns have not basically changed during the past 10 years or more comparing with the results examined by Kao<sup>6)</sup>, Zen-yoji *et al.*<sup>9)</sup>, and Kuwahara *et al.*<sup>8)</sup>. Henceforth, in geographical point of view and also in course of time, drug sensitivity pattern of El Tor vibrio should be examined in each country contaminated with the organism.

In the examination, the time after isolation should be considered, because it is known that the drug sensitivity of *Vibrio cholerae* maintained in laboratory frequently get more sensitive<sup>2)</sup>. There is a fact that antibiotic-resistant strains were isolated at times<sup>7)</sup>, and R-factor was proved in some cases, but R-factor of *Vibrio cholerae* was unstable even on selective media containing antibiotics<sup>10)</sup>.



## ACKNOWLEDGEMENT

The authors wish to express their gratefulness to Dr. K. Kobari who made effort to get the strains.

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## コレラ菌の各種抗菌剤に対する感受性

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エルトルコレラは1961年以降世界的に流行を続けているにも拘らず、分離株の各種抗菌剤に対する年時的・地域的な感受性パターンに関する調査は余り行われていない。我々は1975年ケニアにおいて分離したエルトルコレラ菌97株と、1973年から1978年の間にフィリピンで分離された52株について12種の抗菌剤に対する感受性を調査した。最も抗菌力の強かったものはミノサイクリンであり、全株が0.39 mcg/ml またはそれ以下の濃度で発育が阻止された。ナリディキシックアシッドもこれに準ずる抗菌力を示したが、約10%の株に対する最小発育阻止濃度（MIC）は0.78 mcg/ml であった。これらに続くものはクロラムフェニコール、リファンピシン、および最近開発されたセファロスポリン系薬剤の SCE-963であり、約80%の株が0.78 mcg/ml の濃度で発育を阻止された。ゲンタマイシン、スルベニシリンでは MIC のピークがそれぞれ1.56 mcg/ml, 3.13 mcg/ml を示し、カナマイシン、エリスロマイシン、セファレキシン、セファゾリン、アモキシシリンはさらに効果が劣っていた。ケニア株とフィリピン株の比較ではナリディキシックアシッド、ミノサイクリン、カナマイシン、ゲンタマイシンでわずかな差がみられたほかは、極めて類似した感受性パターンを示していた。約10年前の善養寺らによるフィリピン株の成績と比べても、感受性パターンに殆ど変化はみられず、耐性化の傾向も窺われなかった。

熱帯医学 第21巻 第1号 11-20頁, 1979年3月