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Isolation of methicillin-resistant *Staphylococcus aureus* from a Shih-Tzu dog with canine distemper virus infection

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Abstract : A methicillin-resistant *Staphylococcus aureus* (MRSA) isolate was recovered from a 9-month-old female Shih-Tzu dog with canine distemper virus infection. We performed *in vitro* antimicrobial susceptibility test to determine the most effective antimicrobial drug against the isolate and thus, to emphasize its potential clinical importance in animal practices. Isolate was confirmed MRSA by oxacillin agar screening test. The isolate was fully resistant to all β-lactam antibiotics and was susceptible to glycopeptides. Of the other antibiotics, mupirocin, TMP/SMZ (trimethoprim-sulfamethoxazole), and chloramphenicol showed inhibitory effect at the concentration of 4x MIC. The MICs ranged 0.25->128μg/ml, and MBCs ranged 0.5->128μg/ml. The combined TMP/SMZ with cefamandole or novobiocin showed synergistic effect, whereas the combination of novobiocin plus cefamandole or teicoplanin resulted in antagonistic effects. Although MRSA in animals so far has been reported in the geographically limited countries, at least theoretically, it could be occurred in the future more frequently through either human or animal origin. The use of this combination may be of value in this situation. As with all antimicrobial agents, inappropriate or unnecessarily prolonged therapy may contribute to the emergence of resistance strains and loss of efficacy.

Key words : methicillin-resistant *Staphylococcus aureus*, antibiotic susceptibility, dog.

Introduction

Infections caused by staphylococci remain an important cause of morbidity and mortality in human and animal population[1-7]. The costs of treating such infections can be high, and recently the increasingly frequent outbreaks of multiple drug resistant strains of staphylococci continue to be leading cause of hospital-acquired infection. In particular, methicillin-resistant *Staphylococcus aureus* (MRSA) epidemics

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have presented a challenge to hospitals worldwide and became a clinically significant problem in high frequency[8,9]. Nosocomial infections complicate the treatment of patients, prolonged hospitalization, increase the cost of medical care, and are sometimes life threatening[10]. MRSA isolates have been shown to be fully virulent, causing staphylococcal endocarditis and septicemia at a frequency similar to that of methicillin-sensitive *S aureus* isolates[11]. Furthermore, these isolates are usually resistant to multiple additional antibiotics, including cephalosporins, aminoglycosides, macrolides, and lincosamides[10~12].

In veterinary medicine, the first report of the isolation of MRSA was in 1972, in which it was reported to have been isolated from the milk of mastitic cows[13]. At that time the MRSA isolates were suspected of being human contaminants because of their bacteriologic characteristics, and since then, the existence of MRSA in animals had been considered improbable. However, it could not be denied the fact that no serious effort had been made to detect MRSA from animals. Recently, 16 MRSA isolates from mares with metritis and from a stallion with dermatitis were reported in Japan[14]. Hartmann *et al* reported a horse case of postoperative MRSA wound infection[15], and implied that MRSA infections in animals may also become more frequent.

The aim of our report was to analyze the susceptibility patterns of the MRSA to various antimicrobial agents alone and a combined with another antimicrobial agent, and thus, to emphasize its potential clinical importance for clinicians.

Materials and Methods

Isolation and biochemical tests of bacteria : To isolate and identify *S aureus*, suspected colonies on blood agar plates were selected and underwent following biochemical tests; Gram staining, catalase test, coagulase test, 1% mannitol utilization test, aerobic and anaerobic oxidation from glucose, nitrate reduction, DNase test, and susceptibility to novobiocin (5μg) and polymyxin B (300μg) susceptibility. Methicillin resistance was confirmed by the agar screening test, using Mueller-Hinton agar containing 4% NaCl and 6μg/ml oxacillin[16].

Antimicrobial Agents : Antimicrobial agents used in this study were kindly provided from the pharmaceutical manufacturers, or purchased from a commercial source (Sigma): amikacin, ampicillin, cefamandole, cefazolin, cefmetazole, cefoperazone, chloramphenicol, ciprofloxacin, clindamycin, enrofloxacin, fosfomycin, fusidic acid, gentamicin, imipenem, minocycline, mupirocin, novobiocin, ofloxacin, teicoplanin, tobramycin, trimethoprim-sulfamethoxazole(TMP/SMZ), vancomycin. All laboratory standard powders were kept desiccated at -20℃ until use. Dilutions of the compounds were prepared on the day of use following the manufacturer's specifications.

Antibiotic susceptibility Tests : The susceptibility of the isolate to the various antimicrobial agents was determined in triplicate by using a macrobroth dilution method following the specifications of the National Committee for Clinical Laboratory Standards (NCCLS, 1993) reference methods[17] by using cation-supplemented Mueller-Hinton broth (Difco) containing 2% NaCl. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of antimicrobial that inhibited the visible growth of the test organism in 24h of incubation at 35℃[18]. The minimum bactericidal concentration (MBC) was defined as the lowest concentration of drug that inhibited ≥99.9% of initial inoculum, followed by another 24h of incubation at 35℃. *S aureus* ATCC 29213 (American Type Culture Collection, Rockville, Md., USA) was used as a control. Susceptibility to each antimicrobial was defined using the breakpoint categories of the NCCLS(1998)[19].

Kinetics of growth inhibition : With the macrodilution method, different antimicrobial combinations were evaluated in plates, with incubations at 35℃ for 24h. Growth inhibition curves were constructed with various concentrations of antibiotics alone or in combination. All test tubes were examined at standard time intervals of 0, 3, 5, and 24h postinoculation.

Results

Case summary : A 9-month-old female Shih-Tzu dog weighted 3.4kg was referred to the Veterinary Medical

Teaching Hospital, Seoul National University with high fever (40.1℃) and intermittent convulsion for the last 4 days. On admission, she had intermittent seizures, ocular opacity bilaterally, but responded to menace reflex. On hospital day 6, MRSA was isolated in ocular swabbing specimens, and he was finally diagnosed as canine distemper virus infection by polymerase chain reaction complicated with keratitis. she was given fluid therapy, as was the unit's routine. Despite immediate treatment with topical vancomycin, as the corneal ulcer progresses severely, the cornea melted and the stroma entirely destroyed, resulting in extrusion of Descemet's membrane by the intraocular pressure ; descemetocele.

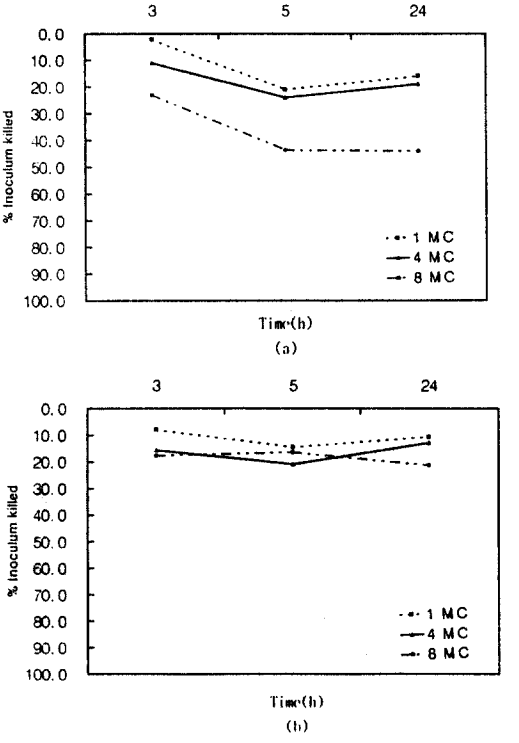
Table 1. MIC and MBC of the MRSA isolate against 22 antimicrobial drugs

Antimicrobial agent	MIC (μg/ml)	MBC (μg/ml)	Susceptibility[a]
Amikacin	16	64	R
Ampicillin	16	32	R
Cefamandole	64	⟩64	R
Cefazolin	⟩128	-	R
Cefmetazole	⟩64	-	R
Cefoperazone	⟩64	-	R
Chloramphenicol	8	8	S
Ciprofloxacin	16	⟩32	R
Clindamycin	⟩32	-	R
Enrofloxacin	32	⟩128	R
Fosfomycin	⟩32	-	R
Fusidic acid	0.25	4	S
Gentamicin	16	64	R
Imipenem	⟩16	-	R
Minocycline	1	2	S
Mupirocin	0.25	0.5	S
Novobiocin	1	2	S
Ofloxacin	⟩64	-	R
Teicoplamin	1	1	S
Tobramycin	⟩32	-	R
TS, 1 : 19	1	⟩8	S
Vancomycin	1	2	S

[a] S : susceptible, R : resistant.

Antibiotic resistance patterns : The MIC and MBC values obtained at 24h are shown in Table 1. The strain was susceptible to vancomycin and teicoplanin, whereas fully resistant to β-lactam antibiotics such as ampicillin, cefamandole, cefazolin, cefmetazole, cefoperazone, and imipenem. Of the other antibiotics, only mupirocin showed inhibitory effect. Among the aminoglycosides, amikacin, gentamicin and tobramycin showed no inhibitory activity. In terms of MICs, mupirocin was the most active drug, followed by polypeptides ; with regard to MBCs, mupirocin was also the most potent drugs, followed by fusidic acid, teicoplanin, and vancomycin(Fig 1).

Kinetics of growth inhibition : Combined time-killing kinetics showed any additive, synergistic, or indifferent effect with respect to kill organisms (Fig 2). In a series of experiments, combination of two antimicrobial drugs with selected concentrations (0.5MIC of a first agent plus 0.5MIC of a



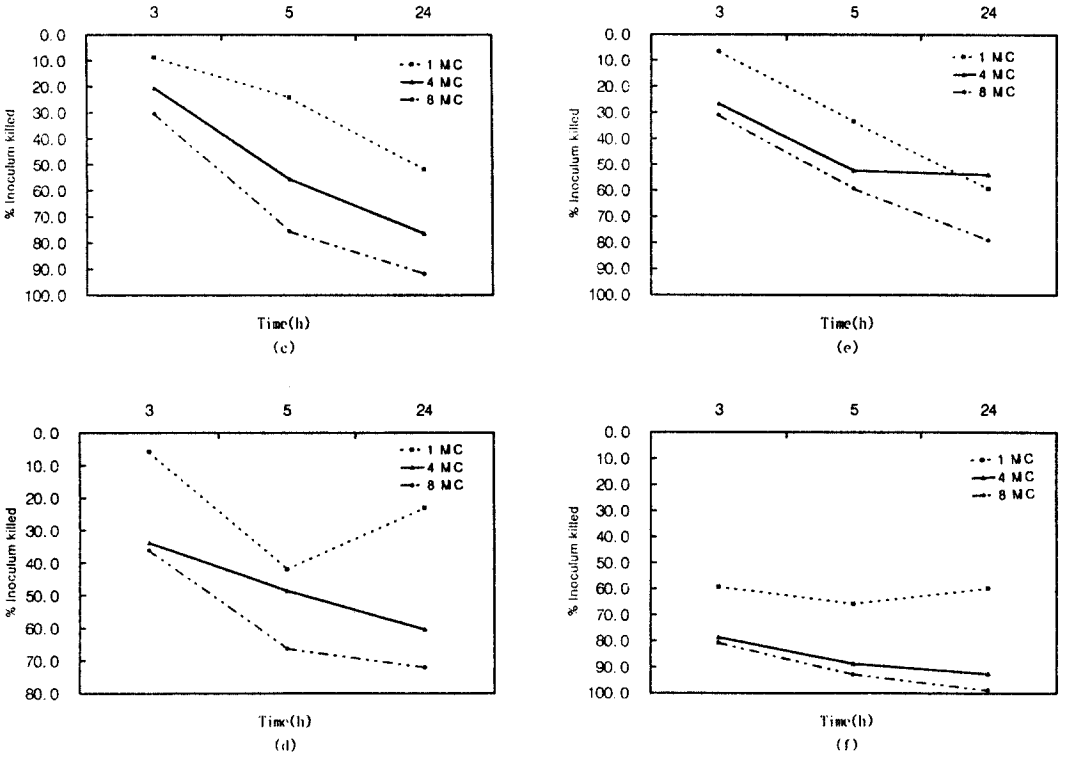


Fig 1. Kinetics of time-killing of the activity (a) chloramphenicol, (b) mupirocin, (c) TMP/SMA, and (k) vancomycin. Each antimicrobial was tested at 1×MIC(■-■), 4×MIC(▲-▲), and 8×MIC(●-●) based on the individual MIC values of the four test strains. Mean values of the percent of inoculum killed for the four strains are plotted.

second agent, 0.5×1MIC, 1×0.5MIC, and 1×1MIC) were examined by means of time-kill curves in an effort to discern various effects. As evident from figure, the combination of vancomycin-mupirocin yielded a synergistic effect from 5h postinoculation. The combination of teicoplanin-TMP/SMZ yielded synergistic effect, while novobiocin combined with cefamandole or teicoplanin resulted in antagonistic effect (data not shown).

Discussion

Ever since the emergence of methicillin resistance among *S aureus*, the problem continues unabated. During the last two decades, the prevalence of MRSA in human medicine has had an enormous impact on antibiotic prescribing practices and on infection control policies in hospitals throughout the world, and they also tend to have a trans-country, inter-continental or even global spread. To limiting and controlling nosocomial infection caused by these organisms, it is essential to understand the properties of MRSA strains. Coleman[20] pointed out some common characteristics which to a large extent are common to all MRSA : first, they often express resistance to a wide spectrum of antimicrobial agents and heavy metal ions, which can result in serious therapeutic problems ; second, they are extremely efficient colonizers of patients and hospital staff and may spread readily, often resulting in infection[21] ; third, as a result of colonization/carriage, MRSA strains can be transferred between one hospital and another, often over great distances, resulting in the introduction of 'new' strains[22,23] ; and fourth, their prevalence and properties are often difficult to monitor in the hospital environment, resulting in problems with in-

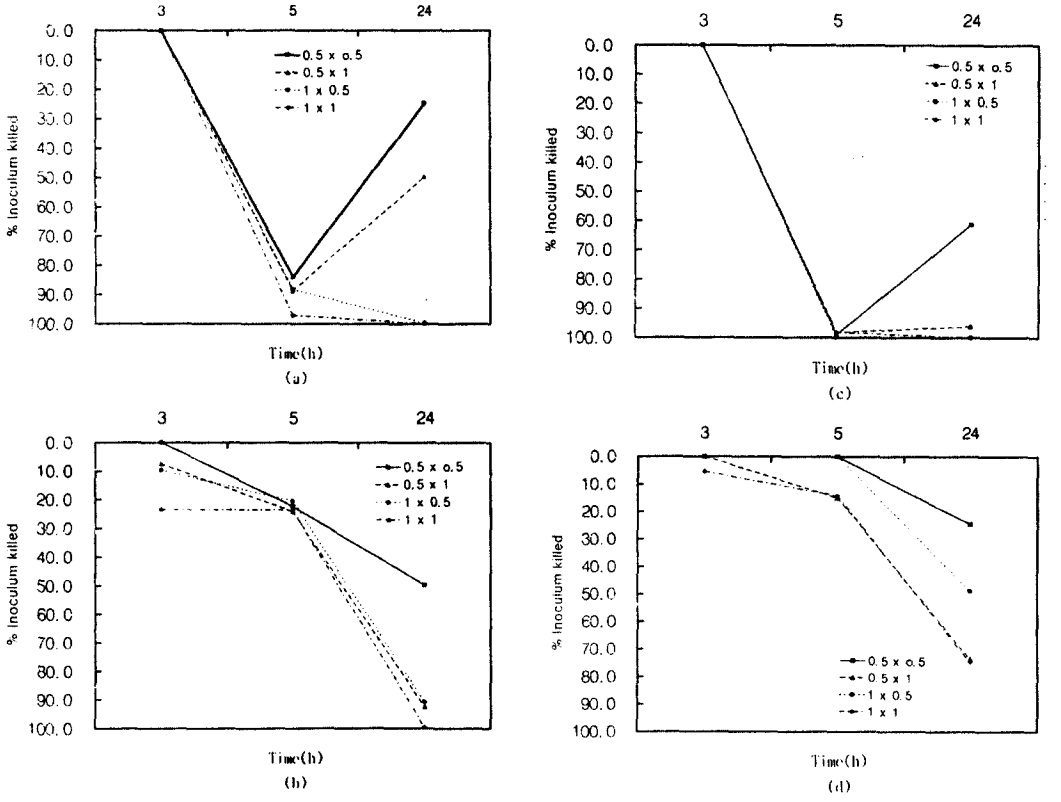


Fig 2. Kinetics of time-killing of the MRSA strains exposed to a variety combination of (a) vancomycin-mupirocin, (b) vancomycin-TMP/SMZ, and (c) cefamandole-TMP/SMZ. Each antimicrobial combination was tested at 0.5×0.5MIC(■-■), 0.5×1MIC(▲-▲), 1×0.5MIC(●-●) and 1×1MIC(◆-◆) based on the indicidual MIC values of the four test strains. Mean values of the percent of inoculum killed for the four strains are plotted.

fection control. MRSA is an unusual opportunistic pathogen which displays features of a virulent pathogen. It seems, however, not easy to disclose the source of infection of MRSA. Many researchers have failed to find the origin of MRSA isolates from animal population[24-27].

The therapy of infections caused by MRSA or the eradication of colonization is particularly difficult because of its characteristics of multiply resistance and limited choice of therapeutic options. Since Moss *et al*[28] first attempted to eradicate nasal carriage of *S aureus*, a lot of antimcirobials have been applied to this purpose, but no single topical or systemic antimicrobial has been successful in permanently eliminating the nasal reservoir; rifampicin[29], combinations cotrimoxazole with rifampicin[30] or ciprofloxacin[31], and local application of chlorhexidine, with or without neomycin[32].

Based on *in vitro* susceptibility testing, mupirocin, TMP/SMZ, teicoplanin, and vancomycin could be candidates for therapy of MRSA infections. Combination therapy could be one of strategies for delaying or reducing the emergence of resistant strains. Experiments on the growth inhibition revealed that the combination of TMP/SMZ with cefamandole or novobiocin showed an enhanced killing effect, compared to the effect of single use. These findings deserve further clinical application in situations in which these strains are involved. The antibiotic resistance of *S aureus* strains allows increased colonization of patients in areas of high antibiotic use and impedes effective antibiotic therapy for infections[33]. Therefore, it is important to be able to document the spread

of an epidemic MRSA isolate so that effective control measures can be rapidly instituted.

Concerns about the rising of resistance to new antimicrobial agents such as fluoroquinolones make epidemiologic surveillance with the utilization of special antimicrobial susceptibility testing and molecular typing essential for the control of nosocomial transmission of these microorganisms[34]. The indiscriminate utilization of newer antimicrobial agents may be one factor responsible for the high incidence of nosocomial MRSA and now, quinolone-resistant *S aureus*.

This case suggested that clinicians should be aware that strains of staphylococci extremely resistant to penicillinase-resistant penicillin might cause ocular infection. Rapid diagnosis and treatment of bacterial keratitis are essential to limit stromal scarring and minimize visual loss. Therefore, an antibiotic with broad activity against gram-positive organisms, such as a vancomycin should be included as part of the initial therapy for corneal ulcer that might be caused by staphylococci. To our best knowledge, this was the first such case in the veterinary literature in Korea, and may indicate the emergence of MRSA as ocular pathogens.

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