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# Efficacy of Antibacterial Drugs in Mice with Complex Infection by *Candida albicans* and *Escherichia coli*

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We investigated the effect of seven antibacterial antibiotics: kanamycin, gentamicin, tetracycline, minocycline, ampicillin, piperacillin and cefotaxime, on survival of mice infected sequentially with a lethal dose of *Candida albicans* and a sublethal dose of *Escherichia coli*. The mortality of *C. albicans*-infected mice was facilitated by the superinfection with *E. coli*. When administered to mice with *C. albicans*/*E. coli* complex infection, aminoglycosides and tetracyclines significantly prolonged the survival period as compared with the infected and untreated controls. The recovery of viable counts of *E. coli* from the renal tissues was rapidly reduced by the treatment with gentamicin or minocycline, compared to the untreated control. Thus it was concluded that nullification by the treatment with aminoglycosides or tetracyclines of the enhancing effect of *E. coli* superinfection on the lethality of *C. albicans*-infected mice is due to early elimination of *E. coli* from the kidney.

Invasive candidiasis developed in immunocompromised patients, especially those with acute leukemia is often complicated with various bacterial infections. Complex infection with *Candida albicans* and Gram-negative bacteria including *Escherichia coli* has been reported to promote significantly morbidity and mortality of hospitalized compromised patients[1~6]. BURD *et al.* reported that synergistic lethality of mice was caused by complex infection with *C. albicans* and *E. coli* and that an endotoxin or lipopolysaccharide (LPS) component of *E. coli* played a major role in lethal responses of such mice[7].

We earlier reported that the lethality of *C. albicans*-infected mice was facilitated by the superinfection with *E. coli* or the administration of *E. coli* LPS, along with increased growth of *Candida* in renal tissues which were heavily invaded and damaged by the fungal organism[8]. This may be due to the increased production of tumor necrosis factor $\alpha$ (TNF$\alpha$) in mice with the complex infection. These studies suggest that for the management of patients with such complex infections those antibacterial drugs which minimize the enhancing effect of bacteria on fungal infections should be used, along with some antifungal agents.

The present study was undertaken to examine whether those antibacterial drugs which potentially decontaminate *E. coli* from the invaded tissues of the host with complex infection are effective in nullifying the promoting effect of the bacterial microorganism on the morbidity and mortality of the host. For this purpose, experiments were conducted using mice infected with a lethal dose of *C. albicans* and a sublethal dose of *E. coli* in combination.

## Materials and Methods

### Animals

Specific pathogen-free (SPF), 4 week-old, Crj:CD-1(ICR) female mice weighing $23 \pm 1$ g were purchased from Japan Clea Co., Ltd. (Atsugi, Japan) and used in all the experiments after being housed for 1 week under SPF conditions at the Laboratory Animal Center of Teikyo University.

Microorganisms

*C. albicans* TIMM1768, a clinically isolated strain preserved at the Teikyo Institute for Medical Mycology (Tokyo, Japan) was used. It was inoculated into Sabouraud dextrose broth supplemented with 0.5% yeast extract and cultures were grown at 37°C for 24 hours in a shaking water bath. Yeast cells were harvested by centrifugation at $1,500 \times g$ for 10 minutes and washed 3 times in sterile physiological saline. The cell number was counted with a hemocytometer, and a cell suspension containing $1 \times 10^6$ organisms/ml was prepared.

*E. coli* Ewing 4932/53 obtained from the Institute of Medical Science, the University of Tokyo, was grown at 37°C for 24 hours in brain heart infusion broth (Difco Laboratories, USA) without agitation. Cultures at the early stationary phase of growth were centrifuged at $1,500 \times g$ for 10 minutes to harvest the bacterial cells. They were washed 3 times and finally suspended in sterile saline. The concentration of cell suspension was adjusted to $1 \times 10^8$ cfu/ml spectrophotometrically and viable counts of suspension were determined on brain heart infusion agar plates in each experiment.

Antibacterial Agents and Susceptibility Testing

The following 7 antibacterial agents were used: 3 $\beta$-lactams, ampicillin (ABPC, Meiji Seika), piperacillin (PIPC, Toyama Chemical), and cefotaxime (CTX, Hoechst); 2 aminoglycosides, kanamycin(KM, Meiji Seika) and gentamicin (GM, Schering Plough); and 2 tetracyclines, tetracycline (TC, Sigma) and minocycline (MINO, Lederle). The *in vitro* susceptibility of *E. coli* Ewing 4932/53 to these antibiotics was tested by a conventional agar dilution method.

Infection of Mice and Administration of Antibiotics

Mice were infected with *C. albicans* and *E. coli*, singly or in combination, in a lethal intravenous dose ($1 \times 10^6$ cells/mouse) and a sublethal intraperitoneal dose ($2 \times 10^8$ cfu/mouse), respectively. To produce a murine model of complex infection with *C. albicans* and *E. coli*, mice were infected first with the fungal organism and then with the bacterial organism 3 hours later as described[8]. Survival of animals was checked daily during the experimental period of 2 or 3 weeks after *C. albicans* inoculation. Mice were given intraperitoneally a testing antibiotic immediately after the challenge of *E. coli* at a dosage of 1 mg/mouse, which was chosen after considering other papers[9,10]. Six experiments were conducted to examine the therapeutic efficacy of antibiotics using the above-mentioned animal model for superinfection. The efficacy of antibiotics was expressed as T/C ratios where T was the average survival period in days of antibiotic-treated groups of mice and C was that of an untreated control group of mice.

Enumeration of *C. albicans* and *E. coli* in Infected Tissues

Mice were sacrificed by cervical dislocation at 2, 12, 24 and 48 hour after superinfection with *E. coli*. The kidneys were removed, weighed and homogenized in sterile saline aseptically. An aliquot of one-tenth ml of the homogenates was inoculated onto *Candida* GS agar plates and DHL agar plates (both from Eiken Kagaku, Tokyo) for counting viable numbers of *C. albicans* and *E. coli*, respectively, after incubation at 37°C for $24 \sim 48$ hours.

Statistical Analyses

Statistical differences of survival periods between groups of mice superinfected with *C. albicans* and *E. coli* and mice infected with either microorganism alone were analyzed by Wilcoxon signed-ranks test and those of microbial viable counts in kidneys by Students' *t*-test. The effects of antibiotics on average survival period ratios (T/C) were analyzed by Wilcoxon signed-ranks test. Significant differences were assumed with $p < 0.05$.

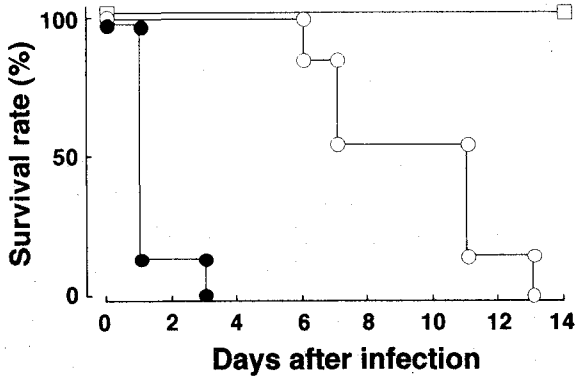
## Results

### Mortality of and Renal Invasion in Mice Infected with *C. albicans* and *E. coli*, Singly and in Combination

Throughout the present study, a lethal dose($1 \times 10^6$ cells/mouse) of *C. albicans* and a sublethal dose ($2 \times 10^8$ cfu/mouse) of *E. coli* were used for infecting mice. Figure 1 shows that when infected alone, *C. albicans* killed all the animals by day 13 postinfection with the mean survival period of $9.1 \pm 2.6$ days, while none of the mice infected with *E. coli* alone was dead by the end of the experimental period. The complex infection of *C. albicans* and *E. coli* killed 90% of animals within 24 hour postinfection with the mean survival period of $1.3 \pm 0.7$ days, indicating that a significant decrease in the survival period of *C. albicans*-infected mice was induced by superinfection with *E. coli* ($p < 0.05$) (Fig. 1).

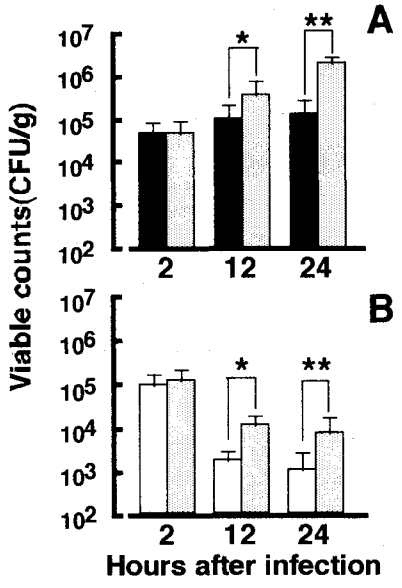
To understand the mechanism by which superinfection with *E. coli* facilitated the lethality of *C. albicans*-infected mice, viable counts of *C. albicans* and *E. coli* recovered from the kidney were compared among

Fig. 1. The survival rate of mice with complex infection by *C. albicans* and *E. coli*.




Survival rate for mice infected with *C. albicans* alone (○), *E. coli* alone (□), and both microorganisms in combination (●). In all groups ($n=8$), mice were intravenously inoculated with a lethal dose of *C. albicans* ($1\times10^6$ organisms/mouse), and/or intraperitoneally with a sublethal dose of *E. coli* ($2\times10^8$ cfu/mouse). The mice were inoculated first with *C. albicans* and 3 hours later with *E. coli* in the indicated dose. *: Statistical significance against a group of mice infected with *C. albicans* or *E. coli* alone: $p<0.01$.

Fig. 2. Recovery of *C. albicans* and *E. coli* from the kidney.




Recovery of *C. albicans* and *E. coli* from the kidney of mice infected with *C. albicans* alone (group-1, ■); *E. coli* alone (group-2, □); and *C. albicans* and *E. coli* in combination (group-3, ▨) 2, 12, and 24 hour postinfection. In Fig. 2A recovery of *C. albicans* was compared between group-1 ($n=5$) and group-3 ($n=5$); in Fig. 2B that of *E. coli* between group-2 ($n=5$) and group-3 ($n=5$). * and ** represent statistical significance at the $p$-value of $<0.05$ and $<0.001$, respectively. Detectable threshold was $10^2$ cfu/g.

mice infected with both and either of these two different categories of microorganisms. The kidney was chosen for culture study because it is known as the major target organ of *C. albicans* in mice. Figure 2 shows that viable counts of *C. albicans* in the kidney from the *C. albicans*/*E. coli*-infected mice were significantly higher than those from the *C. albicans*-infected mice 12 and 24 hour postinfection. Moreover, viable counts of *E. coli* in the kidney from the *C. albicans*/*E. coli*-infected mice were also significantly higher than those from the *E. coli*-infected mice 12 and 24 hour postinfection.

These results suggest the possibility that increased growth of *C. albicans* in the kidney of *C. albicans*/*E. coli*-infected mice would be associated with prolonged persistence of *E. coli* in this organ. This tempted us to investigate whether the treatment of the *C. albicans*/*E. coli*-infected mice with those antibacterial drugs which are active against *E. coli* but not *C. albicans* lower not only the viable counts of *E. coli* but also those of *C. albicans* in the kidney of the infected animals, resulting in cancellation of *E. coli*-induced enhancement of *C. albicans* infection.

## Effect of Antibacterial Drugs on the Recovery of *E. coli* from the Kidney in Mice Infected Only with *E. coli*

Seven antibacterial drugs comprising 3 β-lactams (ABPC, PIPC and CTX), 2 aminoglycosides (KM and GM), and 2 tetracyclines (TC and MINO) were examined for their activity to eliminate *E. coli* from the kidney in animals infected singly with the organism. The MIC values of ABPC, PIPC, CTX, KM, GM, TC and MINO against *E. coli* Ewing 4932/53 were 10, 25, 1.56, 3.13, 0.78, 1.56 and 0.78 μg/ml, respectively. All these drugs at a single dosage of 1 mg were administered intraperitoneally to mice immediately after the injection with $2\times10^8$ cfu of the organism.

As shown in Table 1, in *E. coli*-infected animals viable counts of the organism recovered from the kidney 2, 12 and 24 hour postinfection decreased significantly after

Table 1. Effect of three different classes of antibacterial drugs ($\beta$-lactams, aminoglycosides and tetracyclines) on viable counts of *E. coli* recovered from the kidney of mice infected with *E. coli* alone.

| Treatment with | Viable counts of *E. coli* recovered from the kidney (Log cfu/g) | | |
|---|---|---|---|
| | Hours after infection with *E. coli* | | |
| | 2 | 12 | 24 |
| Exp. 1 | | | |
| none | 5.22±0.96 | 4.52±0.67 | 3.31±0.28 |
| ampicillin | 4.61±0.85 | 4.79±0.10 | 3.85±0.27 |
| piperacillin | 4.71±0.45 | 4.44±1.46 | 3.96±0.67 |
| cefotaxime | 3.95±0.21* | 3.81±0.46 | 3.61±0.73 |
| Exp. 2 | | | |
| none | 4.28±0.13 | 3.71±0.15 | 3.05±0.08 |
| kanamycin | <2*** | <2*** | <2*** |
| gentamicin | <2*** | <2*** | <2*** |
| tetracycline | 2.29±0.35** | 2.09±0.30*** | <2*** |
| minocycline | 3.18±0.25* | <2*** | <2*** |

All groups consisted of 4 mice. Statistical significance against an untreated control group of mice: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.0001$

the treatment with KM, GM, TC or MINO, while they were only slightly lowered by the treatment with ABPC, PIPC or CTX. All of these antibacterial drugs were virtually without effect on the yield of viable counts of *C. albicans* from the kidney when administered to mice infected with $1 \times 10^6$ cells of the yeast (data not shown).

## Effect of Antibacterial Drugs on Survival of Mice with Complex Infection with *C. albicans* and *E. coli*

The above-mentioned 7 antibacterial drugs comprising 4 which were effective in accelerating the elimination of bacteria from the kidney in *E. coli*-infected mice (KM, GM, TC and MINO) and 3 without such efficacy (ABPC, PIPC and CTX) were studied. Because the average survival period of the untreated infection control group of mice varied 1.3 to 2.5 days from experiment to experiment, the efficacy of each antibacterial agent tested was shown by T/C ratio which represents the relative survival period as described in Materials and Methods. The results of 7 experiments are summarized in Table 2. All of the aminoglycosides (KM and GM) and tetracyclines (TC and MINO) significantly prolonged the survival period ($p < 0.01$ or $p < 0.05$) compared to that of the untreated control. By contrast, none of the $\beta$-lactams (ABPC, PIPC and CTX) prolonged but, rather, shorten, although not significantly, the survival period. As a result, the 7 antibacterial drugs tested were classified into two groups depending on whether or not they were effective in prolonging the survival of mice with complex infection with *C. albicans* and *E. coli*. The effective drug group comprised aminoglycosides and tetracyclines and the ineffective one $\beta$-lactams.

## Effect of Antibacterial Drugs on Renal Invasion of *C. albicans* and *E. coli* in Mice with Complex Infection by These Fungal and Bacterial Organisms

Based on the results of the above experiments, 2 drugs were chosen from the effective drug group, *viz*, GM and MINO and 2 from the ineffective drug group, *viz*, ABPC and CTX and investigated for their effectiveness (or ineffectiveness) to reduce viable counts of *C. albicans* and/or *E. coli* in the kidney of mice with the complex infection. Mice infected with a lethal dose of *C. albicans* were superinfected with a sublethal dose of *E. coli* 3 hour later, and then the antibacterial drug being tested at a single dosage of 1 mg/kg was injected immediately. Table 3 illustrates the recovery of *E. coli* from the kidney of mice treated with the antibacterial drugs 2, 12 and 24 hour after the *E. coli* inoculation. As compared with the untreated control mice showing recovery at the level of

Table 2. Therapeutic efficacy of three different classes of antibacterial drugs in mice with complex infection by *C. albicans* and *E. coli*.

| Antibacterial drug | The mean survival days ± SD | T/C ratio[b] | Statistical significance[c] |
|---|---|---|---|
| **β-lactams :** | | | |
| ampicillin(n=8)[a] | 1.6±0.5 | 0.64 | N.S.[d] |
| piperacillin(n=8) | 1.5±0.5 | 0.60 | N.S. |
| cefotaxime(n=8) | 2.9±2.5 | 1.16 | N.S. |
| **Aminoglycosides :** | | | |
| kanamycin(n=8) | 4.3±1.0 | 1.95 | $p<0.01$ |
| gentamicin(n=6) | 4.8±1.2 | 2.29 | $p<0.05$ |
| **Tetracyclines :** | | | |
| tetracycline(n=6) | 3.7±1.4 | 2.18 | $p<0.05$ |
| minocycline(n=6) | 5.1±1.3 | 2.43 | $p<0.05$ |

[a] Number of mice tested.
[b] Therapeutic efficacy of antibacterial drugs was expressed as T/C ratio as described in Materials and Methods.
[c] Statistical significance against an untreated control group of mice( β -lactams; n=8, Aminoglycosides; n=10, Tetracyclines; n=10)
[d] N.S.: not significant.

Table 3. Effect of two β-lactams, ampicillin and cefotaxime, an aminoglycoside, gentamicin, and a tetracycline, minocycline, on viable counts of *E. coli* recovered from the kidney of mice with complex infection by *C. albicans* and *E. coli*.

| Treatment with: | Viable counts of *E. coli* recovered from the kidney (Log cfu/g) | | |
|---|---|---|---|
| | Hours after infection with *E. coli* | | |
| | 2 | 12 | 24 |
| none | 4.74±0.42 | 4.90±0.14 | 3.41±0.37 |
| ampicillin | 4.78±0.43 | 5.09±0.25 | 3.74±0.37 |
| cefotaxime | 4.24±0.14 | 4.21±0.17 | 4.22±0.16 |
| gentamicin | <2.5*** | < 2.5*** | < 2.5** |
| minocycline | 3.01±0.15** | 3.07±0.48** | 2.72±0.26* |

All groups consisted of 4 mice. Statistical significance against an untreated control group of mice: * $p<0.05$, ** $p<0.01$ and *** $p<0.001$

$10^{4.7}\sim10^{5}$ and $10^{3.5}$ cfu/g tissues 2 to 12 and 24 hour postinfection, respectively, the value was rapidly reduced to level as low as $<10^{2.5}$ and $10^{3}$ cfu/g tissues in animals treated with GM and MINO, respectively , even 2 hour postinfection. By contrast, the *E. coli* recovery in ABPC- and CTX-treated mice was scarcely or not significantly lowered 2 and 12 hour postinfection, but instead increased as compared with that for untreaed control mice. None of these 4 drugs had significant effect on the recovery of *C. albicans* in comparison with untreated controls (data not shown).

## Discussion

We recently reported that the survival period of mice lethally infected with *C. albicans* was significantly shortened by a superinfection with a sublethal dose of

*E. coli*[8]. In consistence, the present study demonstrated that such a superinfection with *E. coli* also enhances mortality of *C. albicans*-infected mice (Fig.1). This tempted us to investigate following issues: (1) what role does *E. coli* play in *C. albicans*/*E. coli* complex infection in mice?, and (2) can some anti-*E. coli* antibiotics cancel the enhancing effect of *E. coli* on a preceding *C. albicans* infection in mice?

Regarding the first issue, experiments were conducted to measure viable counts of challenged microorganisms in the kidney, which is known as the primary target organ for *Candida* invasion, among *C. albicans*-infected, *E. coli*-infected and *C. albicans*/*E. coli*-infected mice. The results demonstrated that viable counts of *C. albicans* recovered from animals infected with *C. albicans*/*E. coli* were significantly greater than those from mice infected with *C. albicans* alone, and that viable counts of *E. coli* recovered from mice infected with *C. albicans*/*E. coli* were also significantly greater than those from mice infected with *E. coli* alone. Furthermore, it was also demonstrated that mice with complex infection survived a shorter period than those with a single infection with *C. albicans*. It therefore looks likely that both *C. albicans* and *E. coli* invading renal tissues enhance the infection of the other. There are several papers which show that lethality of *C. albicans* is synergistically promoted in the presence of *E. coli*[2~4,7,8]. However, so far as we know, this is the first paper demonstrating that such a promotion is related with enhanced growth or persistence of both species of organisms in the renal tissues of mice with complex infection.

These results led us to the possibility that some anti-*E. coli* antibiotics with an activity to eliminate *E. coli* from the kidney are effective in canceling the promotional effect of *E. coli* on lethality of *C. albicans*, while some other antibiotics without such an activity are ineffective. To investigate this postulation, 4 antibiotics, comprising 2 aminoglycosides, KM and GM and 2 tetracyclines, TC and MINO, active in eliminating *E. coli* from the kidney of mice infected with this bacterial microorganism, and 3 β-lactams, ABPC, PIPC and CTX, without such an activity were tested for their effect on the survival of mice with *C. albicans*/*E. coli* complex infection. As expected, all of the aminoglycosides and tetracyclines were effective in prolonging the survival period of infected mice, while all the β-lactams were ineffective. Our postulation was also supported by the finding obtained from the experiments using 4 antibiotics chosen from an aminoglycoside GM, a tetracycline MINO and 2 β-lactams, ABPC and CTX, that the former two drugs rapidly decreased viable counts of *E. coli* in the renal tissues while the latter 2 did not in mice with complex infection, as was the case for mice infected with *E. coli* alone, compared with untreated controls.

It therefore looks likely that the effect of aminoglycosides and tetracyclines to prolong the survival of mice with *C. albicans*/*E. coli* complex infection is mainly due to elimination of *E. coli* from the kidney at the early stage of superinfection. Although not to a significant extent, administration of β-lactams to mice with complex infection somewhat shortened the survival period. SHENEP and MOGAN[10,11] reported that in *E. coli*-infected rabbits endotoxin was released during antibacterial therapy and that a prominent endotoxin release was induced by treatment with some β-lactams. It was also shown that endotoxin is liberated from *E. coli* incubated *in vitro* in the presence of β-lactams or some other classes of antibiotics[12]. Moreover, the mortality of *C. albicans*-infected mice was observed to be enhanced by exogenous administration of *E. coli* endotoxin[7,8]. It thus seems that the apparent effect of β-lactams to shorten the survival of mice with complex infection is at least partly related with the release of bacterial endotoxin induced by the antibiotics.

Recently, WEINSTEIN *et al.*[13] noted that infection with combination of *C. albicans* and *E. coli* in hospital became a serious problem in their epidemiological study. In such situation, our study described above confirms the importance of choosing appropriate antibacterial drugs, along with an antifungal drug, to treat patients with *C. albicans*/*E. coli* complex infection.

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## References

1) BAKALETZ, L. O.: Viral potentiation of bacterial superinfection the respiratory tract. Trends in Microbiology 3: 110~114, 1995
2) BILLETER, M.: Rationale and experience in treating suspected hospital based mixed infections. Pharmacotherapy 15 (1pt 2): 22S~26S, 1995
3) DIPIRO, J. T.: Considerations for therapy of mixed infections: Focus on intraabdominal infection. Pharmacotherapy 15 (1 pt 2): 15S~21S, 1995
4) DYESS, D. L.; R. N. GARRISON & D. E. FRY: *Candida* sepsis : Implications of polymicrobial blood-borne infection. Arch. Surg. 120: 345~348, 1985

5) SAUNDERS, G. L.; J. M. J. HAMMOND, P. D. POTGIETER, H. A. PLUMB & A. A. FORDER: Microbiological surveillance during selective decontamination of the digestive tract. J. Antimicrob. Chemother. 34: 529～544, 1994
6) STOUTENBEEK, C. P.; H. K. F. VAN SAENE, D. R. MIRANDA, D. F. ZANDSTRA & B. BINNENDIJIK: The prevention of superinfection in multiple trauma patients. J. Antimicrob. Chemother. 14 (Suppl. B): 203～211, 1984
7) BURD, R. S.; C. S. RAYMOND & D. L. DUNN: Endotoxin promotes synergistic lethality during concurrent *Escherichia coli* and *Candida albicans* infection. J. Surg. Res. 52: 537～542, 1992
8) AKAGAWA, G.; S. ABE & H. YAMAGUCHI: Mortality of *Candida albicans*-infected mice is facilitated by superinfection of *Escherichia coli* or administration of its lipopolysaccharide. J. Infect. Dis. 171: 1539～1544, 1995
9) KERR, J.: Inhibition of fungal growth by *Pseudomonas aeruginosa* and *Pseudomonas cepacia* isolated from patients with cystic fibrosis. J. Infect. 28: 305～310, 1994
10) SHENEP, J. L. & K. A. MOGAN: Kinetics of endotoxin release during antibiotic therapy for experimental Gram-negative bacterial sepsis. J. Infect. Dis. 150: 380～388, 1984
11) SHENEP, J. L.; R. P. BARTON & K. A. MOGAN: Role of antibiotic class in the rate of liberation of endotoxin during therapy for experimental Gram-negative bacterial sepsis. J. Infect. Dis. 151: 1012～1018, 1985
12) GOTO, H. & S. NAKAMURA: Liberation of endotoxin from *Escherichia coli* by addition of antibiotics. Japan J. Exp. Med. 50: 35～41, 1980
13) WEINSTEIN, M. P.; M. L. TOWNS, S. M. QUARTEY, S. MIRRETT, L.G. REIMER, G. PARMIGIANI & L.B. RELLER: The clinical significance of postive blood cultures in the 1990s: A prospective comprehensive evalution of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. Clin. Infect. Dis. 24: 584～602, 1997