

## Drug Sensitivity of Enteric Bacteria and Genus *Pseudomonas* Isolated from Drinking Water in Indonesia

Yoshiyuki OKUWAKI<sup>1</sup>, Hisae YANAI<sup>1</sup>, Kyoko YUTAKA<sup>1</sup>,  
Koichiro FUJITA<sup>2</sup>, Setsuko TSUKIDATE<sup>2</sup> and Masatoshi SUGIYAMA<sup>3</sup>

<sup>1</sup>Department of Microbiology, Kagawa Nutrition College

<sup>2</sup>Department of Medical Zoology, Nagasaki University School of Medicine

<sup>3</sup>Department of Hygiene, Juntendo University School of Medicine

**Abstract:** On sixty-five strains of enteric bacteria and twenty-five strains of Genus *Pseudomonas* isolated from the drinking water in the City of Jakarta, Indonesia between July and August 1983, sensitivity to fourteen chemotherapeutic agents including PCB, PIPC, MZPC, CER, CET, CFX, CMZ, CPZ, GM, KM, CP, TC, CL and NA was tested by the disk agar diffusion method using three levels of concentration. MIC was measured by the dilution method in strains of resistance, and following results were obtained. 1. All strains of enteric bacteria showed highest sensitivity to GM. More than 98% of the strains showed favorable sensitivity to PIPC, MZPC, CPZ, KM, CP, TC, CL and NA. Resistance, however, was encountered to CER (23%) and CET (28%), the first generation cephem drugs, and CFX (47%), the second generation cephem drugs. 2. All strains of Genus *Pseudomonas* showed higher sensitivity to GM and CL. About 90% of the strains showed favorable sensitivity to PIPC, TC, and CPZ, the third generation cephem drug. Resistance, on the other hand, was encountered to NA (96%), CER (82%), CET (96%), CFX (96%) and CMZ (63%). 3. In three of seven strains of *Escherichia coli*, resistance to the first generation cephem drugs was noted. Such tendency was remarkable in *Klebsiella pneumoniae*, with resistance of all strains to CER, CET and CMZ. 4. Each one strain of *Salmonella* C<sub>1</sub> and *Salmonella* E<sub>1</sub> groups were sensitive to all the drugs tested. 5. The resistant strains to double to quadruple drugs were found in 83% in *Klebsiella pneumoniae* and 63% in *Enterobacter cloacae*. In *Escherichia coli*, on the other hand, the resistant strains to triple drugs appeared in only 16%.

**Key words:** Drug sensitivity, Enteric bacteria, *Pseudomonas*, Drinking water, Indonesia

## INTRODUCTION

Among many diseases in Indonesia, infectious diarrhea diseases have remained as one of the most frequent ones. Chemotherapy with high reliability is mainly used for infectious diseases. The history of chemotherapy, however, also clearly has shown various restrictions and limitations in recent years (Yokota, 1984). The most important problems among these would be the increase of resistant strains.

From such viewpoint, we have conducted tests for sensitivity of enteric bacteria and Genus *Pseudomonas* isolated from drinking water in Jakarta, in order to assess the mode of appearance of resistant bacteria in Indonesia at this point.

## MATERIALS AND METHODS

**Test strains.** In the survey during July and August, 1983, sixty-five strains of enteric bacteria and twenty-five strains of Genus *Pseudomonas* were isolated from the drinking water in the City of Jakarta. The details on these bacteria are summarized in Table 1.

Table 1. Strains to sensitivity test

| enteric bacteria                       | number | <i>Pseudomonas</i> sp.          | number |
|--|--------|---------------------------------|--------|
| <i>Escherichia coli</i>                | 7      | <i>Pseudomonas aeruginosa</i>   | 10     |
| <i>Klebsiella pneumoniae</i>           | 6      | <i>Pseudomonas fluorescens</i>  | 7      |
| <i>Enterobacter cloacae</i>            | 23     | <i>Pseudomonas putida</i>       | 6      |
| <i>Enterobacter aerogenes</i>          | 12     | <i>Pseudomonas pseudomallei</i> | 2      |
| <i>Citrobacter freundii</i>            | 15     |                                 |        |
| <i>Salmonella</i> E <sub>1</sub> group | 1      | Total                           | 25     |
| <i>Salmonella</i> C <sub>1</sub> group | 1      |                                 |        |
| Total                                  | 65     |                                 |        |

Table 2. Chemotherapeutic agents to sensitivity test

| chemotherapeutics     |                     |
|-----------------------|---------------------|
| Benzylpenicillin: PCB | Gentamycin: GM      |
| Piperacillin: PIPC    | Kanamycin: KM       |
| Mezlocillin: MZPC     | Chloramphenicol: CP |
| Cephaloridine: CER    | Tetracycline: TC    |
| Cephalothin: CET      | Colistin: CL        |
| Cefoxitin: CFX        | Nalidixic acid: NA  |
| Cefmetazole: CMZ      |                     |
| Cephoperazon: CPZ     |                     |

**Sensitivity test.** Sensitivity test was conducted according to the ordinary method of disk agar diffusion using modified Müller-Hinton agar (Eiken chem. Co.,) and disks representing three levels of concentrations (Eiken chem. Co.,). Table 2 summarizes the names of the disk for the chemotherapeutic agents and their abbreviations. In the strains which seem to have resistance, MIC was measured by the dilution method according to the Japan Society of Chemotherapy (Japan Society of Chemotherapy, 1981).

## RESULTS

Figure 1 showed the distribution of drug sensitivity against the isolated enteric bacteria. All strains showed highest sensitivity (3+) to GM. All strains also showed higher sensitivity than 1+ to MZPC, CPZ, CP and TC. Higher sensitivity than 1+ to PIPC, KM, CL and NA was also found in 98 % of the strains. However, resistance to cephem antibiotics was noted in 23 % (CER), 28 % (CET) and 47 % (CFX) of the strains.

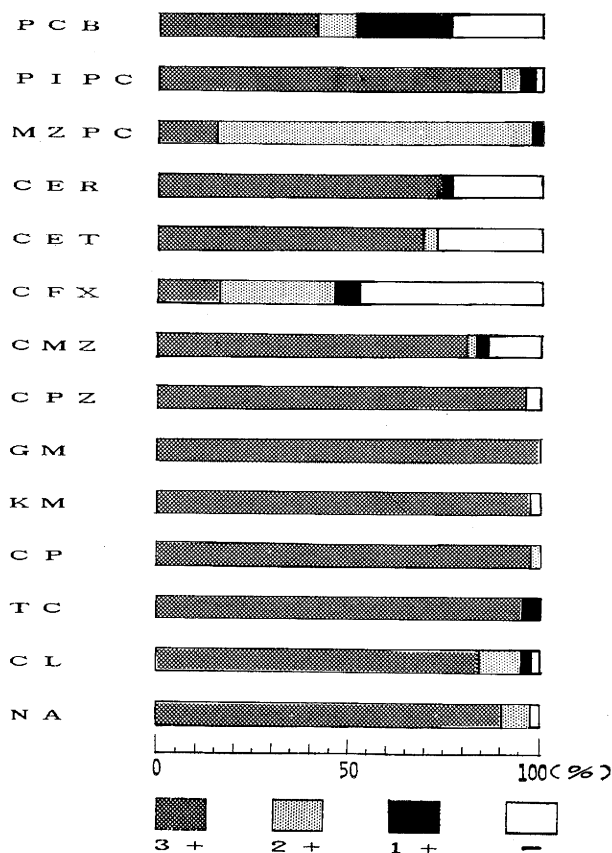


Fig. 1. Distribution of drug sensitivity against isolated enteric bacteria.

Figure 2 showed the distribution of drug sensitivity against the isolated strains of Genus *Pseudomonas*. A similar tendency to the distribution of that against the enteric bacteria was noted. All strains showed highest sensitivity (3+) to GM. All strains also showed higher sensitivity than 1+ to CL. More than 90 % of the strains showed sensitivity to PIPC, CPZ and TC. Resistance to NA, however, was seen in 96 % of the strains. Resistance to cephem antibiotics was noted in 82 % (CER), 96 % (CET), 96 % (CFX) and 63 % (CMZ) of the strains.

Figure 3 and 4 showed the results of tests for sensitivity of each species to the chemotherapeutic agents. In three of seven strains of *Escherichia coli* were resistant to CER and CET. One strain was resistant to CFX. All strains, however, showed sensitivity to other drugs. In the case of *Klebsiella pneumoniae*, all six strains showed resistance to CER, CET and CMZ, and four of six strains were resistant to CFX. All twenty-three strains of *Enterobacter cloacae* were resistant to NA and all twelve strains of *Enterobacter aerogenes* were resistant to CER and CL. Each one strain of *Salmonella* C<sub>1</sub> and *Salmonella* E<sub>1</sub> groups were sensitive to all the drugs tested.

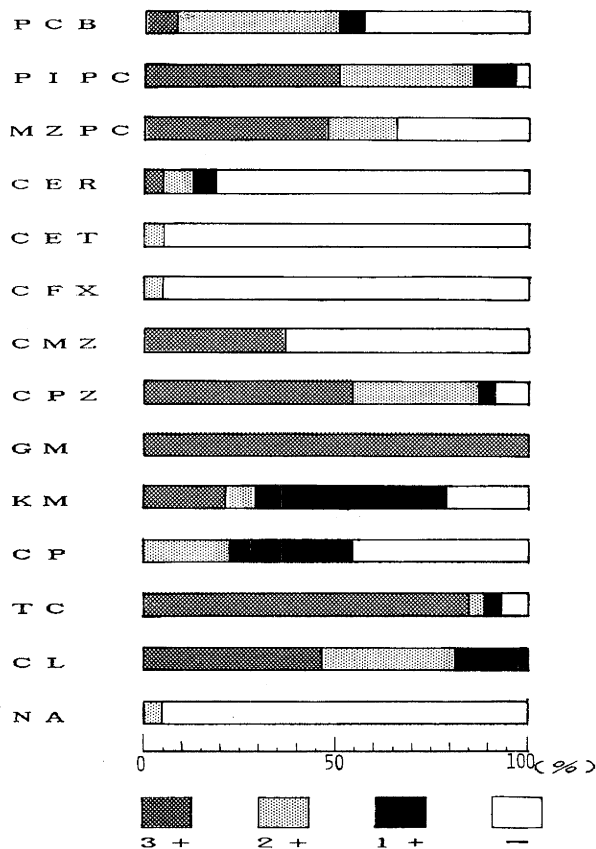


Fig. 2. Distribution of drug sensitivity against isolated Genus *Pseudomonas*.

Results of the sensitivity tests on strains of Genus *Pseudomonas* revealed similar tendency in each species. All strains were sensitive to GM and CL. Scarcely any sensitivity, however, was shown to CER, CET, CFX and CMZ. Almost all strains were sensitive to CPZ.

Table 3 showed the result of resistance of the enteric bacteria to multiple chemotherapeutic agents. The resistant strains to double to quadruple drugs were found in 83 % in *Klebsiella pneumoniae* and 63 % in *Enterobacter cloacae*. In *Escherichia coli*, on the other hand, the resistant strains to triple drugs appeared in only 16%.

MIC was measured in those strains which seemed to be resistant by the sensitivity test, as shown in Fig. 5. *Escherichia coli* was resistant to PCB and CET, *Klebsiella*

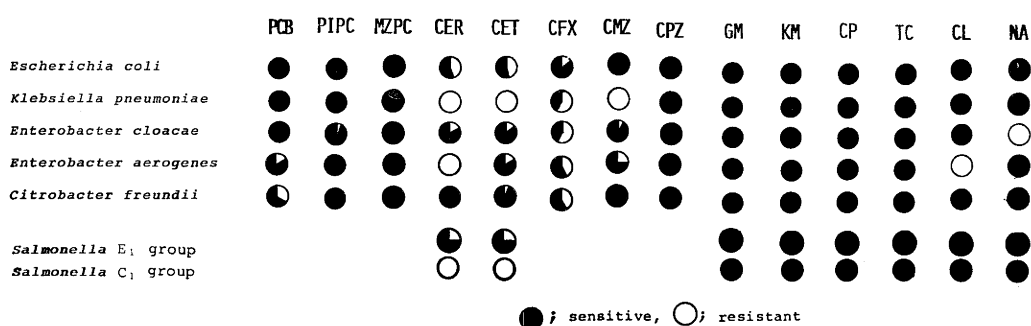


Fig. 3. Sensitivity of each enteric bacteria to chemotherapeutic agents.

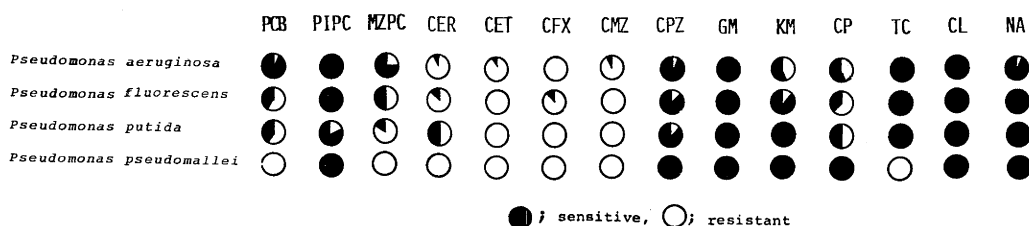


Fig. 4. Sensitivity of each *Pseudomonas* sp. to chemotherapeutic agents.

Table 3. Result of resistance of enteric bacteria to multiple chemotherapeutic agents

|                               | No. of strain | sensitive | resistant |        |        |           |
|-------------------------------|---------------|-----------|-----------|--------|--------|-----------|
|                               |               |           | single    | double | triple | quadruple |
| <i>Escherichia coli</i>       | 7             | 76(%)     | 8(%)      | 0(%)   | 16(%)  | 0(%)      |
| <i>Klebsiella pneumoniae</i>  | 6             | 0         | 17        | 33     | 17     | 33        |
| <i>Enterobacter cloacae</i>   | 23            | 5         | 32        | 32     | 26     | 5         |
| <i>Enterobacter aerogenes</i> | 12            | 50        | 17        | 17     | 8      | 8         |
| <i>Citrobacter freundii</i>   | 15            | 56        | 25        | 13     | 6      | 0         |

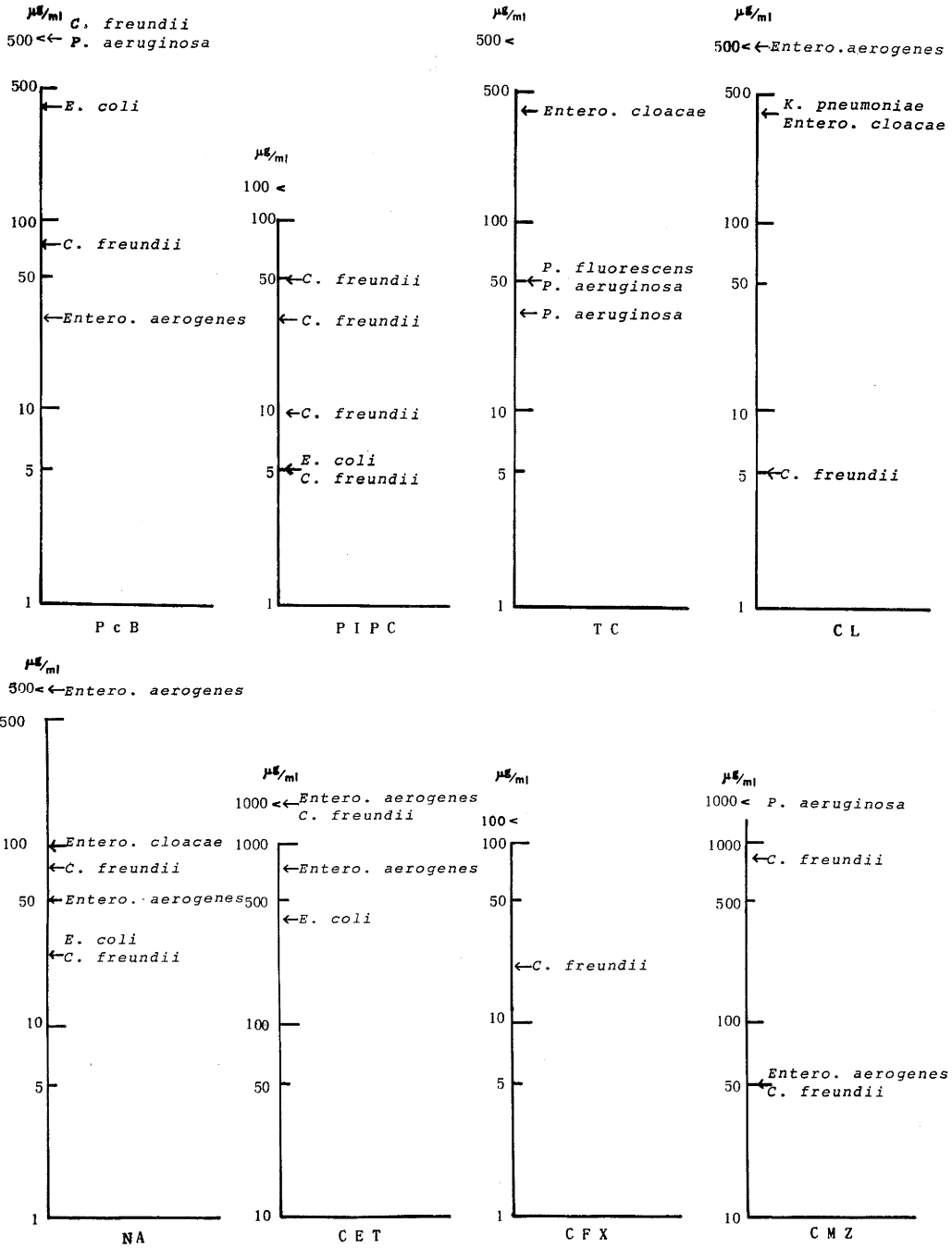


Fig. 5. Minimum inhibitory concentration of chemotherapeutic agents to resistant strains.

*pneumoniae* to CL and *Enterobacter cloacae* to TC and CL, at concentrations as high as 400 µg/ml. Resistance of *Enterobacter aerogenes* and *Citrobacter freundii* to CET and that of *Pseudomonas aeruginosa* to CMZ was found at very high concentrations exceeding 1,000 µg/ml.

## DISCUSSION

The sensitivity of bacteria isolated from drinking water of Jakarta to fourteen representative chemotherapeutic agents was tested. The enteric bacteria and Genus *Pseudomonas* were subjected to the test for sensitivity in the present study. Recently, two noteworthy facts concerning these bacteria were discussed in Japan and other advanced countries in Europe and United States. One is the appearance of strains resistant to aminoglycoside antibiotics such as GM, and the other is the appearance of strains resistant to  $\beta$ -lactum antibiotics especially the cephem drugs (Tanaka and Nakamura, 1977). These are explained by the administration of large doses of these antibiotics to mild infectious diseases or to preventive measures (Yokota, 1984).

According to results of present study, no resistance to GM was seen at all in the enteric bacteria and Genus *Pseudomonas*. In Japan, the resistance of *Klebsiella pneumoniae* to cefazolin (CEZ) was the first example of resistance to cephem drugs receiving attention of the clinicians for the first time in 1975 (Ueda and Mashita, 1981). According to our results, *Klebsiella pneumoniae* showed the highest resistance to cephem drugs among the strains of enteric bacteria. *Escherichia coli* was also found to be resistant to the first and second generation cephem drugs in about half of the strains tested.

Among glucose nonfermentative Gram-negative rods, *Pseudomonas aeruginosa* is thought to be important from the viewpoints of both frequency of detection and pathogenicity. These species are widely distributed in soil and water in the nature and frequently high resistant to chemotherapeutic agents to which other general Gram-negative rods are sensitive. Such resistance is thought to depend both on natural resistance by structural gene and on artificial resistance to multiple drugs controlled by plasmid (Ueda, 1984). For this reason, the third generation cephem drugs such as CPZ and latamoxef (LMOX) resistant to cephalosporinase of this species have been used. Among the strains isolated in Indonesia, almost no sensitive ones was seen at all to the first and second generation cephem and, on the contrary, no resistant strain was seen against GM. This fact suggests that relatively new chemotherapeutic agents are under current use in Indonesia.

In the 1970s, Gram-negative rods with mild virulence received attention as the causative microorganisms of intractable infectious diseases. Gram-negative rods of mild virulence including *Pseudomonas aeruginosa* frequently hold R-factor, and these rods are known to be highly resistant to the first generation cepheims, aminoglycosides, TC, CP and sulfonamides and synthetic antimicrobial agents such as NA (Tomioka *et al.*, 1977;

Mitsuhashi, 1982). Vast number of reports have subsequently appeared on the resistance of bacteria found in humans and animals to multiple drugs. As to the bacteria detected from the drinking water, Armstrong *et al.* (1981) reported the distribution of resistant strains which were isolated 2,653 strains from the drinking water of six cities in the State of Oregon. Resistant strains to double drugs was found in 61.0 %, those to triple drugs in 23.4 %, those to quadruple drugs in 12.7 % and those to quintuple drugs in 2.9 %. According to our results, resistant strains to multiple drugs were prominent, as seen in Table 3, 33 % of the strains of *Klebsiella pneumoniae* resistant to double drugs, 17 % to triple drugs and 33 % to quadruple drugs. In the results on MIC, resistant strain to high concentrations exceeding 1,000 µg/ml was seen in *Enterobacter aerogenes* and *Citrobacter freundii* against CET and *Pseudomonas aeruginosa* against CMZ. The fact will suggest that the grade of resistance of individual strains increase to each chemotherapeutic agent.

In view of these results, a great deal of attention has now been focussed on the future use of chemotherapeutic agents and the changes of infections in Indonesia.

#### REFERENCES

- 1) Armstrong, J. L., Shigeno, D. S., Calomiris, J. J. & Seidler R. J. (1981): Antibiotic-resistant bacteria in drinking water. *Applied and Environmental Microbiology*, 42 (2), 277-283.
- 2) Japan Society of Chemotherapy (1981): The second revision of the method of measurement of MIC Chemotherapy, 29 (1), 76-79. (in Japanese).
- 3) Mitsuhashi, S. (1982): Method of drug susceptibility test. 1-11. Kodansha Co., Tokyo. (in Japanese).
- 4) Tanaka, N. & Nakamura, S. (1977): Outline of antibiotics. 296-317, University of Tokyo press, Tokyo. (in Japanese).
- 5) Tomioka, S., Kobayashi, Y., Uchida, H. & Kameoka, Y. (1977): Susceptibilities of glucose nonfermentative Gram-negative rods to antimicrobial agents. *The SAISHIN-IGAKU*, 32 (8), 1454-1459. (in Japanese).
- 6) Ueda, Y. & Mashita, K. (1981): Modern chemotherapy. 3-13, Life science Co., Tokyo. (in Japanese).
- 7) Ueda, Y. (1984): Infectious diseases and chemotherapy. 19-41, Life science Co., Tokyo. (in Japanese).
- 8) Yokota, T. (1984): How to use of the new antibiotics. 1-5, Life science Co., Tokyo. (in Japanese).

---

インドネシアの飲料水より分離された腸内細菌および *Pseudomonas* 属の薬剤感受性について

奥脇義行, 矢内寿恵, 豊経子 (女子栄養大学微生物学研究室), 藤田紘一郎, 月館説子 (長崎大学医学部医動物学教室), 杉山雅俊 (順天堂大学医学部衛生学教室)



インドネシアでの疾病は、微生物感染症が常に上位を占めている。この感染症に対しては、主に化学療法が用いられるが、近年耐性菌の増加などの問題点が提起されている。そこで我々は、インドネシアの首都ジャカルタ市内の飲料水から分離した腸内細菌 65 菌株及び *Pseudomonas* 属 25 菌株に対して、PcB, PIPC, MZPC, CER, CET, CFX, CMZ, CPZ, GM, KM, CP, TC, CL, NA の 14 剤の化学療法剤について感受性試験を行い、インドネシアにおける耐性菌の出現状況などを知ることにした。その結果、以下のような成績が得られた。1. 腸内細菌では、全ての菌で GM に高度に感受性が示された。また、PIPC, MZPC, CPZ, KM, CP, TC, CL, NA でも良好な感受性がみられた。しかし、第 1 世代 cephem 剤の CER (23%), CET (28%), 第 2 世代 cephem 剤の CFX (47%) には抵抗性がみられた。2. *Pseudomonas* 属では、全ての菌で GM と CL に高度に感受性が示された。また、PIPC, TC 及び第 3 世代 cephem 剤の CPZ でも良好な感受性がみられた。一方 NA (96%) と CER (82%), CET (96%), CFX (96%), CMZ (63%) の cephem 剤には高い抵抗性が示された。3. 各々の菌種では、*E. coli* で第 1 世代 cephem 剤に約半数が抵抗性であった。*K. pneumoniae* でこの傾向が顕著にみられ、第 1 及び第 2 世代 cephem 剤に対して全てが抵抗性を示した。4. *Salmonella* C<sub>1</sub> 群 1 株、*Salmonella* E<sub>1</sub> 群 1 株は、それぞれすべての薬剤に感受性を示した。5. *Pseudomonas* 属は、第 1 及び第 2 世代 cephem 剤にほとんどの菌種が抵抗性を示したが、GM と CL には高い感受性がみられた。6. 多剤耐性を示す成績では、*E. coli* は 3 剤に 16% と比較的低い値であったが、*K. pneumoniae* は 2 剤 (33%), 3 剤 (17%), 4 剤 (33%), *En. cloacae* では 2 剤 (32%), 3 剤 (26%), 4 剤 (5%) などというように、すでに高い割合で多剤耐性が認められた。7. MIC の測定結果では、*E. coli* で PcB と CET, *K. pneumoniae* で CL, *En. cloacae* で TC と CL に対して、それぞれ 400 µg/ml に、さらには *En. aerogenes* と *C. freundii* で CET, *P. aeruginosa* で CMZ に対して、それぞれ 1000 µg/ml という非常に高濃度に耐性が示された。