% Similarity

50 60 70 80 90 100

TUM1965
TUM1966
TUM1933
TUM1934
TUM1935
TUM1967
TUM1936
TUM1937

Fig. 1. PFGE profiles obtained with *Sfi*I chromosomal digestion of *Prov. rettgeri* carrying the IMP-1 metallo-β-lactamase.

detected in *Klebsiella oxytoca*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *M. morganii* and *Prov. rettgeri* by PCR analysis. However, their study analysed only ceftazidime-resistant strains for β-lactam resistance factors and integron structure.

In this study 495 indole-positive proteae strains were isolated. Among these, 54·9 % were *M. morganii*. It appears that this species is quite common in this part of the world, having a high incidence in Korea as well (Kim *et al.*, 2003), even though it has been reported as rare in other places (Murray *et al.*, 2003). *Prot. vulgaris* was second in frequency of isolation (25·7 %) in the current study and followed by *Prov. rettgeri* (12·5 %) (Table 1). Of the proteae, 43 % (213/495) were urinary-tract isolates (Table 2). Of the *Prov. rettgeri* isolates, 69·3 % (43/62) were isolates from the urinary tract. Proteae have been recognized as pathogens in urinary-tract infections, and the majority of these urinary-tract infections are a consequence of urinary-tract catheterization and instrumentation (Warren, 2001). Stickler *et al.* (1998) reported that *Prov. rettgeri* can form crystalline biofilms that rapidly encrust and block catheters. This study did not distinguish catheter specimens from other urine specimens. For future surveillance, it will be necessary to specify the origin and to evaluate biofilm formation as one of the pathogenic factors in this species.

The isolation frequency of MβL-producing *Prov. rettgeri* strains was 1·6 % (8/495). Kimura *et al.* (2005) reported an isolation frequency of MβL-producing *Ps. aeruginosa* of 1·9 % (11/594) in 2002 using strains from the same surveillance programme as the current study. It is of interest that no MβL-producing *Prov. rettgeri* were isolated from hospitals where MβL-producing *Ps. aeruginosa* were isolated. Eight MβL-producing *Prov. rettgeri* strains were isolated from only two hospitals, Mie and Nagasaki, which are separated by over 600 km. The genetic relatedness was evaluated by pulsed-field gel electrophoresis, integron structure and plasmid incompatibility group. These data show that the resistant *Prov. rettgeri* strains had two different origins, which coincided with the two different hospitals where they were isolated. The strains isolated in each hospital shared the same integron structure and also the same incompatibility group. These

results strongly suggest that nosocomial infection by *Prov. rettgeri* occurred in the two different hospitals. Moreover, these *bla*$_{IMP-1}$-encoding plasmids could transfer from *Prov. rettgeri* isolates to other species and their incompatibility groups could expand to other *Enterobacteriaceae*. This result suggests that the spread of this imipenem-resistance factor to other *Enterobacteriaceae* is not very difficult.

In conclusion, we report the finding of *Prov. rettgeri* isolates that harbour a conjugative plasmid containing an integron on which *bla*$_{IMP-1}$ is encoded. Our results very strongly suggest that nosocomial infections by IMP-1-producing *Prov. rettgeri* occurred at two hospitals. IMP-1-producing *Enterobacteriaceae* could become a serious problem in the future. Thus, it is important to continue surveillance and monitoring of carbapenem resistance and reduced susceptibility *Enterobacteriaceae* including indole-positive proteae.

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ORIGINAL ARTICLE

Semi-quantitative analysis of Streptococcus pneumoniae urinary antigen: Kinetics of antigen titers and severity of diseases

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Abstract

Detection of urinary antigen by a rapid immunochromatographic membrane test (Binax NOW) was widely accepted as a powerful tool for diagnosis of Streptococcus pneumoniae pneumonia. This is a qualitative kit, so the value of quantitative analysis of urinary antigen, especially correlation of antigen titers and severity of diseases, remained to be determined. We examined semi-quantitative antigen titer in urines collected from urinary antigen-proven S. pneumoniae pneumonia on admission, and analyzed the kinetics of antigen titer and its relation to severity of diseases. After serial 2-fold dilution of urine, the highest dilution for positive results was determined, and this was designated as maximum dilution factor (MDF). MDFs varied from 1 to 4096 in 29 patients examined (mean MDF, 317.8). Importantly, severe cases of S. pneumoniae pneumonia were higher values of MDFs (mean MDF: 760.5) than those of non-severe cases (mean MDF: 5.4). The patients with high MDFs (≥ 64) demonstrated higher values of LDH, CRP and lower values of WBC and PaO_2 compared to those of low MDFs group (≤ 32). There was no clear correlation between CRP values and antigen titers, and conversely the majority of severe cases showed relatively weak CRP responses, despite high levels of bacterial antigen. Kinetic analysis of urinary and serum antigen titers in 4 cases of S. pneumoniae pneumonia exhibited consistently higher values of antigen titers in urine than those in serum. The half lives of urinary and serum antigen titers were calculated to be 1.0–3.4 and 1.1–2.3 weeks, respectively. These data suggest that quantitative analysis of urinary antigen may be a useful indicator for severity of disease and course of S. pneumoniae pneumonia. Our results demonstrate an application for S. pneumoniae antigen titer determination in urine and serum, which may be crucial not only for diagnostic measures, but also may provide a better understanding of the pathogenesis of S. pneumoniae infection.

Introduction

Streptococcus pneumoniae has been consistently shown to be the most common cause of community-acquired pneumonia (CAP) in both adults and children. This organism accounts for about two-thirds of cases where an etiologic diagnosis is made [1]. In particular, systemic pneumococcal infection is a major cause of morbidity and mortality, especially for young children, people with underlying diseases, and the elderly. Rapid diagnosis and proper antibiotic chemotherapy, in addition to correct evaluation of severity of disease, may be crucial for determining course and outcome of S. pneumoniae infection [2,3].

Recently, a new, rapid immunochromatographic membrane test, the NOW S. pneumoniae urinary antigen test (Binax, Inc., Portland, Maine), has been developed for the detection of antigens of S. pneumoniae in urine samples [4]. This test is simple to perform, detects the C-polysaccharide cell wall antigen common to the majority of S. pneumoniae strains, and provides results within 15 min. The utility of this assay has been repeatedly demonstrated, in which sensitivity and specificity of this kit was shown to be 65.9–82.0% and 89.7–100%, respectively [5–9]. It is likely that recovery of bacterial antigen from urine may be associated with invasion of bacteria or dispersion of bacterial components and products into the blood stream.

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Thus, it is reasonable to query whether intensity of urinary antigen reflects intravascular loading of bacteria, and more importantly, severity of disease.

To answer this question, we have examined intensity of urinary antigen by diluting urine samples in a serial 2-fold fashion. Our data suggest a correlation between urinary antigen titers and severity of pneumonia. Furthermore, the kinetics and the half lives of urinary and serum antigens were demonstrated in patients with S. pneumoniae pneumonia.

Materials and methods

Collection of clinical data and samples in S. pneumoniae pneumonia cases

Urine samples from CAP patients admitted to Toho University Hospital were tested for pneumococcal antigen using the NOW S. pneumoniae urinary antigen test. Urinary antigen positive cases (29 cases; age range 18–86 y; 66% male) were recruited as part of the present study. Clinical and laboratory data were recorded, and urine samples were stored at $-80^{\circ}C$ until used. In cases presenting with dyspnea, arterial blood-gas analysis was performed before and after oxygen supplementation. All enrolled patients had an acute illness with clinical features of pneumonia and radiographic pulmonary shadowing that was at least segmental or present in 1 lobe and was neither pre-existing nor due to some other known causes. Patients were excluded when pneumonia was not the principal reason for admission. Also, patients with history of pneumonia within past 3 months were not included in this study.

In 4 cases of urinary-antigen proven S. pneumoniae pneumonia, urine and serum samples were consecutively collected over 17 weeks after admission, and these samples were also stored at $-80^{\circ}C$ until used.

Evaluation of severity of diseases

There are no universally accepted criteria for severe or non-severe CAP [2,3,10–12]. According to previous reports, we have used 1 set of variables that has been proposed as a reliable predictor defining severe CAP: the presence of 2 out of 3 possible minor criteria (systolic blood pressure <90 mmHg, multilobar disease, $PaO_2/FiO_2 < 250$), or 1 of 2 major criteria (need for mechanical ventilation or septic shock).

Qualitative and semi-quantitative analysis of pneumococcal antigen

The urine samples were tested using the immunochromatographic assay Binax NOW S. pneumoniae antigen (Binax). This test detects the C-polysaccharide antigen from the cell wall of S. pneumoniae that is believed to be specific for a majority of pneumococcal serotypes. The test was performed in accordance with the manufacture's instructions. A swab was dipped into the urine sample and then inserted into the test device. A buffer solution was added, and the device was closed, bringing the sample into contact with the test strip. The test was read at 15 min and was interpreted by noting the presence or absence of visually detectable pink lines. A positive test result was indicated by the detection of both sample and control lines, and a negative result was indicated by the detection of a control line only. The results were read by 2 observers and consensus data were used for following analysis.

For semi-quantitative analysis in urine and serum, serial 2-fold dilution was performed with phosphate-buffered saline, and then presence of pneumococcal antigen was examined by the NOW S. pneumoniae urinary antigen test, as described above. The dilution at which urinary antigen is positive, but negative at the next 2-fold dilution, was designated to be maximum dilution factor (MDF) in the present study.

Statistical analysis

We used Student's t-test to compare quantitative variables. A 2-tailed p-value of 0.05 was considered to be statistically significant.

Results

Urinary antigen titers on admission

MDFs of 29 cases of S. pneumoniae pneumonia were examined in urine samples, which were obtained and stocked on admission (Figure 1). MDFs varied from 1 to 4096, with the median value calculated to be 317.8. Next, we examined the correlation of urinary antigen titers and severity of disease, as defined in Materials and methods. MDFs of 17 cases with non-severe diseases ranged from 1 to 32, with a median MDF value of 5.4. In contrast, MDFs of 12 cases with severe pneumonia were widely distributed from 1 to 4096, with the median MDF value calculated to be 760.5 (p <0.05). In the present study, 2 lethal cases were included, and MDFs of urines in these cases were 4 and 4096. These data demonstrated that severe cases of S. pneumoniae pneumonia appear to have higher MDFs in urine than those of non-severe cases.
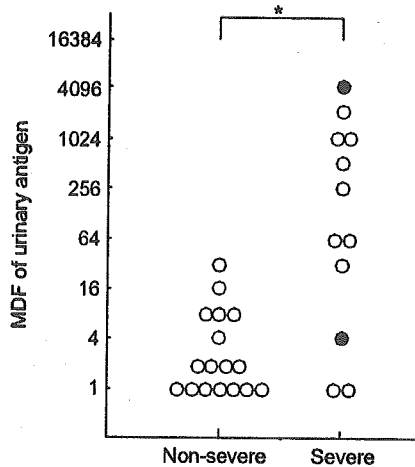
Figure 1. Urinary antigen titers on admission. MDFs of 29 cases of S. pneumoniae pneumonia were examined in urine samples, which were obtained and stocked on admission. MDFs of 17 cases with non-severe diseases ranged from 1 to 32, with a median MDF value of 5.4. In contrast, MDFs of 12 cases with severe pneumonia were widely distributed from 1 to 4096, with the median MDF value calculated to be 760.5. Closed circles demonstrated lethal cases. *p <0.05.

MDF group exhibited slightly lower PaO_2 than those of patients with the low MDF group.

CRP is an acute phase protein which is produced in the liver in response to a variety of stimuli, including bacterial components and products [13]. C-polysaccharide of S. pneumoniae, which is a major component of urinary antigen, is a major binding target for CRP. We examined a correlation of urinary MDFs and CRP levels in severe and non-severe cases of pneumonia (Figure 3). In non-severe cases, CRP values were widely distributed from 1 to 46 mg/dl, although urinary MDFs were all less than 64. On the other hand, CRP values in 6 of 10 severe cases were between 20 and 30 mg/dl, and MDFs of all these cases were equal or higher than 64. In the present data, we did not observe a correlation between CRP values and urinary MDFs in pneumococcal pneumonia. Conversely, our results suggest relatively weak CRP responses in the majority of severe cases of pneumonia, despite the high levels of bacterial antigen.

Comparison of laboratory data in patients with low (≤32) and high (≥64) urinary MDF

We compared laboratory data on admission in patients with low MDF (≤32) and the high MDF group (≥64) (Figure 2). Of the laboratory markers examined, the median values of CRP and LDH were slightly elevated in high MDF group. Conversely, the median WBC count in the high MDF group was lower than those of patients in the low MDF group, although WBC counts in the high MDF patients segregated into 2 groups: high (more than 20,000/mm^3, 2 cases) and low (less than 7,000/mm^3, 6 cases) WBC counts. Arterial blood-gas analysis of patients on room-air upon admission showed that the patients with the high

Kinetic analysis of urinary and serum pneumococcal antigen

We examined kinetic changes of urinary and serum MDFs in 4 patients, in which serial samples were stocked from their admission to 17 weeks after the onset of pneumonia (Figure 4). In case 1, for example, urinary MDF of 4096 was observed on admission, which gradually declined over the period of observation. The half life of urinary antigen was calculated to be approximately 3.2 weeks. In the serum of this case, MDF on admission was 256, which decreased to 2 at 16 weeks after the onset of pneumonia (half life, 2.3 weeks). In case 2, the kinetic data of urinary and serum antigens were similar to that observed in case 1. In addition, the present data demonstrated that urinary MDFs are 8- to 16-fold higher than those of serum. Collectively
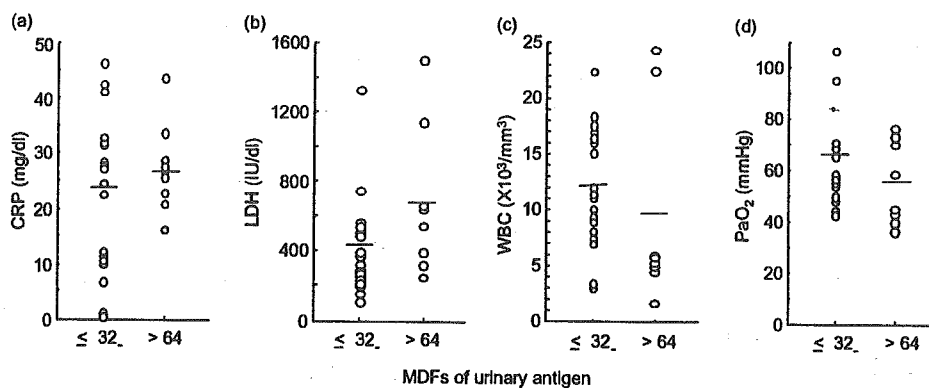

Figure 2. Comparison of laboratory data in patients with low (≤32) and high (≥64) urinary MDF. We compared laboratory data on admission in patients with low MDF (≤32) and the high MDF group (≥64). a) CRP: b) LDH: c) WBC: d) PaO_2.
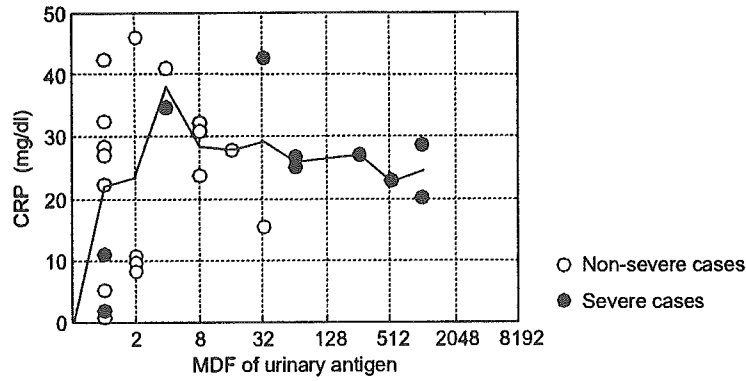
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Figure 3. Correlation of urinary MDFs and CRP levels in severe and non-severe cases of pneumonia.

from these data, the half lives of urinary and serum antigen titers were calculated to be 1.0–3.4 and 1.1–2.3 weeks, respectively.

Discussion

The present study demonstrated, for the first time, a correlation between pneumococcal urinary antigen and severity of infection, as well as kinetic changes of antigen titers in urine and serum. Although careful observations of clinical findings may be a best indicator for severity of diseases, our data suggest a potential usefulness of quantitative analysis of urinary antigen for supplementary information.

Although several clinical indicators, such as changes of vital signs, laboratory and radiographic findings, are useful for evaluation of severity of diseases, these markers remain imprecise, especially

in patients with rapidly progressive diseases, such as Legionella and S. pneumoniae pneumonia [2,3,10–12]. In our quantitative analysis of urinary antigen, MDFs of 29 cases of S. pneumoniae pneumonia were widely distributed from 1 to 4096. Interestingly, the mean MDF of severe cases of pneumonia was clearly higher than that of non-severe cases, although MDFs of 12 cases with severe pneumonia displayed considerable patient-to-patient variability. Typical examples were observed in 2 lethal cases, in which MDFs of their urines were 4 and 4096. In addition, the present data indicated a trend of higher values of CRP and LDH, whereas the values of WBC and PaO_2 were lower in patients with high MDFs. These data demonstrated that urinary antigen titers may be an indicator for determining severity of pneumococcal infection, while also suggesting the involvement of other factors. In this
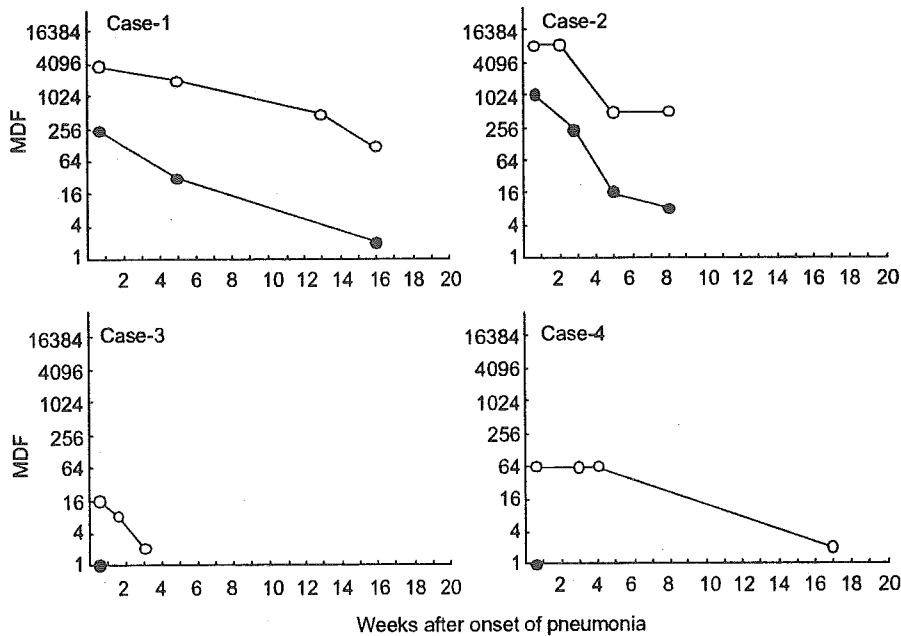

Figure 4. Kinetic analysis of urinary and serum pneumococcal antigen. Open circles: urinary MDFs; Closed circles: serum MDFs.

regard, host reactions, such as cytokine/chemokine productions and acute phase responses, to bacteria and bacterial antigens may be a crucial factor, which is largely unknown in pneumococcal infection.

C-polysaccharide of S. pneumoniae is a major ligand for CRP, and binding of CRP to ligands activates the classical complement pathway and stimulates phagocytosis [13]. In addition, pneumococcal C-polysaccharide is known to trigger inflammatory cytokine releases, including IL-1, IL-6, IL-8 and TNF-α, which in turn induce production of CRP [14,15]. Recently, Almirall et al. have reported a role for CRP in assessment of severity of CAP [16]. In the present analysis of a correlation between CRP values and MDF titers, relatively weak CRP responses were observed in the majority of severe cases of pneumonia, in which high levels of bacterial antigen were demonstrated. It is generally accepted that severe infections are frequently associated with multiple organ failure, including liver injury, which may suppress production of acute phase proteins [17,18]. In this regard, liver diseases, such as cirrhosis, are known to be a major risk factor for life-threatening pneumococcal infections [19]. Recently, Roy et al. have reported that mutations in mannose-binding lectin, an acute-phase reactant, are a crucial factor determining severity of pneumococcal infections in a case-control study [20].

The semi-quantitative analysis uncovered kinetic changes of pneumococcal urinary and serum antigen titers in infected individuals. The present data support previous results describing continuous secretion of bacterial antigen in urine [21,22], and further demonstrated the half lives of urinary and serum antigen to be 1.0–3.4 and 1.1–2.3 weeks, respectively. Long-lasting excretion of urinary antigen (probably 12–36 weeks) may be expected in certain cases (Figure 4), as an MDF value of 4096 was observed in the urine of 1 such patient on admission. Additionally, the present data indicated consistently higher values of urinary antigen titer compared to that of serum. These data suggest that concentration and clearance of bacterial antigen during the excretion step in kidney may be crucial. Other investigators have extended the application of this assay to the detection of pneumococcal antigen in nasopharyngeal samples, effusions of otitis media and cerebrospinal fluids [23–26]. Regarding the sensitivity of this kit, we observed approximately 10^5 CFU/ml of bacteria as a detection limit in Binax NOW kit (data not shown).

Collectively, these data suggest that quantitative analysis of urinary antigen may be a useful indicator for severity of disease and course of S. pneumoniae pneumonia. Our results illustrate a new application for S. pneumoniae antigen titer determination in urine and serum, which may be crucial not only for diagnostic measure, but also for better understanding of the pathogenesis of S. pneumoniae infection.

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Identification of biochemically atypical *Staphylococcus aureus* clinical isolates with three automated identification systems

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Between January and April 2002, a total of 271 strains of *Staphylococcus aureus* were isolated from clinical specimens at Toho University Omori Hospital, Japan, including 201 (74·2 %) which were identified as meticillin-resistant *S. aureus* (MRSA). However, 34 (12·5 %) were biochemically atypical, because they did not produce acid on mannitol salt agar or did not agglutinate in Staphaurex testing but were categorized as MRSA by PCR analysis and by antibiotic susceptibility. Three automatic identification systems, AutoScan-4® (Dade Behring), BD Phoenix® (Becton Dickinson) and Vitek® 2 (bioMérieux), were evaluated by testing these atypical *S. aureus* isolates. The AutoScan-4® and Phoenix® systems identified all 34 isolates as *S. aureus*. Without additional tests such as Staphaurex, observation of colony pigment and haemolysins on sheep blood agar, Vitek® 2 identified only 16 isolates (47·1 %) as *S. aureus* with good or better confidence levels and misidentified one of the remaining isolates as *Staphylococcus chromogenes*. This study shows that it is possible to identify these physiologically atypical *S. aureus* isolates correctly by using the Phoenix® and AutoScan-4® fully automatic identification systems.

INTRODUCTION

Staphylococci are widespread in nature, although they are mainly found living on the skin and mucous membranes. The coagulase-positive species *Staphylococcus aureus* is well known as a human pathogen. Serious infections produced by *S. aureus* include bacteraemia, pneumonia, osteomyelitis, acute endocarditis, myocarditis, pericarditis, encephalitis, meningitis, choriamnionitis, mastitis, scalded skin syndrome and abscesses of the muscle, urinogenital tract and various intra-abdominal organs (Murray *et al.*, 2003). The species is identified on the basis of a variety of conventional physiological or biochemical characters. The key characters for *S. aureus* are colony pigment, free coagulase, clumping factor, protein A, heat-stable nuclease and acid production from mannitol (Murray *et al.*, 2003). In addition, *S. aureus* can be identified by PCR methods. Sequences targeted by PCR include *tst* (encoding the toxic shock syndrome protein), *eta* and *etb* (encoding exfoliative toxins A and B, respectively), staphylococcal enterotoxin genes such as *sea*, *sec*, *sed*, *seg*, *seh*, *sei*, *sej* and *sel*, *nuc* (encoding thermostable nuclease) and the Sa442 DNA fragment (Becker *et al.*, 2003; Martineau *et al.*, 1998; Pinto *et al.*, 2005). Since the 1980s,

meticillin-resistant *S. aureus* (MRSA) has spread widely to become a major clinical and epidemiological problem in many medical centres (Maple *et al.*, 1989; Matsuhashi *et al.*, 1986). Some MRSA isolates may be biochemically atypical compared to meticillin-susceptible *S. aureus*, particularly in coagulase production or acid production from carbon sources (Berke & Tilton 1986; Smole *et al.*, 1998; Wilkerson *et al.*, 1997). In clinical microbiology laboratories, it is very important to distinguish *S. aureus* from other staphylococci, because *S. aureus* is an important nosocomial pathogen (Murray *et al.*, 2003).

A total of 271 *S. aureus* isolates were isolated by the Clinical Laboratory Department in Toho University Omori Hospital, Japan, from January to April 2002. These isolates, including 34 biochemically atypical isolates, were analysed using Vitek® 2 and its optional tests. Thirty-four (12·5 %) were found to be biochemically atypical, because they did not produce acid from mannitol salt agar or did not agglutinate in Staphaurex testing but were categorized as MRSA by PCR analysis and antibiotic susceptibility. Because of an increased frequency of isolation of physiologically atypical *S. aureus* and the need to identify them accurately, this collection of isolates was used to evaluate three automatic identification systems, AutoScan-4®, BD Phoenix® and Vitek® 2.

METHODS

Bacterial strains. The date of isolation and origin of the clinical isolates used in this study are given in Table 1. All atypical isolates were isolated at Toho University Omori Hospital between February and April 2002. Data from pulsed-field gel electrophoresis provided by the hospital infection control team indicated that the 34 *S. aureus* isolates used did not have a common origin (data not shown).

Identification of *S. aureus*. All isolates were initially evaluated by Vitek® 2 with additional tests and then stored at −80 °C using 30 % glycerol. Additionally, Staphaurex (Murex Biotech) was used to detect clumping factor, as membrane-bound coagulase, and protein A. Free coagulase production was detected by aggregation of rabbit plasma (Eiken Chemical Co.) by the tube method, according to the supplier's instruction manual. Mannitol fermentation was tested on mannitol salt agar (Murray *et al.*, 2003). Haemolysis was detected on sheep blood agar after 20 h incubation at 35 °C; yellow pigment production was defined as the visual detection of carotenoid pigments by two or more people after 24 h incubation at 35 °C (Murray *et al.*, 2003).

Confirmation of *S. aureus* by species-specific PCR and detection of the *mecA* gene. DNA amplification of the 34

Table 1. Atypical *S. aureus* isolates used in the evaluation of three automatic identification systems and their biochemical characteristics

All isolates produced free coagulase, detected by aggregation of rabbit plasma. Acid production from mannitol was observed by a change in colour of the mannitol-salt agar plate. Haemolysis of colonies and yellow pigment were observed on trypticase soy agar II with 5 % sheep blood. BAL, Bronchoalveolar lavage.

Isolate	Isolation date	Specimen	Aggregated by Staphaurex	Acid production from mannitol	Haemolysin activity	Yellow pigment
1[a]*	10 April 2002	Pharyngeal swab	+	−	+	+
2	29 March 2002	Umbilical swab	+	−	+	+
3[b]	29 March 2002	Skin	+	−	+	+
4[b]	1 April 2002	Pharyngeal swab	+	−	+	+
5[b]	1 April 2002	Pharyngeal swab	+	−	+	+
6[b]	1 April 2002	Pharyngeal swab	+	−	+	+
7	22 February 2002	Faeces	−	+	+	+
8†	1 April 2002	Intravenous catheter	+	−	−	+
9	1 February 2002	Faeces	−	+	+	+
10[a]	18 March 2002	Pharyngeal swab	+	−	+	+
11[a]	23 March 2002	Nasal swab	+	−	−	−
12	26 March 2002	BAL fluid	+	−	−	+
13	26 March 2002	BAL fluid	+	−	−	+
14	25 March 2002	Urinary catheter	−	+	+	+
15[a]	5 March 2002	BAL fluid	+	−	−	−
16[a]	7 March 2002	Pharyngeal swab	+	−	+	+
17[a]	8 March 2002	Pharyngeal swab	+	−	+	+
18[a]	8 March 2002	Pharyngeal swab	+	−	+	+
19	8 March 2002	Vaginal swab	+	−	−	+
20[a]	8 March 2002	Vaginal swab	+	−	+	+
21	4 February 2002	Sputum	−	+	+	+
22	8 March 2002	Pharyngeal swab	+	−	+	+
23[a]	8 March 2002	Vaginal swab	+	−	+	+
24	8 March 2002	Pharyngeal swab	+	−	−	−
25[a]	8 March 2002	Pharyngeal swab	+	−	+	+
26	15 March 2002	Blood	−	+	+	+
27	26 February 2002	Intravenous catheter	−	+	+	+
28	4 March 2002	Vaginal swab	+	−	+	+
29[a]	4 March 2002	Vaginal swab	+	−	+	+
30	5 March 2002	Skin	−	+	+	+
31[a]	5 March 2002	BAL fluid	+	−	+	+
32[a]	4 March 2002	Intravenous catheter	+	−	−	−
33	8 February 2002	Vaginal swab	−	+	+	+
34	20 February 2002	Faeces	−	+	+	+

*Resolved by additional tests: *a*, coagulase production; *b*, coagulase production, yellow pigment and haemolysis.
†Identified as *S. chromogenes* by the Expert system of Vitek[x] 2.

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isolates was performed with colony direct PCR (Tsuchizaki *et al.*, 2000). A small portion of a colony was picked up by a toothpick, transferred directly to 50 μl of PCR mixture containing 50 pmol of each oligonucleotide primer from the Sa442 set (Martineau *et al.*, 1998), 25 μl SYBR® Green Master Mix (Applied Biosystems) and autoclaved MilliQ water. *S. aureus* FDA 209P and *Staphylococcus epidermidis* ATCC 14990 were used as positive and negative controls, respectively, for the PCR. The thermal cycling protocol was as follows: 5 min at 95 °C for hot start of DNA polymerase and initial denaturation followed by 40 cycles of two steps consisting of 1 s at 95 °C for denaturation and 55 °C for the annealing and extension steps. Real-time detection of the PCR product was performed on an ABI PRISM 7000 Sequencing Detection System (Applied Biosystems) by measuring the fluorescence signal. Specificity of the fluorescence signal was estimated by a denaturation protocol to compare with a theoretical T_m value of the PCR product after 40 cycles.

The *mecA* gene was used as the gold standard for detection of meticillin resistance by PCR assay. The specific primer set for *mecA* reported by Reischl *et al.* (2000) was used. PCR conditions used were as previously described (Martineau *et al.*, 1998). *S. aureus* N315 (Hiramatsu *et al.*, 1992) and *S. aureus* FDA 209P were used as positive and negative controls for the *mecA* gene, respectively.

Biochemical identification. Inocula for the following studies were prepared using a nephelometric device to adjust the turbidity to McFarland standard 0·5. *S. aureus* FDA 209P and *S. epidermidis* 14990 were used as positive and negative controls, respectively. Analysis of the results was based on the computerized reports from each identification system.

Identification with AutoScan-4® system. Preparation of the AutoScan Pos ID panel (Dade Behring), inoculum preparation, panel rehydration and inoculation, biochemical overlays (Pos ID only), incubation, reading of the panels and quality control were performed according to the manufacturer's instructions. Pos ID panels were read visually after 24 h incubation at 35 °C. The test reactions were read by the AutoScan-4® (Dade Behring) and the results were converted to compare with the AutoScan updated database.

Identification with Vitek® 2. The test panels (ID-GPC; bioMérieux) were automatically filled by a vacuum device, sealed and inserted into the Vitek® 2 reader-incubator module (bioMérieux) and subjected to a kinetic fluorescence measurement every 15 min. The results were interpreted by the ID-GPC database and final results were obtained automatically.

Identification with the Phoenix® system. The Phoenix® system (Becton Dickinson) was used according to the manufacturer's instructions with PMIC/ID14 panels (Becton Dickinson) for strain identification and oxacillin-susceptibility testing. Test suspensions were prepared from pure bacterial cultures grown on trypticase soy agar II with 5 % defibrinated sheep blood (Becton Dickinson). The ID suspension was inoculated within 30 min into the panel, which was then loaded into the instrument for incubation at 35 °C and continuous reading. The results were interpreted by the ID-GPC database and final results were obtained automatically.

RESULTS AND DISCUSSION

One of the most important strategies to prevent and control the spread of MRSA is early and correct identification of positive strains, including those coming from diseased or colonized areas. Samples used in this study came from both. Several samples were taken as a precaution due to a high incidence of MRSA infections in the neonatal intensive care

unit (e.g. vaginal samples taken from pregnant women prior to delivery, pharyngeal samples or umbilical samples taken from newborns) (Table 1). Once an isolate has been positively identified, cases of disease can be treated appropriately, and other procedures such as patient isolation, decontamination of exposed areas and increased hygiene measures can take place. Thus, identification is crucial.

All 34 isolates in this study were confirmed as *S. aureus* by PCR using the Sa442 primer set. Free coagulase production was confirmed for all isolates by the tube method with rabbit plasma. Nine of 34 isolates did not aggregate in the Staphaurex test, 25 of 34 isolates did not produce acid from mannitol, 26 of 34 had haemolysin activity and only 30 of these clinical isolates produced yellow pigment (Table 1). In addition, these 34 strains were confirmed as MRSA (*mecA* gene positive) by PCR analysis. Antibiotic susceptibility testing also confirmed all isolates as MRSA by the Phoenix® system.

Out of 34 isolates tested, a concordant identification to the species level was obtained by the Phoenix® system, AutoScan-4® (Table 2) and genetic determination by PCR for all the isolates tested. On the other hand, only 16 (47·1 %) isolates were identified as *S. aureus* by Vitek® 2, with good or better confidence levels, without the use of supplementary tests such as Staphaurex and/or haemolysin activity and pigment of colony on sheep blood agar (Table 2). One strain was identified incorrectly as *Staphylococcus chromogenes* by the Vitek® 2 instrument (Table 1).

The 34 isolates used formed 24 clusters by pulsed-field gel electrophoresis when the data were analysed by the criteria of Tenover *et al.* (1995) (data not shown). Therefore, these atypical MRSA isolates have 24 or more origins.

Toho University Omori Hospital uses the Vitek® 2 system for identification of clinical isolates. This system had previously proved to provide accurate and acceptable identification and antibiotic susceptibility for Gram-positive cocci (Ligozzi *et al.*, 2002). Recently, the frequency of isolation of *S. aureus* with atypical physiological characteristics has increased to approximately 12·5 % (data not shown) in Toho University Omori Hospital, and it is very difficult to identify atypical *S. aureus* by the Vitek® 2 system unless extra tests are used. Furthermore, there have been several reports on the limitations of this identification system in distinguishing staphylococcal species (Becker *et al.*, 2004; Ben-Ami *et al.*, 2005).

Several genes have been targeted for PCR analysis of *S. aureus* but among these genes, *tst* genes, *eta* and *etb* and staphylococcal enterotoxin genes are not always detected in *S. aureus* (Becker *et al.*, 2003; Pinto *et al.*, 2005). The *nuc* gene has been widely used for species-specific detection, although it has also been reported in *Staphylococcus intermedius* strains (Becker *et al.*, 2005). Thus, the Sa442 DNA fragment was used to confirm *S. aureus* by PCR in the present study. Recently, Klaassen *et al.* (2003) reported that

Table 2. S. aureus isolates with dissenting or ambiguous results in identification by Vitek® 2, AutoScan-4® or Phoenix®

Isolate	Identification by Vitek® 2 (T index, confidence level)	Confidence value (%)	
		AutoScan-4®	Phoenix®
1	S. chromogenes (0·58, low); S. aureus (0·46, low)	99·9	99
2	S. aureus (0·31, good)	99·9	99
3	S. chromogenes (0·58, low); S. hyicus (0·50, low); S. aureus (0·49, low)	99·8	99
4	S. aureus (0·59, low); S. chromogenes (0·58, low); S. hyicus (0·50, low)	90·5	99
5	S. aureus (0·59, low); S. chromogenes (0·58, low); S. hyicus (0·50, low)	99·9	99
6	S. chromogenes (0·58, low); S. hyicus (0·50, low); S. aureus (0·49, low)	90·5	99
7	S. aureus (1·00, excellent)	99·9	98
8	S. chromogenes (0·50, good)	92·6	99
9	S. aureus (0·88, excellent)	99·9	99
10	S. chromogenes (0·58, low); S. aureus (0·56, low)	92·6	99
11	S. chromogenes (0·58, low); S. aureus (0·56, low)	99·9	99
12	S. aureus (0·56, very good)	99·9	99
13	S. aureus (0·56, very good)	99·9	99
14	S. aureus (0·90, excellent)	99·9	99
15	S. chromogenes (0·58, low); S. aureus (0·56, low)	99·9	99
16	S. chromogenes (0·58, low); S. hyicus (0·50, low); S. aureus (0·49, low)	99·8	99
17	S. chromogenes (0·58, low); S. aureus (0·56, low)	99·9	99
18	S. chromogenes (0·58, low); S. aureus (0·46, low)	99·9	99
19	S. aureus (0·56, very good)	99·8	99
20	S. chromogenes (0·58, low); S. aureus (0·46, low)	99·9	99
21	S. aureus (0·90, excellent)	99·9	99
22	S. aureus (0·84, excellent)	90·5	99
23	S. chromogenes (0·58, low); S. aureus (0·46, low)	99·8	99
24	S. aureus (0·56, very good)	99·9	99
25	S. chromogenes (0·58, low); S. aureus (0·56, low)	99·9	99
26	S. aureus (0·90, excellent)	99·9	99
27	S. aureus (0·65, very good)	99·9	99
28	S. aureus (0·56, very good)	99·8	99
29	S. chromogenes (0·58, low); S. aureus (0·46, low)	99·9	99
30	S. aureus (0·65, very good)	99·9	99
31	S. chromogenes (0·58, low); S. aureus (0·46, low)	99·8	99
32	S. chromogenes (0·58, low); S. aureus (0·46, low)	99·9	99
33	S. aureus (1·00, excellent)	99·9	99
34	S. aureus (1·00, excellent)	99·9	99

the Sa442 primer set did not work against the clinical isolate S. aureus 550226. They concluded that a number of S. aureus strains may have been misidentified in the past or the presence of S. aureus in clinical isolates may have been overlooked when identification was based solely on the Sa442 PCR assay. However, in our study, all 34 atypical S. aureus isolates were identified by PCR using this primer set.

Extra tests were required to confirm the identity of the strains when using the Vitek® 2 system: Staphaurex for membrane-bound coagulase and protein A, haemolysin activity and production of pigment on sheep blood agar plate. Staphaurex is a method commonly used for S. aureus identification, even though it only detects clumping factor and protein A. Staphaurex has also been reported as too insensitive for reliable detection of MRSA (Rappaport et al., 1988). We obtained negative results with Staphaurex for

several isolates. Fortunately, isolates negative for clumping factor and/or protein A produced acid from mannitol, and all 34 isolates produced free coagulase, were identified by PCR and as oxacillin resistant by antibiotic susceptibility testing.

The semi-automatic identification system of AutoScan-4® conforms to the requirements of the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) and requires a longer incubation period than Vitek® 2 or Phoenix®. However, it permits technologists to check biochemical reactions visually by observing the panels. We believe that this point is very important for clinical technologists, because some like to reconfirm the results of biochemical reactions by eye. The AutoScan-4® correctly identified the 34 S. aureus strains in this study without any extra tests.

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The Phoenix™ system also correctly identified all *S. aureus* strains without extra testing within 6 h. This was faster than the 18 h needed by the AutoScan-4® system and the more than 24 h needed by the Vitek® 2 system when additional tests were necessary. The confidence levels of identification were above 90 % for AutoScan-4® and Phoenix™. On the other hand, Vitek® 2 had low discrimination levels for 17 strains (Table 2).

It should be noted that molecular biological techniques, such as DNA sequencing (Becker *et al.*, 2004), hybridization (Sogaard *et al.*, 2005; Trindade *et al.*, 2003) or the use of DNA microarray technology (Charbonnier *et al.*, 2005), could provide a more accurate identification and classification tool, but such techniques are difficult to apply in a routine clinical laboratory. A rapid, conventional and automated identification method, based on phenotypic characters, is a more practical approach for daily clinical laboratory procedures.

In conclusion, the Phoenix™ system and AutoScan-4® could provide accurate information for the identification of *S. aureus* with atypical physiological characteristics without any extra tests. The merit of AutoScan-4® is that technologists can check biochemical reactions by observing the panels. This study shows that biochemically atypical *S. aureus* strains were not identified as *S. aureus* by Vitek® 2 unless extra tests were used. The Phoenix™ system identified all strains correctly within 6 h. Accordingly, this report suggests that Phoenix™, a fully automatic system, can be used for rapid identification in the clinical laboratory.

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ICU 患者における APACHE スコアと感染症発症率の関係

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Association between APACHE Score and Infection Rates in ICU Patients

別　　刷

〈報　告〉

ICU 患者における APACHE スコアと感染症発症率の関係

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Association between APACHE Score and Infection Rates in ICU Patients

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要　　旨

2000 年 7 月～2002 年 5 月，厚生労働科学研究参加 34 施設から収集された ICU 患者データをもとに，年齢 16 歳以上，ICU 在室 24 時間以上 1000 時間未満，APACHE スコアの情報が得られ，他院 ICU 転出例を除いた 13630 名を対象にして，ICU 在室中の感染症発症を調べた．観察期間は ICU 入室から最初の感染症発症を確認された日または ICU 退室日または ICU 入室後 22 日目までにした．対象者を APACHE スコアにより 0-10 群（6116 名），11-20 群（5304 名），21 以上群（2210 名）の 3 群にわけ，各群の感染症発症率を求めた．さらに，観察期間を 5 区間（0～2 日，3～7 日，8～12 日，13～17 日，18～22 日）にわけ，各群の区間別感染症発症率を求めた．

観察期間内の感染症発症者は 1412 名（10.4％）であった．APACHE スコアによる 3 群を比較すると，0-10 群で 249 名（4.1％），11-20 群で 653 名（12.3％），21 以上群で 510 名（23.1％）であり，APACHE スコアが高いほど感染症発症率が高い傾向を認めた（p＜0.001）．APACHE スコア 0-10 群は，観察期間が長いほど区間別感染症発症率が高い傾向を認め（p＜0.001），区間別感染症発症率を結んだ回帰直線の傾きは 5 区間では 0.009（95％ 信頼区間：－0.009～0.027）であったが，人数が少ない 18-22 日を除いた 4 区間では 0.018（95％ 信頼区間：0.015～0.022）であった．APACHE スコア 11-20 群は，観察期間と区間別感染症発症率の明らかな増減傾向を認めず（p＝0.4），区間別感染症発症率を結んだ回帰直線の傾きは－0.005（95％ 信頼区間：－0.008～－0.001）であった．APACHE スコア 21 以上群は，観察期間が長いほど区間別感染症発症率が低い傾向を認め（p＜0.001），区間別感染症発症率を結んだ回帰直線の傾きは－0.018（95％ 信頼区間：－0.029～－0.007）であった．

ICU 患者における感染症発症率は，APACHE スコア 0-10 では ICU 在室日数が長いほど増加，APACHE スコア 21 以上では ICU 在室日数が長いほど減少，APACHE スコア 11-20 では ICU 在室日数の影響を受けず，ほぼ一定であることが明らかにされた．

Key words：多施設共同研究，ICU, APACHE スコア，感染症，ICU 在室期間

はじめに

集中治療室（Intensive Care Unit; ICU）は，重症患者が収容される，侵襲的処置が行われるなどの理由から，院内感染が発生しやすい部署である[1,2]．ICU 患者の院内感染のリスク要因については，これまで数多くの研究が行なわれ，外部要因として手術，ディバイス，その他の侵襲的処置，内部要因として重症度や免疫抵抗力などの存在が指摘されている[1,3]．各施設・部署の感染症発症率を評価するにあたり，これらリスク要因の調整が重要である．しかし，アメリカのサーベイランスシステム（National Nosocomial Infection Surveillance; NNIS）を含めて，既存のサーベイランスシステムの多くは重症度の情報を収集しておらず，内部要因の調整が十分行なわれていないという問題が指摘されている[3~5]．

厚生労働省院内感染対策サーベイランス事業

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(Japanese Nosocomial Infection Surveillance; JANIS)
は 2000 年 7 月から開始され，ICU 部門，検査部門，全
入院部門の 3 部門を設定して，独自のサーベイランス
システムの構築を進めている．ICU 部門の特徴の 1 つ
に，Acute Physiology and Chronic Health Evaluation
II[6]（以下，APACHE スコア）の情報を収集している点
が挙げられる．APACHE スコアは ICU 患者の重症度
の評価と予後の予測を目的につくられた指標である．
1985 年に Knaus らが提唱して以来，ひろく世界中で
つかわれている．12 種類のバイタルサインに関するポ
イント（acute physiology score），年齢に関するポイン
ト（age points），慢性疾患と手術に関するポイント
（chronic health points）の合算でもとめられ，スコアが
高いほど重症度が高いと判断される．さらに，指定され
た計算式にあてはめれば，予測死亡率を計算できる．

　APACHE スコアと死亡率との関連については，これ
まで数多くの研究が行なわれ，予測死亡率と観察死亡率
の相関などが示されている．しかし，APACHE スコア
と感染症発症率との関連については，十分検討されてお
らず，APACHE スコアが院内感染の予測指標になりう
るかという点において必ずしも見解が一致していな
い[7,8]．サーベイランスから得られた結果を正しく評価
するために，リスク要因の扱いを明確にする必要があ
り，内部要因を代表する APACHE スコアと感染症発症
率との関連を明らかにすることは感染症発症率の評価方
法を検討する基礎資料を提供すると考えられる．本研究
では，JANIS の ICU 部門の研究班のデータベースを用
いて，ICU 患者における APACHE スコアと感染症発
症率の関係を調べた．APACHE スコアは ICU 在室日
数を左右すると考えられ，ICU 在室の長期化が院内感
染のリスクを高める可能性が指摘されている[9~11]こと
から，とくに ICU 在室日数を考慮した場合の両者の関
係の違いに注目した．

対象と方法

　ICU 患者データは，JANIS の実施マニュアルにもと
づいて，厚生科学研究参加 34 施設から収集した[12]．詳
細は別稿[13,14]にあるが，全 ICU 患者を対象にして，属
性（性，年齢，主病名，APACHE スコア，ICU 入・退
室日時と経路），リスク要因（手術，ディバイス，特殊治
療，合併症），感染症（肺炎，尿路感染症，カテーテル関
連血流感染症，敗血症，創感染症，その他の感染症），
転帰（ICU 退室時診断，退院時診断，診療報酬点数）な
どの情報を JANIS 開発の入力支援ソフトを利用して入
力した．APACHE スコアは ICU 入室後 24 時間以内に
判定した．感染症は厚生科学研究班の基準[15]により診
断した．

　2000 年 7 月～2002 年 5 月の ICU 患者 27625 名のう

ち，年齢 16 歳以上，ICU 在室 24 時間以上，APACHE
スコアの情報が得られたものは 13838 名である．追跡
不可能例として他院 ICU 転出を除外，特殊例として
ICU 在室 1000 時間以上を除外，残された 13630 名（男
性 8829 名，女性 4801 名）を対象にした．

　ICU 在室中の感染症発症を調べた．対象者（13630
名）の 97.0％ は ICU 入室後 23 日未満で ICU を退室し
ていた．また，感染症発症者（1433 名）の 98.5％ は ICU
入室後 23 日未満で感染症を発症していた．そこで，観
察期間は ICU 入室から最初の感染症発症を確認された
日または ICU 退室日または ICU 入室後 22 日目までに
した．

　対象者を APACHE スコアにより 0-10 群（6116 名），
11-20 群（5304 名），21 以上群（2210 名）の 3 群にわ
け，各群の感染症発症率を求めた．APACHE スコアの
高さによる感染症発症率の増減傾向を調べるために，
Cochran-Armitage の傾向性の検定[16]を実施した．さら
に，観察期間を 5 区間（0～2 日，3～7 日，8～12 日，
13～17 日，18～22 日）にわけ，各群の区間別感染症発
症率を求めた．観察期間の長さによる区間別感染症発症
率の増減傾向を調べるために，Cochran-Armitage の傾
向性の検定および回帰分析を実施した．統計学的解析は
Statistical Analysis System (SAS Version 8.2)を用いた．

　なお，本研究を実施するにあたり，個人情報の保護を
配慮して，データの匿名化をはかり，データの収集・解
析の各段階において機密保持につとめた．

結　　果

　表 1 に本研究対象の属性を示した．表 2 に APACHE
スコアによる 3 群の観察期間の分布を示した．観察期
間内の感染症発症者は 1412 名（10.4％）であった．感染
部位の内訳は，多いほうから，肺炎 902 名，敗血症 250
名，創感染症 175 名，尿路感染症 64 名，カテーテル関
連血流感染症 49 名，その他 156 名（重複を含む）であ
り，肺炎が最多の 64％ を占めた．APACHE スコアに
よる 3 群を比較すると，0-10 群で 249 名（4.1％），11-
20 群で 653 名（12.3％），21 以上群で 510 名（23.1％）で
あり，APACHE スコアが高いほど感染症発症率が高い

表 1　本研究対象の属性

		全　体 (n=13620)	男　性 (n=8829)	女　性 (n=4801)
年齢 （平均±標準偏差）		62.4±15.7	62.1±14.9	63.0±17.0
APACHE スコア	0-10	6116(44.9%)	4091(46.3%)	2025(42.2%)
	11-20	5304(38.9%)	3362(38.1%)	1942(40.4%)
	21+	2210(16.2%)	1376(15.6%)	834(17.4%)

数値：人数

表2　APACHE スコアによる3群の観察期間の分布

APACHE スコア		観察 期 間						全体
		0-2	3-7	8-12	13-17	18-22	23+$	
全体	全 体	5742	5760	1205	519	202	202	13630
	非感染者	4965	5340	1067	462	182	202	12218
	感 染 者	777	420	138	57	20	0	1412
0-10	全 体	3002	2547	340	149	40	38	6116
	非感染者	2874	2467	315	136	37	38	5867
	感 染 者	128	80	25	13	3	0	249
11-20	全 体	1991	2380	525	217	99	92	5304
	非感染者	1615	2188	472	195	89	92	4651
	感 染 者	376	192	53	22	10	0	653
21+	全 体	749	833	340	153	63	72	2210
	非感染者	476	685	280	131	56	72	1700
	感 染 者	273	148	60	22	7	0	510

数値：人数
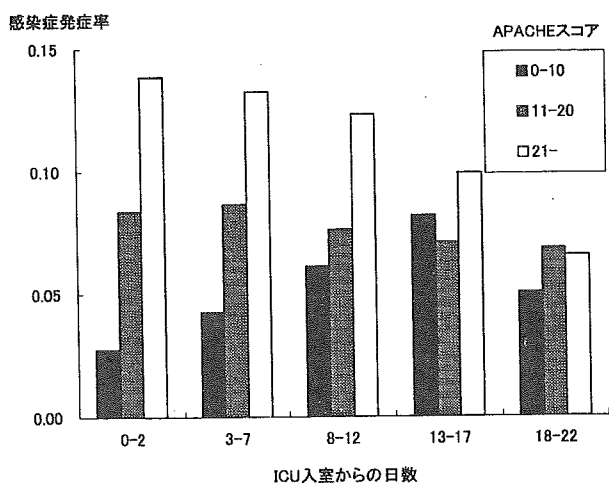観察期間は ICU 入室から最初の感染症発症を確認された日または ICU 退室日または ICU 入室後 22 日目まで（$ は打ち切り例）

図1　区間別感染症発症率

傾向を認めた（p＜0.001）.

　非感染症発症者において，打ち切り例を除いて，APACHE スコアと観察期間の関係を調べたところ，以下のような回帰式が得られ，APACHE スコアが高いほど ICU 在室日数が長い傾向を認めた（p＜0.001）.

　　観察期間（日数）＝2.98＋0.11×APACHE スコア
　　　（傾きの 95％ 信頼区間：0.10〜0.12；p＜0.001）

　ICU 在室日数の長期化が院内感染のリスクを高める可能性が指摘されている[9〜11]ことから，ICU 在室日数を考慮した APACHE スコアと感染症発症率の関係を明らかにするために，観察期間を 5 区間にわけ，各群の区間別発症率を調べた.

　図 1 に区間別感染症発症率を示した．APACHE スコア 0-10 群は，観察期間が長いほど区間別感染症発症率

が高い傾向を認めた（p＜0.001）．区間別感染症発症率を結んだ回帰直線の傾きは 5 区間では 0.009（95％ 信頼区間：−0.009〜0.027；p＝0.2）であったが，人数が少ない 18〜22 日を除いた 4 区間では 0.018（95％ 信頼区間：0.015〜0.022；p＜0.001）であった．APACHE スコア 11-20 群は，観察期間と区間別感染症発症率の明らかな増減傾向を認めず（p＝0.4），区間別感染症発症率を結んだ回帰直線の傾きは−0.005（95％ 信頼区間：−0.008〜−0.001；　p＝0.02）であった．APACHE スコア 21 以上群は，観察期間が長いほど区間別感染症発症率が低い傾向を認めた（p＜0.001）．区間別感染症発症率を結んだ回帰直線の傾きは−0.018（95％ 信頼区間：−0.029〜−0.007；p＝0.01）であった.

考　　察

　JANIS の ICU 部門の研究班のデータベースを用いて，ICU 患者における APACHE スコアと感染症発症率の関係，とくに ICU 在室日数を考慮した場合の両者の関係の違いを調べた．ヨーロッパ 17 ヵ国の多施設共同研究（EPIC スタディ）は ICU 在室日数が長いほど感染率が高いことを示しており，多重ロジスティック解析においても ICU 在室日数の影響を有意に認めた[9]．ICU 在室日数の影響については，フランスやメキシコの多施設共同研究からも同様の結果が報告されている[10,11]．このような過去の研究の結果から，本研究においても，APACHE スコアのレベルに関わらず，ICU 在室日数が長いほど感染症発症率が高いと予想された．しかし，本研究の結果から，ICU 患者における感染症発症率は，APACHE スコア 0-10 では ICU 在室日数が長いほど増加，APACHE スコア 21 以上では ICU 在室日数が長い

ほど減少，APACHE スコア 11-20 では ICU 在室日数の影響を受けず，ほぼ一定であることが明らかにされた．本研究は対象者を APACHE スコアにより 3 群にわけ，さらに，観察期間を 5 区間にわけ，各群の区間別感染症発症率を求めたことで，これまで知られていない隠れた傾向を検出しえたといえる．

　APACHE スコア 21 以上の全身状態が悪い患者は早期死亡が多く ICU 在室日数が短い，このような関連から ICU 在室日数が長いほど感染症発症率が減少して見えたのでないか，すなわち，見かけ上の効果にすぎないのでないかという指摘もあるかもしれない．しかし，APACHE スコア 21 以上の患者の区間死亡率は，0〜2 日が 9.2％，3〜7 日が 13.8％，8〜12 日が 13.7％，13〜17 日が 11.1％，18〜22 日が 13.9％であり，死亡による脱落が区間死亡率の著しい偏りを生じていないことを確認している．

　図 1 の解釈に関して，ICU 在室日数が長いほど APACHE スコアの影響が小さくなり，APACHE スコアのレベルに関わらず，感染症発症率が一定レベルに収束する様子を表しているという意見もあるかもしれない．ICU 在室日数が長くなれば，APACHE スコアを含めて，ICU 入室時の要因の影響は小さくなると推察される．また，ICU 入室時の状態から変化する可能性もある．APACHE スコアの経時的変化を考慮した解析をおこなうか，異なる集団において本研究の結果を再確認する必要があるだろう．

　ICU 在室日数の区間別感染症発症率の傾向が APACHE スコアのレベルで異なる理由は不明であり，今後，追求すべき課題である．ただ，現時点において，外部要因と内部要因のバランスの違いを反映している可能性が考えられる．APACHE スコアが 20 を超えると予測死亡率が 50％ を超えるため，20 がひとつの目安になると言われている[17]．また，JANIS データベースを用いて，APACHE スコア 0-10 群を基準にした解析をおこない，11 を超える各群は死亡リスクが有意に高いことを報告している[18]．そこで，本研究は APACHE スコアを 0-10，11-20，21 以上にわけた．APACHE スコア 0-10 群は全身状態が良く死亡リスクが低い軽症の患者，APACHE スコア 21 以上群は全身状態が悪く死亡リスクが高い重症の患者，APACHE スコア 11-20 群は両者の中間を表わしている．APACHE スコア 0-10 の全身状態が良い患者では，院内感染はおもに外部要因（環境）に依存して発生するため，ICU 在室日数が長いほど外部要因の曝露の機会が多くなり，院内感染が増加したと考えられる．それに対して，APACHE スコア 21 以上の全身状態が悪い患者では，院内感染はおもに内部要因（宿主）に依存して発生するため，ICU 在室日数が長いほど病態が改善され，内部要因が少なくなり，院内
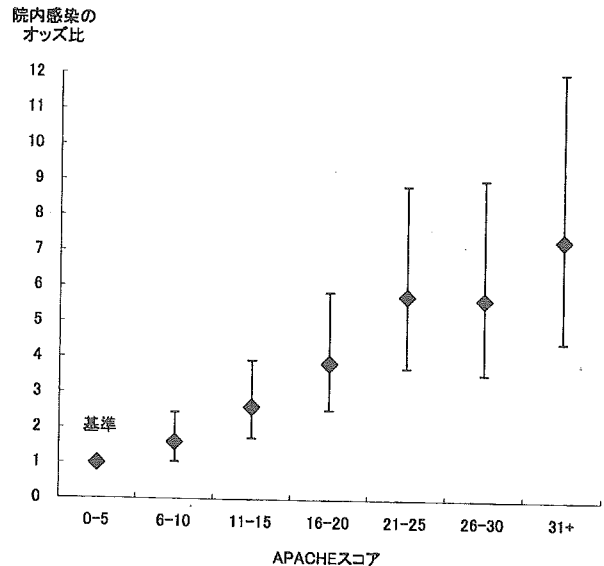
院内感染の
オッズ比

図 2　院内感染のオッズ比と 95％信頼区間（APACHE スコア 0-5 を基準にした場合）
ICU 在室 2〜14 日の 7999 名について，多重ロジスティック解析から，性，年齢，手術，ディバイスを調整した院内感染のオッズ比を求めた．

感染がむしろ減少したと考えられる．

　本研究の結果から，APACHE スコアが高いほど感染症発症率が高い傾向を認めた．さらに，性，年齢，手術，ディバイスなどの交絡因子を調整した多重ロジスティック解析を実施した結果から，APACHE スコアが高いほど感染リスクが高いことを確認している（図 2）[19]．ICU の院内感染の予防対策において，APACHE スコア 21 以上の全身状態が悪い患者は重点対象になる．ただ，内部要因にともなう易感染性を軽減することは難しいため，これらの患者の感染を予防することは困難であろうと考えられる．それに対して，APACHE スコア 0-11 の全身状態の良い患者は ICU 入室当初からの重点対象にならないかもしれない．しかし，本研究の結果によれば，ICU 在室日数が長いほど感染症発症率が高く，外部要因による感染の増加が疑われた．外部要因による感染は適切な対策を講じれば予防可能であり，ICU 入室時点の全身状態が悪くなく APACHE スコアが高くない患者においても，ICU 在室日数が長くなる場合，院内感染を予防する適切な対策を講じる必要があることが示唆された．

　JANIS の ICU 部門の研究班のデータベースを用いたことで，標準化されたデータによる，より信頼性のある検討[5,20]を実現しえた．その一方，本研究対象の厚生科学研究参加 34 施設はおもに国立大学から構成され，高度先進医療を実施する施設であることから，本研究対象は日本全体を代表すると言い難い．しかし，日本の三次医療機関の ICU 患者における APACHE スコアと感染

症発症率の関係をしめす貴重なエビデンスである．本研究の結果を踏まえ，サーベイランスにおいては，得られた結果を正しく評価するために，対象者を APACHE スコアや ICU 在室日数で層別化するなど，評価方法を工夫が求められる．また，院内感染のリスク要因を探索する研究においては，APACHE スコアと ICU 在室日数を考慮した解析が求められる．

本研究では，全般的傾向を把握するために，基礎疾患や感染部位を限定せずに APACHE スコアと感染症発症率の関係を調べたが，対象を特定の疾患の患者に限定した研究[21]や感染症を特定の感染部位に限定した研究[22,23]なども行われている．ICU 在室日数を考慮した場合の APACHE スコアと感染症発症率の関係についても，今後，基礎疾患や感染部位別の解析をおこない，詳細を明らかにしたい．

結 論

JANIS の ICU 部門の研究班のデータベースを用いた検討から，ICU 患者における感染症発症率は，APACHE スコア 0-10 では ICU 在室日数が長いほど増加，APACHE スコア 21 以上では ICU 在室日数が長いほど減少，APACHE スコア 11-20 では ICU 在室日数の影響を受けず，ほぼ一定であることが明らかにされた．

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