Why Do Antimicrobial Agents Become Ineffectual?

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and Ryouichi Okamoto

Antibiotic resistance has evolved over the past 50 years from a merely microbiological curiosity to a serious medical problem in hospitals all over the world. Resistance has been reported in almost all species of gram-positive and -negative bacteria to various classes of antibiotics including recently developed ones. Bacteria acquire resistance by reducing permeability and intracellular accumulation, by alteration of targets of antibiotic action, and by enzymatic modification of antibiotics. Inappropriate use of an antibiotic selects resistant strains much more frequently. Once resistant bacteria has emerged, the resistance can be transferred to other bacteria by various mechanisms, resulting in multiresistant strains. MRSA is one of the typical multiresistant nosocomial pathogens. A study of the PFGE pattern of endonuclease-digested chromosomal DNA showed that MRSA of a few clones were disseminated among newborns in the NICU of a Japanese hospital. In this regard, it is important to choose appropriate antibiotics and then after some time, to change to other classes to reduce the selection of resistant strains. Since the development of epoch-making new antibiotics is not expected in the near future, it has become very important to use existing antibiotics prudently based on mechanisms of antibiotic action and bacterial resistance. Control of nosocomial infection is also very important to reduce further spread of resistant bacteria.

Key Words: Emergence of resistance, nosocomial spread, Cmax/MIC80, MRSA, coagulase type, PFGE patterns

The use of broad-spectrum antimicrobial agents has had a profound effect on the trend of bacterial infection in patients and on the emergence of such gram-positive bacteria as methicillin-resistant Staphylococcus aureus (MRSA), penicillin-resistant Streptococcus pneumoniae (PRSP) and multiresistant Enterococcus spp. (Tomasz, 1994; Bax et al. 1998).

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Also, the use of antimicrobial agents has resulted in the decrease of β-lactam- and quinolone-sensitive Pseudomonas aeruginosa, Enterobacter cloacae, and Serratia marcescens. These phenomena may be explained by the similar chemical structures of many antimicrobial agents, the prolonged clinical use of antimicrobial agents and the failure to control the nosocomial spread of infections.

The recent emergence of new antimicrobial resistance has drawn our attention: for example cephalosporin-resistant Escherichia coli and Klebsiella pneumoniae and vancomycin-resistant enterococci in other countries (Bush et al. 1995; Livermore and Yuan, 1996; Bell et al. 1998), carbapenemase-producing gram-negative bacilli (Watanabe et al. 1991; Ito et al. 1997), erythromycin-resistant Helicobacter pylori (Kobayashi et al. 1997) and vancomycin-intermediate MRSA in Japan (Hiramatsu et al. 1997a; Hiramatsu et al. 1997b).

Volume 39
The development of new antimicrobial agents requires a lengthy processes, including various evaluation procedures, before their approval for clinical use by the health authority. Moreover, a recent decrease in the efforts of pharmaceutical companies resulted in a reduction in the development of new antimicrobial agents. Therefore, it has become important to use existing antibacterial agents discreetly to be able to extend their usefulness as long as possible.

An antimicrobial agent which is suspected to lose its effectiveness because of emergence of resistant bacteria, must probably have dropped out during its developmental stage. Therefore, it can be considered that among the more than 150 antibacterial agents developed and currently available in Japan, there must have been no worry about the emergence of resistance at the time of development. If resistant strains to these antibacterial agents emerged, it should be considered that the users or some other factors, but not the drugs themselves, were responsible. We need to understand the mechanisms of action of antimicrobial agents and the resistance of bacteria before we attempt to prevent the selection and spread of resistant bacteria.

Factors which determine activities of antibacterial agents

When we want to clarify why antimicrobial agents become inactive, we must first understand how they act against bacteria. Different classes of antibiotics, such as cefalosporins, penicillins, quinolones, aminoglycosides and macrolides have different chemical structures. Also, bacteria have different cell structure depending on species.

The charge of a drug molecule affects the reaching of antibiotics to bacterial cell surface significantly. For example, most cefalosporins are negatively charged, while lipopolysaccharide and various other carbonic acids (\(-\text{COOH}\) on the surface of bacterial cells also render them negatively charged. Thus, cefalosporins have difficulty in reaching the bacterial cell surface. In contrast, aminoglycosides and carbapenems such as imipenem, panipenem and meropenem can easily reach the bacterial cell surface because of their positive charge.

The antibiotics must penetrate into bacterial cells to exert antimicrobial activities. Several factors affect the penetration rate of antibiotics (Hancock, 1981; Nikaido and Vaara, 1985; Yoshimura and Nikaido, 1985; Satake et al. 1990). The permeability of third-generation cephalosporin, such as cefotaxime, ceftazidime, the monobactam class drug aztreonam and carbapenems into cell of E. coli and P. aeruginosa were compared (Nikaido and Vaara, 1985; Satake et al. 1990). Imipenem can penetrate into cells of E. coli about 20 times easier compared to cefotaxime, ceftazidime and aztreonam. This may be partly due to the fact that imipenem is much smaller in size.

Permeability may also differ depending on bacteria. Satake et al. and others (Hancock et al. 1979; Gotoh et al. 1989; Satake et al. 1990) have suggested lower permeability of ceftazidime, imipenem, panipenem and meropenem through the outer membrane of P. aeruginosa than into E. coli. We also found lower permeability of imipenem, meropenem and a new carbapenem, biapenem into cells of P. aeruginosa than into those of E. coli (Iyobe et al. 1999). Fig. 1 schematically shows the penetration of drugs into bacterial cells. The cell wall of gram-positive bacteria such as S. aureus and S. epidermidis have a thick peptidoglycan layer. However, as peptidoglycans of these bacteria are loosely linked in a net-like manner, drugs can freely pass through this layer into the bacterial cell. Therefore, the problem of impermeability of antibiotics is almost non-existent in gram-positive bacteria. On the other hand, the hydrophilic property of a drug is also an important factor in determining permeability. The peptidoglycan layer of gram-negative bacteria, such as E. coli and P. aeruginosa, is very thin, but as it is covered with lipophilic phospholipid, lipid A and lipoprotein layer, hydrophilic antibiotics pass through this layer with difficulty. New quinolones are more active than cefalosporins against gram-negative bacteria such as P. aeruginosa because they can pass through the phospholipid bilayer more efficiently than cefalosporins (Hancock et al. 1979; Hancock, 1981).

If the bacterial cell wall is not permeable even to nutrients, such as carbohydrates and amino acids, bacteria cannot multiply. Therefore, there are pores called porin on the outer membrane layer, through which hydrophilic substances can penetrate into the
cell. As some quinolones are lipophilic, they cannot penetrate the hydrophilic route. Since porin has a property to favor passage of positively-charged substances (Yoshimura and Nikaido, 1985; Gotoh et al. 1989), cephalosporins, which are negatively charged, have difficulty passing through.

The size of porins differs depending on bacterial species: for example, the porins of E. coli allow passage of compounds with a molecular weight of 600, while those of P. aeruginosa, which are small in size, permit passage of compounds with a molecular weight of about 350 (Nikaido and Vaara, 1985; Satake et al. 1990). Therefore, cephalosporins with a molecular weight of 380–650 can penetrate easily into cells of E. coli, but not well into cells of P. aeruginosa. Macrolides having a molecular weight of 780–910 cannot pass easily through the porin of gram-negative bacilli. Thus, macrolides are not active against gram-negative bacteria, while they are active against S. aureus. However, some staphylococci carry an mrsA gene, which encodes a cytoplasmic membrane protein that mediates the so-called active macrolide-efflux system (Wondrack et al. 1996; Matsuoka et al. 1998).

Antimicrobial agents-inactivating enzymes

After penetration of the outer membrane, drugs must encounter inactivating enzymes, such as hydrolyzing or modifying ones, which may be present in the periplasmic space (Fig. 1). For example, β-lactams are hydrolyzed by β-lactamases, resulting in inactivation and they cannot react with the target site. Table 1 shows the influence of typical class C β-lactamases on minimum inhibitory concentrations (MICs) of β-lactams for E. coli K12. Without the presence of β-lactamase, MICs of β-lactams for this strain were low (0.1–0.2 μg/mL), indicating that the β-lactams are active. However, the addition of β-lactamase elevated the MICs dose dependently, indicating that the β-lactams were inactivated by the β-lactamases (Ambler, 1980; Hiraoka et al. 1988a; Hiraoka et al. 1988b).

Gram-negative bacteria usually produce class C β-lactamase inducibly (Gootz and Sander, 1983; Maejima et al. 1987; Jones et al. 1997). In the absence of an inducing β-lactam, the level of β-lactamase production is low, but when it is present the production increases. When the inducer is removed, the rate of enzyme production returns to a low basal level. However, derepressed gram-negative bacteria are the result of a genetic change in which the induction mechanism is lost (Livermore et al. 1982; Higashitani et al. 1990). A number of investigators have reported the isolation of derepressed mutants with resistance to one or more of the β-lactams, including piperacillin, cefotaxime and ceftazidime following therapy with various β-
Table 1. Influence of the class C β-lactamase on MICs of β-lactams for E. coli K-12 strain

<table>
<thead>
<tr>
<th>β-lactams</th>
<th>MIC (μg/mL)* in the presence of β-lactamase (U/well)*:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Cephaloridine</td>
<td>0.39</td>
</tr>
<tr>
<td>Cefotiam</td>
<td>0.2</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0.1</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0.2</td>
</tr>
<tr>
<td>Cefozopran</td>
<td>0.1</td>
</tr>
<tr>
<td>Cefepime</td>
<td>0.1</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.1</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*: β-lactamase originated from E. cloaca GN467.  
*: MIC was determined with inoculum of 1.0 x 10^5 CFU/well.

![Fig. 2. MICs of β-lactams against E. cloaca KU69 mutants which produce various levels of class C β-lactamase. IMP, imipenem; PAPM, panipenem; CFPM, cefepime; CTM, cefotiam; CAZ, ceftazidime; CER, cephaloridine.](image)

lactams. These stably derepressed multiple β-lactam-resistant gram-negative bacilli emerge in clinical settings as a result of the selection of a minority population of pre-existing derepressed mutants and not as a result of β-lactamase induction (Livermore et al. 1982).

MICs of cephaloridine, cefotiam, ceftazidime, and cefepime, as well as the carbapenems, imipenem and panipenem, for the strains of E. cloaca KU69 with various class C β-lactamase activities are shown in Fig. 2. The older cephalosporins are more susceptible to inactivation by class C enzyme, while the newer ones are more resistant.

Mutant cells usually appear at a rate of 1 in every 10^3 to 10^5 cells when antibiotics are used for long time. For example, MICs of ceftazidime and cefotaxime are usually 0.1 - 0.2 μg/mL for Enterobacter cloaca and Citrobacter freundii. However, MICs for isolates from patients receiving these antibiotics for a prolonged period were elevated by more than 10^3-fold. It is clear that mutants survived by producing 10^3-fold or more inactivating enzymes. In contrast, MICs of imipenem remained almost unchanged, which demonstrated that an increase of cephalosporin-inactivating enzymes does not affect activities of carbapenems.

Target sites of antimicrobial agents

When a drug passes through the outer membrane of bacteria, it can reach the target, unless an inactivating enzyme is present in the periplasm. The target sites are the cell-wall synthesizing enzyme for β-lactams, the biosynthesis system of DNA for quinolones, and protein-producing ribosome for aminoglycosides (Hancock, 1981; Yamagishi et al. 1996). There is no remarkable difference among antibiotics of various classes if they are compared in terms of inhibition of cell-wall synthesizing enzymes. The antibacterial activities of β-lactams against gram-negative bacteria increase with an advance in generation (e.g. from first- to fourth-generation), while no difference is seen in activity at the target site.
Therefore, the higher antibacterial activities of third-generation β-lactams compared to first- and second-generation may be explained by the improvement of permeability and stability to the action of β-lactamases.

It is known that β-lactams not only inhibit cell-wall synthesizing enzymes, but also cause morphological changes of bacteria. For example, carbapenems make bacilli rounded while cephalosporins and penicillins make them elongated. Even if bacteria with morphological changes due to the effect of antibiotics are insensitive to drugs, they will undergo lysis because of the weakened cell wall. Thus, there is little possibility that morphologically-changed bacteria can be isolated from clinical specimens. It is clinically important that the amount of endotoxin released from bacterial cells differs greatly depending on whether bacteria are rounded or elongated. From elongated bacteria due to the action of β-lactams, 20 to 30 times the amount of endotoxin is released compared to that from rounded bacteria (Horii et al. 1998; Trautmann et al. 1998).

Levels of antimicrobial agents and the incidence of emergence of resistant isolates

We determined to which antibiotic bacteria acquire resistance most easily by testing the incidence of resistance mutants of E. cloacae KU69 in various antibiotic concentrations in vitro (Fig. 3). In the presence of 32 x MIC (8 μg/mL) and 512 x MIC (128 μg/mL) of ceftazidime, resistant cells survived at a rate of 10^{-7} and 10^{-8}, respectively. In other words, if 10^5 cells/mL of bacteria were present in urine of a patient with urinary tract infection, one liter of urine must contain at least one surviving bacterium. Therefore, if ceftazidime is administered for a prolonged period, resistant bacteria gradually increase and are finally isolated like a pure culture. On the other hand, when cefepime is present at a concentration of 32 x MIC (2 μg/mL), no resistant strains will be isolated, indicating no selection of resistant strains at this concentration. Since nearly 100 μg/mL of blood level can be achieved by the intravenous injection of cefepime, it is considered that resistant organisms will not appear.

After incubation of bacteria with imipenem, panipenem and meropenem, no resistant mutants were isolated. That was because these drugs can easily penetrate into bacterial cells. With respect to cefoperazone, resistant bacteria could not be isolated any longer when a concentration of 64 x MIC (16 μg/mL) was present. If cefoperazone was present together with the β-lactamase inhibitor sulbactam, no resistant bacteria appeared even when the concentration was 16 x MIC (8 μg/mL), indicating that inhibition of the enzyme renders acquisition of resistance difficult for the bacteria.

The dosage actually used in clinical practice is an important factor in determining whether resistant strains can develop or not. A C_{max}/MIC_{50} value which is calculated by dividing the highest blood level of the drug (C_{max}) by MIC_{50} for each clinical isolate of bacteria influenced the emergence of resistance. For example, if the MIC_{50} of ceftazidime for E. coli is 0.125 – 0.25 μg/mL and the blood level of ceftazidime can be reached to 70 μg/mL, C_{max}/MIC_{50} is 280 – 560. It was considered that resistant strains can hardly be selected even after prolonged use of an antibiotic if the blood concentration of a drug reaches several hundred times the MICs. Therefore, it is assumed that ceftazidime-resistant E. coli strains will not be selected except for special circumstances. In contrast, the C_{max}/MIC_{50} values of ceftazidime for S. marcescens and P. aeruginosa are only 20 – 30. Similar results were

![Fig. 3. Incidence of resistant cells from E. cloacae KU69, depending on the presence of various concentrations of antimicrobial agents. IMP, imipenem; MEPM, meropenem; PAPM, panipenem; AZT, aztreonam; CTM, cefotiam; CAZ, ceftazidime; CTX, cefotaxime; CPZ, cefoperazone; SBT, sulbactam; CFPM, cefepime; CPR, cefpiramide.](image-url)
Why Do Antimicrobial Agents Become Ineffectual?

obtained for cefepime and cefpirome. However, it is assumed that selection of resistant strains will occur even with third- and fourth-generation cephalosporins if they are used for a prolonged period of time.

Transferable and nontransferable antibiotic resistance

The clinical emergence of resistant strains cannot be explained by this selection alone. Then, how can resistant bacteria appear in clinical settings? When strains of E. coli are isolated from patients and examined for MICs, most of the cases show a monophasic distribution pattern, but some cases show biphasic distribution. It can be said that sensitive strains are genetically different from resistant ones from the beginning. Clinically, few resistant cells among many susceptible ones pose little problem since they disappear if high levels of drugs are present. However, if some resistant bacterial cells survive even in the presence of high concentrations of antibiotics, they can become a problem clinically.

It was found in the 1960s and 1970s that bacteria resistant to tetracycline, streptomycin and kanamycin increased through contact between bacterial cells (Mitsuhashi and Inoue, 1980; Mitsuhashi and Inoue, 1981). Currently, it is considered that there are two types of drug resistance: the transferable plasmid

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Transfearable (plasmid) type</th>
<th>Nontransferable (chromosomal) type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycosides</td>
<td>+</td>
<td>(+)*</td>
</tr>
<tr>
<td>Penicillins</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>Carbapenems</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Macrolides</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Sulfanilamide</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>+</td>
<td>(+)*</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Quinolones</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*: Positive in parenthesis indicates occurrence with low frequency.
*: Minocycline.

Fig. 4. Difference in proliferation patterns of resistant isolates depending on the transferability of resistance. Black cell, resistant; open cells, susceptible. In transferable resistance, the resistance is transferred from cell A to all of the cells (B-H). In nontransferable resistance, use of an antibiotic selects resistance cells which survive and multiply, while all of the susceptible cells (B-F) are killed.
type, which is spread through contact between bacterial cells and nontransferable chromosomal type (Table 2). Transferable resistance in gram-positive bacteria was reported to aminoglycosides, penicillins and macrolides, as well as enterococci to vancomycin (Inoue et al. 1994; HICPAC, 1995; Hiramatsu et al. 1997; Johnson, 1998).

Nontransferable resistance is found to cephalosporins and quinolones. Recently, nontransferable resistance became a problem in MRSA and gram-negative bacteria (Inoue et al. 1994; Tomasz, 1994; Livermore and Yuan, 1996). Nontransferable resistance is not spread by bacterial cell contact. Then, how is nontransferable resistance spread? Fig. 4 illustrates the method of proliferation of resistant bacteria. In the case of nontransferable resistance, a few resistant bacterial cells initially exist mixed with a large number of sensitive cells. Sensitive bacterial cells are killed during prolonged contact with antibiotics, leaving resistant ones which proliferate like a pure culture. Isolation rates of ceftazidime-resistant C. freundii at a Japanese hospital in 1989 and 1992 were 71% and 63%, respectively. Since ceftazidime resistance of this bacteria is not spread by bacterial cell contact, the isolates were suspected to originate from one clone. Most strains with plasmid-type resistance at that time showed multiresistance to piperacillin, streptomycin, kanamycin and tetracycline, while most of the chromosomal type strains showed resistance to both ceftazidime and nalidixic acid. Therefore, it was considered that in the hospital, strains with transferable as well as nontransferable resistance existed together. Recently, it became possible to determine genetic relatedness of bacteria based on the patterns of DNA band obtained by pulsed-field gel electrophoresis (PFGE) (Struelens et al. 1992). With this technique, the isolates of 1989 and 1992 were tested and found to have very similar PFGE patterns.

**Problems with nosocomial spread of resistant bacteria**

MRSA is a good example of problem nosocomial pathogen. MRSA is a problem organism of surgical infection. Gentamicin ointment was used in 1978 at a burn center in Japan, with a gradual increase of MRSA isolation. However, isolation of MRSA has increased rapidly since 1983 (Fig. 5). In most Japanese hospitals, the proportion of MRSA accounted for 60% of all S. aureus isolates. There is no doubt that third-generation cephalosporins, which have been used since 1981, triggered the increase of MRSA. A sharp increase of MRSA, which were also resistant to quinolone, has been observed since 1985. It appears that quinolones which were introduced for clinical use in 1985 favored acquisition of quinolone resistance by MRSA. MRSA is isolated in other countries, too, but such a rapid increase of MRSA in Japan was also noted only in a few countries. However, recently an increasing tendency of MRSA

![Fig. 5. Annual changes of the proportion of MRSA isolated in Japan. Rapid increase of MRSA was observed since the early 1980s, reaching a rate of about 60%.

![Fig. 6. Annual changes in the proportion of coagulase types of MRSA isolated in Japan. A significant increase of type II strains and a decrease of other types were noted since the mid-1980s.](image-url)
has been reported in Australia (Turnidge, 1998), France (Ploy et al. 1998), Indonesia (Warsa et al. 1996), Korea (Lee and Chong, 1996) and the United States (Johnson and James, 1997). Coagulase is one of the virulent factors of S. aureus. S. aureus can be grouped to eight different coagulase types (Ushioda, 1981; Kaida et al. 1987). Coagulase type II strain has increased sharply since 1983, while type IV has decreased (Inoue et al. 1994) (Fig. 6). Thus, the increase of MRSA were mostly due to the increase of coagulase type II strains. Coagulase type II strains were also found among the MRSA isolated in other countries (unpublished data). What was the cause of this phenomenon?

As one of the factors related to this, our attention was drawn to enterotoxin. When the production of enterotoxin by methicillin-susceptible S. aureus (MSSA) is examined in relation to the coagulase type, it was found that the rate of enterotoxin production of type II strain was no higher than those of other types. In particular, there was no tendency of increased production of type C enterotoxin by coagulase type II strain (Table 3). Contrary to these findings, the rate of enterotoxin production by coagulase type II MRSA was much higher. Also, it was found that many coagulase type II strains produced type C enterotoxin and toxic shock syndrome toxin-1 (TSST-1) (Table 4). On the other hand, there were few coagulase type IV strains

### Table 3. Enterotoxin production by coagulase types of MSSA

<table>
<thead>
<tr>
<th>Toxin</th>
<th>No. of strains with enterotoxin production by coagulase type:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Enterotoxin A</td>
<td>0</td>
</tr>
<tr>
<td>Enterotoxin B</td>
<td>0</td>
</tr>
<tr>
<td>Enterotoxin C</td>
<td>2</td>
</tr>
<tr>
<td>TSST-1</td>
<td>2</td>
</tr>
<tr>
<td>None</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
</tr>
</tbody>
</table>

*: Mixed type.
*: Total No. of strains tested were 178.

### Table 4. Enterotoxin production by coagulase types of MRSA

<table>
<thead>
<tr>
<th>Toxin</th>
<th>No. of strains with enterotoxin production by coagulase type:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Enterotoxin A</td>
<td>0</td>
</tr>
<tr>
<td>Enterotoxin B</td>
<td>1</td>
</tr>
<tr>
<td>Enterotoxin C</td>
<td>1</td>
</tr>
<tr>
<td>Enterotoxin D</td>
<td>0</td>
</tr>
<tr>
<td>TSST-1</td>
<td>1</td>
</tr>
<tr>
<td>TSST-1 + Enterotoxin A</td>
<td>0</td>
</tr>
<tr>
<td>TSST-1 + Enterotoxin B</td>
<td>0</td>
</tr>
<tr>
<td>TSST-1 + Enterotoxin C</td>
<td>1</td>
</tr>
<tr>
<td>TSST-1 + Enterotoxin A + C</td>
<td>0</td>
</tr>
<tr>
<td>TSST-1 + Enterotoxin B + D</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
</tr>
</tbody>
</table>

Number 6 509
producing type A enterotoxin. Enterotoxin-producing MRSA can cause severe MRSA enteritis.

The high prevalence of MRSA in Japan is probably due to the spread of the same strains. The spread of the same strains can also be assumed from other studies. In a study to determine intranasal carriage of MRSA, we found the rates were high among patients and health care personnel (Table 5). Approximate isolation rates of MRSA from patients in a neonate intensive care unit (NICU) were 70% from the nose, 50% from skin, 75% from throat, and 90% from feces. It was postulated that health care personnel might have carried the same strain of MRSA in their nose. In fact, PFGE patterns of chromosomal DNA of some MRSA isolated from neonates in the NICU were similar and it was considered that probably a few types of MRSA were colonized and disseminated among newborns admitted in the NICU (Fig. 7).

Control measures of nosocomial infection

It is clear that MRSA becomes adapted to a hospital environment and exists persistently. In fact,
MRSA is colonized with a 60% incidence in many hospitals in Japan. In particular, caution should be exercised for nasal carriers. In some hospitals, it is routine pre-surgical procedure to culture nasal specimens to determine the presence of MRSA, and for the carrier, administration of vancomycin and bathing with isodine are recommended for the prevention of MRSA infections. Postoperative infections are mostly considered due to bacteria entering through the skin by way of a catheter.

However, it is not the only nosocomial pathogen in the hospital environment. *P. aeruginosa* was isolated from 40% of patients who were colonized with MRSA (Inoue et al. 1994). Therefore, it is not sufficient to control MRSA alone. To control both MRSA and *P. aeruginosa*, it is necessary to use both anti-MRSA and anti-*P. aeruginosa* drugs. Prolonged prophylactic use of any antibiotics can result in the appearance and spread of resistant bacteria with a high probability. Therefore, it may be necessary to discontinue administration of an antibiotic after 3 or 4 days and switch to drugs of a different chemical class. For prevention of intrahospital infections, it is also very important for health care personnel to perform the disinfection of hands after every contact with a patient.

When the presence of antibiotic resistance is found, it is important to interpret the result correctly. For example, if a strain is resistant to a cephalosporin, it can be assumed that the strain is also resistant to other cephalosporins, since they have a similar chemical structure. Therefore, if bacteria acquire resistance to a drug during prolonged treatment, a clinical cure cannot be expected unless the antibiotic is changed to a different class.

Since MRSA shows resistance to many antibiotics, it is difficult to choose antibiotics for the treatment of the infection. Even vancomycin, teicoplanin, and arbekacin may not be effective clinically. *P. aeruginosa* is isolated together from 40% of patients with MRSA infection, empirical treatment with either vancomycin or teicoplanin and imipenem may be useful, as carbapenems has a potent bactericidal effect against *P. aeruginosa*.

In conclusion, despite recent efforts to develop such antimicrobial agents which allow the evolution of resistant bacteria less likely, resistant bacteria have continued to increase. The most important factor that determines the appearance of resistant strains is the use of antibiotics. Since it is not expected that some epoch-making new antibiotics will be developed in the near future, proper use of existing antibiotics which is based on the mechanisms of action of antimicrobial agents and of resistance of bacteria, and control of nosocomial infection are very important to reduce the further spread of resistant bacteria.

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**REFERENCES**


Higashitani F, Hyodo A, Ishida N, Inoue M, Mitsuhashi

Number 6  

511


Turnidge J: Emerging difficulties in the management of Staphylococcal infections. In Abstract of 6th West-