following thermal cycling profile: initial denaturation at 94°C for 5 min, the denaturation, annealing, and extension reactions shown in Table 1 for 30 cycles (35 cycles for detection of *agrI, agrII, agrIII*, and *agrIV*), respectively, followed by final extension at 72°C for 7 min. PCR products were then analyzed by electrophoresis on a 2% agarose gel. After electrophoresis, gels were stained with ethidium bromide (1 mg/l) and photographed under a UV trans-illuminator. A 100-bp DNA Ladder (New England Biolabs, Beverly, MA, USA) was used as a molecular size marker. The fragment sizes of each PCR product are shown in Table 1.

Retrospective clinical study. We retrospectively reviewed the medical records of the 109 patients and classified their UTI as catheter-related or catheter-unrelated, polymicrobial or monomicrobial, and febrile or non-febrile cases. Febrile UTI was defined as UTI in a patient with a body temperature of ≥38.0°C.

Statistical methods. Data are expressed as mean values ± standard deviation (SD). Comparison of OD_{570} values between groups was carried out using Fisher's exact test or Mann-Whitney's *U* test. All results were considered statistically significant at the $P<0.05$ level.

Results

Biofilm formation. Of the 109 MRSA isolates, 10 (9.2%), 31 (28.4%), 63 (57.8%), and 5 (4.6%) isolates exhibited strong (OD_{570} ≥0.5), medium (OD_{570} ≥0.2 to <0.5), weak (OD_{570} 0 to <0.2), and no biofilm formation, respectively. The mean OD_{570} of the 109 isolates was 0.24±0.18 (mean±SD).

Presence of various genes in MRSA isolates. Of the 109 MRSA isolates, 79 (72.5%), 8 (7.3%), 21 (19.3%), 78 (71.6%), 89 (81.7%), 73 (67.0%), 79 (72.5%), 3 (2.8%),

84 (77.1%), 6 (5.5%), 108 (99.1%), 1 (0.9%), 99 (90.8%), 2 (1.8%), and 0 (0%) isolates possessed *tst, sea, seb, sec, hla, hlb, fnbA, fnbB, clfA, cna, icaA, agrI, agrII, agrIII,* and *agrIV*, respectively (Fig. 1). Eight determinants (*tst, sec, hla, hlb, fnbA, clfA, icaA*, and *agrII*) were found to be predominant among these isolates.

Relationship between biofilm formation and several virulence determinants. We evaluated the relationship between biofilm formation and the six predominant genes of the MRSA isolates (Table 2). The six determinants were as follows: *tst*, encoding the toxic shock syndrome toxin 1; *sec*, encoding the staphylococcal enterotoxin C; *hla*, encoding the α- toxin; *hlb*, encoding the β- toxin; *fnbA*, encoding the fibronectin-binding protein A; and *clfA*, encoding the fibrinogen-binding protein A. The other predominant genes, *icaA* of the intercellular adhesin locus and *agrII* of the accessory gene regulator, were excluded from the evaluation, since more than 90% of the MRSA isolates possessed these genes (Fig. 1). As shown in Table 2, the mean OD_{570} value (mean±SD) was significantly higher in *hlb*- and *fnbA*-positive isolates than in *hlb*- and *fnbA*-negative isolates (P=0.0034 and P =0.0052, respectively). The value was also higher in *hla*-positive isolates than *hla*-negative isolates (P=0.0836). The percentage of *hla*-, *hlb*-, and *fnbA*-positive isolates was 45.6%, 57.4%, and 50% among those with OD_{570} values of 0 to <0.2, 90.3%, 80.7%, and 83.9% among those with OD_{570} values of ≥0.2 to <0.5, and 100%, 90.3%, and 90% among those with OD_{570} values of ≥0.5, respectively (Fig. 2). The percentage of *hla*- and *fnbA*-positive isolates was significantly higher in the strong biofilm-forming group than in the weak group (P=0.012, P =0.020), and the percentage of *hlb*-positive isolates was higher in the strong biofilm-forming group than in the weak group (P=0.079). We also evaluated the biofilm-forming capacities of MRSA isolates in seven groups based on the presence/absence of *hla, hlb*, and *fnbA* genes (Fig. 3). As shown with a box and whisker plot, the MRSA isolates with 3 or 2 combinations of *hla,*

hlb, and *fnbA* genes had greater capacities for biofilm formation than did those lacking these three genes. The Mann-Whitney's *U* test of two mean OD_{570} values (mean±SD), 0.31±0.43 and 0.15±0.01, in 62 *hla*-, *hlb*-, *fnbA*-positive and 13 *hla*-, *hlb*-, *fnbA*-negative isolates, respectively, confirmed that MRSA isolates possessing *hla*, *hlb*, and *fnbA* genes together had significantly greater capacities for biofilm formation than did those lacking these three genes (P=0.0186).

Relationship between biofilm formation and clinical background. The 109 cases of UTI caused by MRSA consisted of 51 catheter-related (46.8%) and 58 catheter-unrelated cases (53.2%), 50 polymicrobial (45.9%) and 59 monomicrobial cases (54.1%), and 21 febrile (19.3%) and 88 non-febrile cases (80.7%). The biofilm-forming capacities of MRSA isolates from catheter-related cases were significantly greater than those from catheter-unrelated cases (P=0.0162) (Table 2). As shown in Fig. 4, the percentage of *hla*-, *hlb*-, and *fnbA*-positive isolates was 88.2%, 72.5%, and 76.5%, respectively, among MRSA isolates from catheter-related cases (n=51) and 75.9%, 60.3%, and 70.7%, respectively, among those from catheter-unrelated cases (n=58).

Discussion

On our urology ward, 20% of UTI caused by MRSA are febrile and patients who are asymptomatic are often observed without any intervention [4]. We previously reported that the presence of both the *tst* and *sec* genes may be associated with the incidence of febrile cases of UTI caused by MRSA [4]. MRSA isolated from urine rarely causes serious infectious symptoms, but once this occurs, therapy is difficult. One reason for this is that MRSA forms biofilms in the urinary tract [16]. It is also difficult to eradicate bacteria completely in patients with an indwelling urinary catheter and/or stent.

Therefore, it is important to understand biofilm formation and the pathogenicity of MRSA infections in the urinary tract.

Biofilms are surface-associated, sessile bacterial communities. A mature biofilm is formed when planktonic cells initially colonize a surface, aggregate and/or grow into multicellular colonies, and embed themselves in an exopolysaccharide matrix [6, 7]. In general, staphylococcal cells embedded in a biofilm or in microcolonies are much more resistant to antimicrobial agents than are planktonic cells [5]. Many patients with a chronic staphylococcal infection have been treated with various antimicrobial agents, mostly without much success. Genetic analyses of staphylococci have shown that the progression of biofilm development consists of 2 steps: initial cell-to-surface interactions followed by cell-to-cell interactions [5, 11, 17].

S. aureus is especially capable of adhering to a large variety of matrix components to initiate colonization [5]. This adherence is frequently mediated by protein adhesins of the family known as MSCRAMM (microbial surface components recognizing adhesive matrix molecules). The collagen-binding protein, fibronectin-binding proteins, and fibrinogen-binding proteins belong to this family. In this study, we analyzed the presence of four determinants (*fnbA, fnbB, clfA*, and *cna*) encoding surface proteins FnBPA, FnBPB, ClfA, and Cna, respectively. Of 109 MRSA isolates, 79 (72.5%), 3 (2.8%), 84 (77.1%), and 6 (5.5%) isolates possessed *fnbA, fnbB, clfA*, and *cna*, respectively. Of the four determinants, *fnbA* and *clfA* genes were predominant in the isolates, and the *fnbA*-positive isolates had significantly greater capacities for biofilm formation than did the *fnbA*-negative isolates (P=0.0052) (Table 2). The percentage of *fnbA*-positive isolates was higher among MRSA isolates from catheter-related cases than those from catheter-unrelated cases (Fig. 4).

The *ica* locus, which is required for the synthesis of the polysaccharide

intracellular adhesin (PIA) of staphylococci, plays a role in cell-to-cell interactions during biofilm formation and is predominantly present in clinical isolates [5]. Our data showed that 108 of 109 (99.1%) of MRSA isolates possessed *icaA*. Only one isolate without the *icaA* gene possessed *tst, sec, hla, hlb, fnbA*, and *clfA*, and the biofilm-forming capacity of the *icaA*-negative isolate was weak (OD_{570} value: 0.02). Even though the isolate does not produce PIA, other surface-associated virulence factors may be overexpressed, functionally compensating for the lack of PIA. Other factors, such as the autolysin, the D-alanine esterification of teichoic acids, the accumulation-associated protein, and the like that contribute to biofilm formation were described in a review article [5]. Previously unknown factors, in particular adhesins, which have been identified by whole genome sequencing of MRSA, may also contribute to biofilm formation [18].

Caiazza *et al.* [8] showed that Hla, a 34-kDa protein that causes host cell lysis by heptamerizing upon insertion into eukaryotic cell membranes, plays a role primarily in cell-to-cell interactions during biofilm formation. The level of Hla correlates with the level of biofilm formation. Caiazza *et al.* [8] were initially surprised to find that a secreted toxin had such a dramatic impact on biofilm formation. In this study, we showed that the biofilm-forming capacities of MRSA isolates were higher in *hla*- and *hlb*-positive isolates than in *hla*- and *hlb*-negative isolates, respectively (P=0.0034, P=0.0836). Other examples exist in which secreted toxins and enzymes may play a role in biofilm formation [19, 20, 21, 22]. These toxins encoded by *hla* and *hlb* genes may be bifunctional enzymes and cause tissue damage of urinary epithelium. The percentage of *hla*- and *hlb*- positive isolates was higher among MRSA isolates from catheter-related cases than those from catheter-unrelated cases (Fig. 4).

Quorum sensing via the accessory gene regulator (*agr*) system has been assigned a central role in the pathogenesis of staphylococci, particularly *S. aureus* [10, 11]. The *agr*

system regulates a wide array of virulence factors, including those involved in surface-associated virulence and biofilm formation [5, 7, 10, 11]. *S. aureus* strains can be divided into 4 major groups based on *agr* variations [10]. The relationship between *agr* groups and clinical features has been reported [10, 23]; for instance, most menstrual TSS strains belong to *agr* group III [24], all the strains causing leucocidin-induced necrotizing pneumonia belong to *agr* group III [25], most intermediate-level glycopeptide resistance strains belong to *agr* group II [26], and most exfoliatin-producing strains belong to *agr* group IV [27]. Our data showed that 99 of 109 (90.8 %) of the MRSA isolates belonged to *agr* group II. We are unable to assess the relationship between *agr* group II and UTI, since *agr* group II may be predominant in MRSA isolated in Japan, based on a database search [18].

The epidemic of UTI caused by MRSA at the Okayama University Hospital appears to be representative of the changing epidemiology of *S. aureus* throughout Japan [4]. Molecular typing of MRSA isolates by random amplified polymorphic DNA and pulsed-field gel electrophoresis analyses revealed no apparent clonality of these isolates in the urology ward over a 10-year period. It is possible that the MRSA in the urology ward originate from other wards in the hospital, other hospitals, or other communities. MRSA isolates that had previously been largely confined to hospitals have recently started emerging in the outside community [28, 29, 30].

In this study, the biofilm-forming capacities of MRSA isolates from catheter-related cases were significantly greater than those from catheter-unrelated cases (P=0.0162). Biofilm formation by staphylococci occurs not only with indwelling devices but also in response to any bacterial factor that mediates adherence to components of the extracellular matrix of the host [5]. Peacock *et al.* [15] reported that seven determinants (*fnbA*, *cna*, *sdrE*, *sej*, *eta*, *hlg*, and *ica*) of *S. aureus* were significantly more common in

invasive isolates. No single factor predominated as the major predictor of virulence, and their effects appeared to be cumulative. The relative importance of host factors versus bacterial virulence determinants in disease pathogenesis is unknown. Host factors for *S. aureus* disease are likely to include a genetic predisposition via one or more susceptibility genes and acquired factors such as the presence of intravenous devices, surgical wounds, and other events that perturb normal host defenses.

Taken together, our studies suggest that MRSA colonization and infection of the urinary tract may be promoted by *hla*, *hlb*, and *fnbA* gene products.

Acknowledgments. This work was supported in part by a Grant-in-Aid for Scientific Research (C) (2003-2004, No. 15591688) from the Japan Society for the Promotion of Science and by a Grant-in-Aid from the Ministry of Health, Labour and Welfare.

References

1. Tenover FC and Gaynes RP: The epidemiology of *Staphylococcus* infections; in Gram-Positive Pathogens, Fischetti VA, Novick RP, Ferretti JJ, Portnoy DA and Rood JI eds, American Society for Microbiology, Washington DC (2000) pp 414-421.
2. Konno M: Nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* in Japan. J Infect Chemother (1995) 1: 30-39.
3. Arakawa Y, Ike Y, Nagasawa M, Shibata N, Doi Y, Shibayama K, Yagi T and Kurata T: Trends in antimicrobial-drug resistance in Japan. Emerg Infect Dis (2000) 6: 572-575.
4. Araki M, Kariyama R, Monden K, Tsugawa M and Kumon H: Molecular epidemiological studies of *Staphylococcus aureus* in urinary tract infection. J Infect Chemother (2002) 8: 167-174.
5. Götz F: *Staphylococcus* and biofilms. Mol Microbiol (2002) 43: 1367-1378.
6. Donlan RM and Costerton JW: Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev (2002) 15: 167-193.
7. Hall-Stoodley L, Costerton JW and Stoodley P: Bacterial biofilms: from the natural environment to infectious diseases. Nature Rev Microbiol (2004) 2: 95-108.
8. Caiazza NC and O'Toole GA: Alpha-toxin is required for biofilm formation by *Staphylococcus aureus*. J Bacteriol (2003) 185: 3214-3217.
9. Dinges MM, Orwin PM and Schlievert PM: Exotoxins of *Staphylococcus aureus*. Clin Microbiol Rev (2000) 13: 16-34.
10. Novick RP: Autoinduction and signal transduction in the regulation of staphylococcal vilulence. Mol Microbiol (2003) 48: 1429-1449.

11. Yarwood JM and Schlievert PM: Quorum sensing in *Staphylococcus* infections. J Clin Invest (2003) 112: 1620-1625.

12. Knobloch JK, Horstkotte MA, Rohde H and Mack D: Evaluation of different detection methods of biofilm formation in *Staphylococcus aureus*. Med Microbiol Immunol (Berl). (2002) 191: 101-106.

13. Johnson WM and Tyler SD: PCR detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin-1 in *Staphylococcus aureus*; in Diagnostic Molecular Microbiology: Principles and Applications, Persing DH, Smith TF, Tenover FC and White TJ eds, American Society for Microbiology, Washington DC (1993) pp 294-299.

14. Booth MC, Pence LM, Mahasreshti P, Callegan MC and Gilmore MS: Clonal associations among *Staphylococcus aureus* isolates from various sites of infection. Infect Immun (2001) 69: 345-352.

15. Peacock SJ, Moore CE, Justice A, Kantzanou M, Story L, Mackie K, O'Neill G and Day NP: Virulent combinations of adhesin and toxin genes in natural populations of *Staphylococcus aureus*. Infect Immun (2002) 70: 4987-4996.

16. Kumon H: Pathogenesis and management of bacterial biofilms in the urinary tract. J Infect Chemother (1996) 2: 18-28.

17. Heilmann C, Gerke C, Perdreau-Remington F and Götz F: Characterization of Tn*917* insertion mutants of *Staphylococcus epidermidis* affected in biofilm formation. Infect Immun (1996) 64: 277-282.

18. Kuroda M, Ohta T, Uchiyama I, Baba T, Yuzawa H, Kobayashi I, Cui L, Oguchi A, Aoki K, Nagai Y, Lian J, Ito T, Kanamori M, Matsumaru H, Maruyama A, Murakami H, Hosoyama A, Mizutani-Ui Y, Takahashi NK, Sawano T, Inoue R, Kaito C, Sekimizu K, Hirakawa H, Kuhara S, Goto S, Yabuzaki J, Kanehisa M, Yamashita A,

Oshima K, Furuya K, Yoshino C, Shiba T, Hattori M, Ogasawara N, Hayashi H and Hiramatsu K: Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*. Lancet (2001) 357: 1225-1240.

19. Kachlany SC, Fine DH and Figurski DH: Secretion of RTX leukotoxin by *Actinobacillus actinomycetemcomitans*. Infect Immun (2000) 68: 6094-6100.
20. Kachlany SC, Planet PJ, Bhattacharjee MK, Kollia E, DeSalle R, Fine DH and Figurski DH: Nonspecific adherence by *Actinobacillus actinomycetemcomitans* requires genes widespread in bacteria and archaea. J Bacteriol (2000) 182: 6169-6176.
21. Vuong C, Saenz HL, Götz F and Otto M: Impact of the *agr* quorum-sensing system on adherence to polystyrene in *Staphylococcus aureus*. J Infect Dis (2000) 182: 1688-1693.
22. Kristich CJ, Li YH, Cvitkovitch DG and Dunny GM: Esp-independent biofilm formation by *Enterococcus faecalis*. J Bacteriol (2004) 186: 154-163.
23. Jarraud S, Mougel C, Thioulouse J, Lina G, Meugnier H, Forey F, Nesme X, Etienne J and Vandenesch F : Relationships between *Staphylococcus aureus* genetic background, virulence factors, *agr* groups (alleles), and human disease. Infect Immun (2002) 70: 631-641.
24. Ji G, Beavis R and Novick RP: Bacterial interference caused by autoinducing peptide variants. Science (1997) 276: 2027-2030.
25. Gillet Y, Issartel B, Vanhems P, Fournet JC, Lina G, Bes M, Vandenesch F, Piemont Y, Brousse N, Floret D and Etienne J: Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet (2002) 359:753-759.
26. Sakoulas G, Eliopoulos GM, Moellering RC Jr, Novick RP, Venkataraman L,

Wennersten C, DeGirolami PC, Schwaber MJ and Gold HS: *Staphylococcus aureus* accessory gene regulator (*agr*) group II: is there a relationship to the development of intermediate-level glycopeptide resistance? J Infect Dis (2003) 187: 929-938.

27. Jarraud S, Lyon GJ, Figueiredo AMS, Gérard L, Vandenesch F, Etienne J, Muir TW and Novick RP: Exfoliatin-producing strains define a fourth *agr* specificity group in *Staphylococcus aureus*. J Bacteriol (2000) 182: 6517-6522.
28. Hiramatsu K, Cui L, Kuroda M and Ito T: The emergence and evolution of methicillin-resistant *Staphylococcus aureus*. Trends Microbiol (2001) 9: 486-493.
29. Cookson BD: Methicillin-resistant *Staphylococcus aureus* in the community: new battlefronts, or are the battles lost? Infect Control Hosp Epidemiol (2000) 21: 398-403.
30. Chambers HF: The changing epidemiology of *Staphylococcus aureus*? Emerg Infect Dis (2001) 7: 178-182.

Legends to Figures

Fig. 1 Percentage of *tst*-, *sea*-, *seb*-, *sec*-, *hla*-, *hlb*-, *fnbA*-, *fnbB*-, *clfA*-, *cna*-, *icaA*-, *agrI*-, *agrII*-, *agrIII*-, and *agrIV*-positive isolates among MRSA isolates.

Fig. 2 Percentage of *hla*-, *hlb*-, and *fnbA*-positive isolates among MRSA isolates that belong to the following three biofilm-forming groups: OD_{570} 0 to <0.2 (weak biofilm former), OD_{570} ≥0.2 to <0.5 (medium biofilm former), and OD_{570} ≥0.5 (strong biofilm former).

Bars: ▨, *hla* ; ⊡, *hlb* ; ⊞, *fnbA*.

**P*=0.012, ***P*=0.079, ****P*=0.020 (Fisher's exact test)

Fig. 3 Biofilm-forming capacities of MRSA isolates in seven groups based on the presence/absence of *hla*, *hlb*, and *fnbA* genes. OD_{570} values of the isolates in seven groups are shown by the box and whiskers plot that is a five-number summary (upper extreme, upper quartile, median, lower quartile, and lower extreme). The mean OD_{570} values (mean±SD) in seven groups are also shown. **P*=0.0186 (Mann-Whitney's *U* test)

Fig. 4 Percentage of *hla*-, *hlb*-, and *fnbA*-positive isolates among MRSA isolates, from catheter-related cases (A), catheter-related polymicrobial cases (B), catheter-related monomicrobial cases (C), catheter-unrelated cases (D), catheter-unrelated polymicrobial cases (E), and catheter-unrelated monomicrobial cases (F).

Bars: ▨, *hla* ; ⊡, *hlb* ; ⊞, *fnbA*.

Table 1 PCR primers and conditions used in this study

Primer specificity	Primer sequences	Product length [bp]	PCR conditions cycling	$MgCl_2$ (mM)	Reference
tst	F: 5'-ATGGCAGCATCAGCTTGATA-3' R: 5'-TTTCCAATAACCACCCGTTT-3'	350	1 min, 94℃; 1 min, 55℃; 1 min, 72℃	1.5	13
sea	F: 5'-TTGGAAACGGTTAAAACGAA-3' R: 5'-GAACCTTCCCATCAAAAACA-3'	120	1 min, 94℃; 1 min, 55℃; 1 min, 72℃	1.5	13
seb	F: 5'-TCGCATCAAACTGACAAACG-3' R: 5'-GCAGGTACTCTATAAGTGCC-3'	478	1 min, 94℃; 1 min, 55℃; 1 min, 72℃	1.5	13
sec	F: 5'-GACATAAAAGCTAGGAATTT-3' R: 5'-AAATCGGATTAACATTATCC-3'	257	1 min, 94℃; 1 min, 55℃; 1 min, 72℃	1.5	13
hla	F: 5'-CTGGCCTTCAGCCTTTAAGG-3' R: 5'-CTGTAGCGAAGTCTGGTGAAA-3'	455	1 min, 94℃; 1 min, 50℃; 1 min, 72℃	1.5	this study
hlb	F: 5'-GCCAAAGCCGAATCTAAG-3' R: 5'-CGCATATACATCCCATGGC-3'	845	1 min, 94℃; 1 min, 50℃; 1 min, 72℃	1.5	14
fnbA	F: 5'-GCGGAGATCAAAGACAA-3' R: 5'-CCATCTATAGCTGTGTGG-3'	1278	1 min, 94℃; 1 min, 50℃; 1 min, 72℃	1.5	14
fnbB	F: 5'-GGAGAAGGAATTAAGGCG-3' R: 5'-GCCGTCGCCTTGAGCGT-3'	811	1 min, 94℃; 1 min, 50℃; 1 min, 72℃	1.5	14
clfA	F: 5'-CGATTGGCGTGGCTTCAG-3' R: 5'-GCCAGTAGCCAATGTCAC-3'	1004	1 min, 94℃; 1 min, 50℃; 1 min, 72℃	1.5	14
cna	F: 5'-AGGATCAGATTCAAGGTGGACAGCA-3' R: 5'-GAGTGCCTTCCCAAACCTTTTGAG-3'	711	1 min, 94℃; 1 min, 50℃; 1 min, 72℃	1.5	this study
icaA	F: 5'-GATTATGTAATGTGCTTGGA-3' R: 5'-ACTACTGCTGCGTTAATAAT-3'	770	1 min, 94℃; 1 min, 50℃; 1 min, 72℃	4	15
agr subgroup I	F: 5'-ATCGCAGCTTATAGTACTTGT-3' R: 5'-CTTGATTACGTTTATATTTCATC-3'	739	1 min, 94℃; 1 min, 50℃; 1 min, 72℃	3	15
agr subgroup II	F: 5'-AACGCTTGCAGCAGTTTATTT-3' R: 5'-CGACATTATAAGTATTACAACA-3'	691	1 min, 94℃; 1 min, 50℃; 1 min, 72℃	3	15
agr subgroup III	F: 5'-TATATAAATTGTGATTTTTTATTG-3' R: 5'-TTCTTTAAGAGTAAATTGAGAA-3'	712	1 min, 94℃; 1 min, 50℃; 1 min, 72℃	3	15
agr subgroup IV	F: 5'-GTTGCTTCTTATAGTACATGTT-3' R: 5'-CTTAAAAATATAGTGATTCCAATA-3'	683	1 min, 94℃; 1 min, 50℃; 1 min, 72℃	3	15

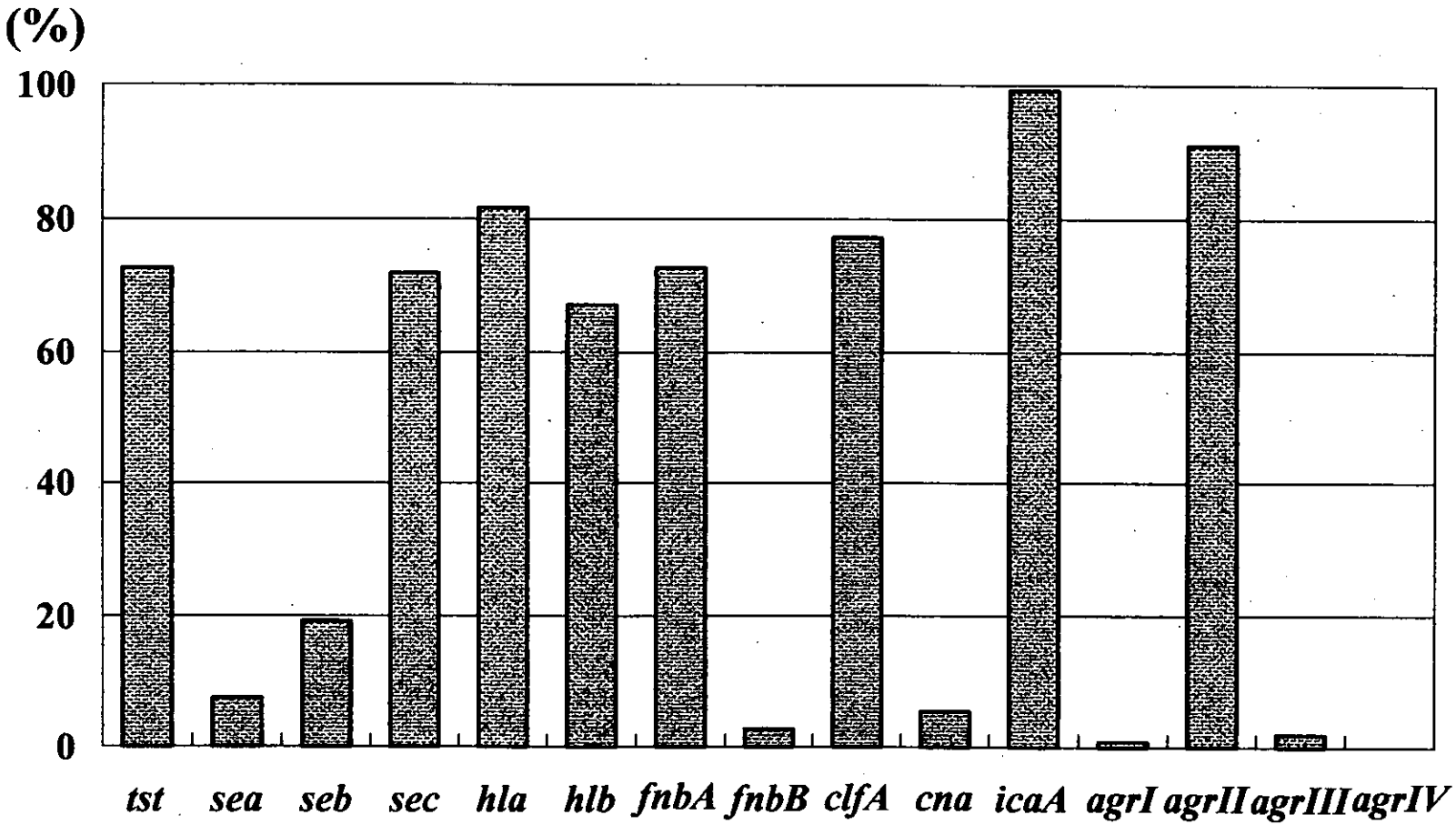

(%)
100
80
60
40
20
0
tst
sea
seb
sec
hla
hlb
fnbA
fnbB
clfA
cna
icaA
agrI
agrII
agrIII
agrIV

Fig. 1

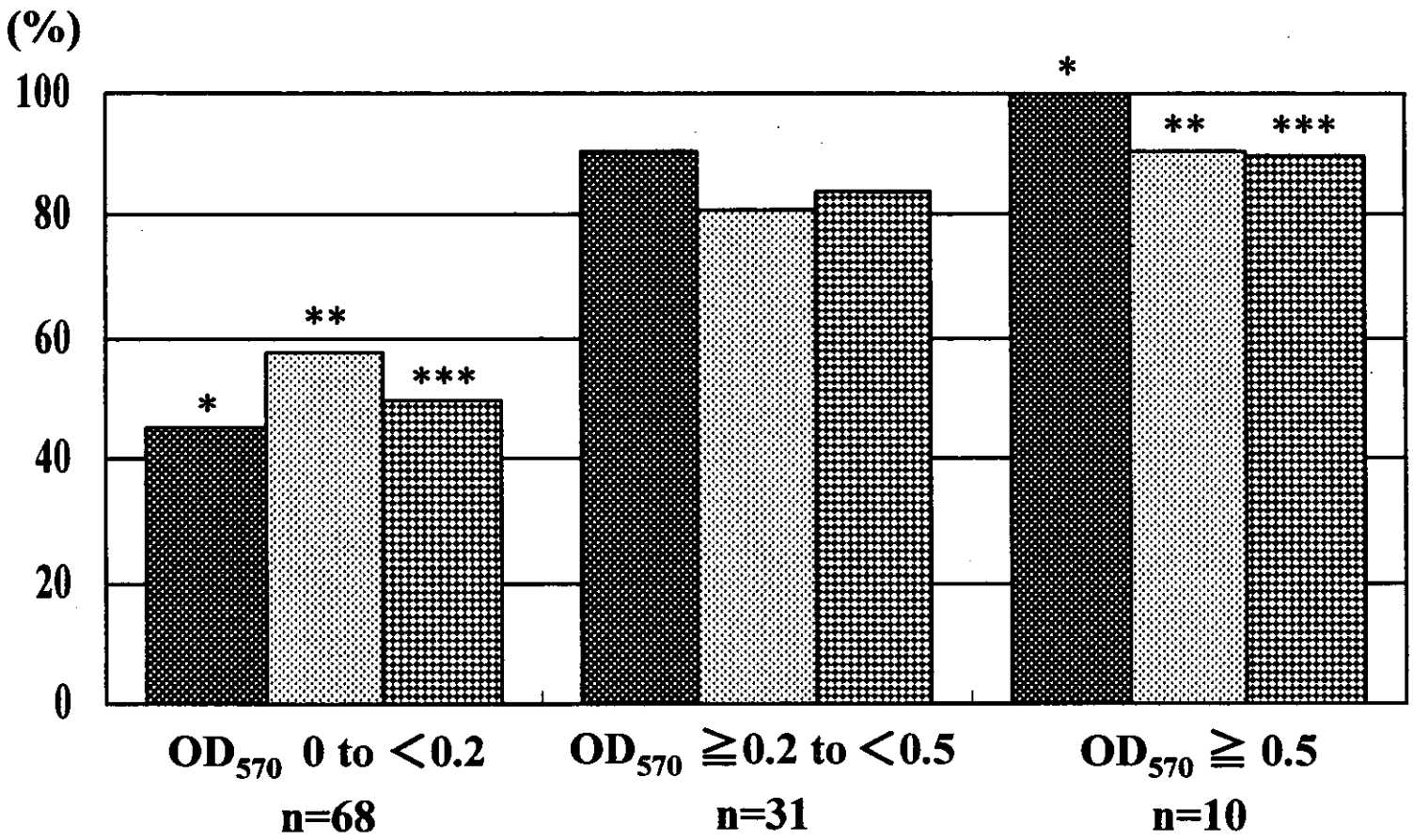


Fig. 2

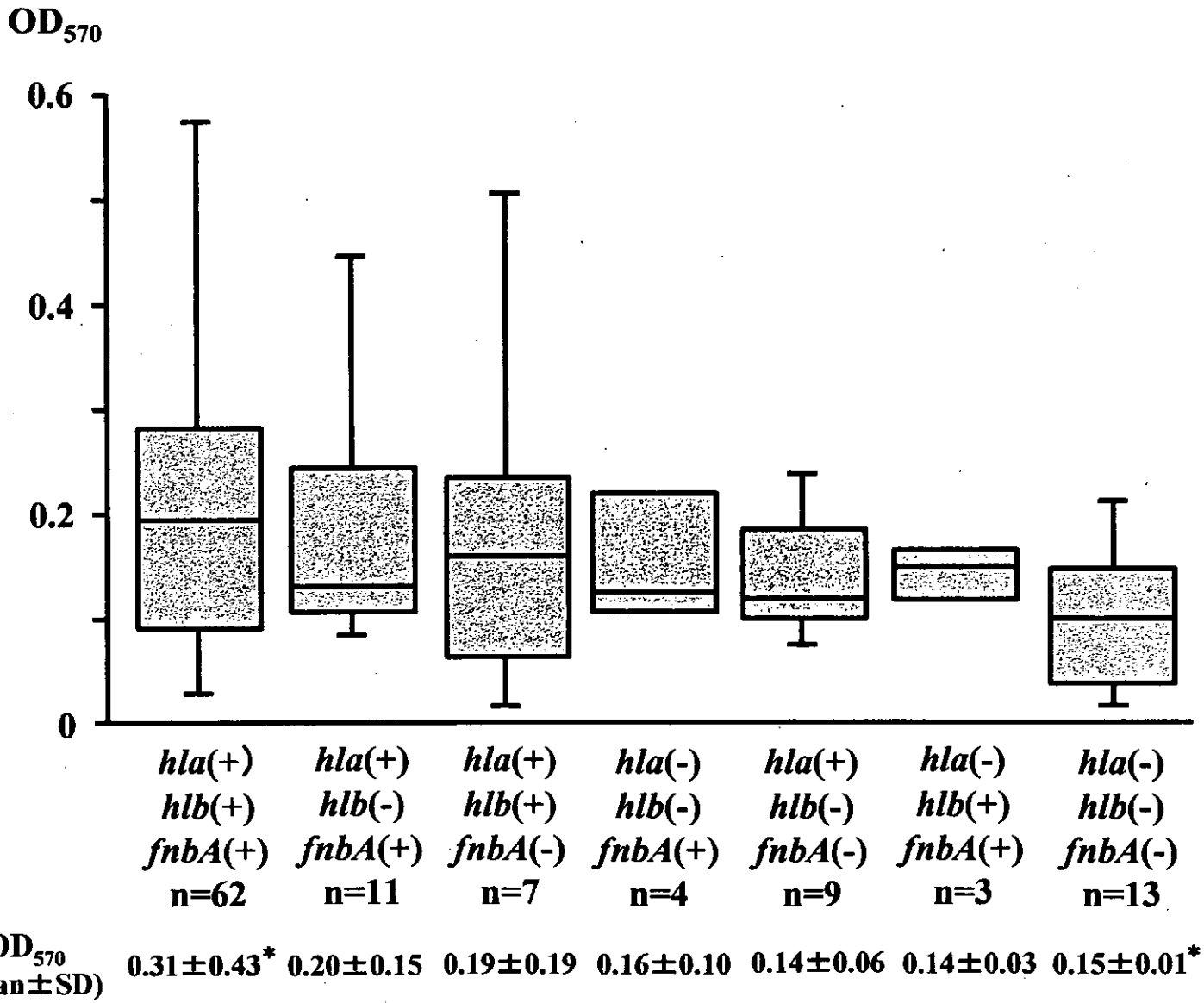

OD570
0.6
0.4
0.2
0
hla(+) hlb(+) fnbA(+) n=62
hla(+) hlb(-) fnbA(+) n=11
hla(+) hlb(+) fnbA(-) n=7
hla(-) hlb(-) fnbA(+) n=4
hla(+) hlb(-) fnbA(-) n=9
hla(-) hlb(+) fnbA(+) n=3
hla(-) hlb(-) fnbA(-) n=13
OD570 (mean±SD)
0.31±0.43*
0.20±0.15
0.19±0.19
0.16±0.10
0.14±0.06
0.14±0.03
0.15±0.01*

Fig. 3

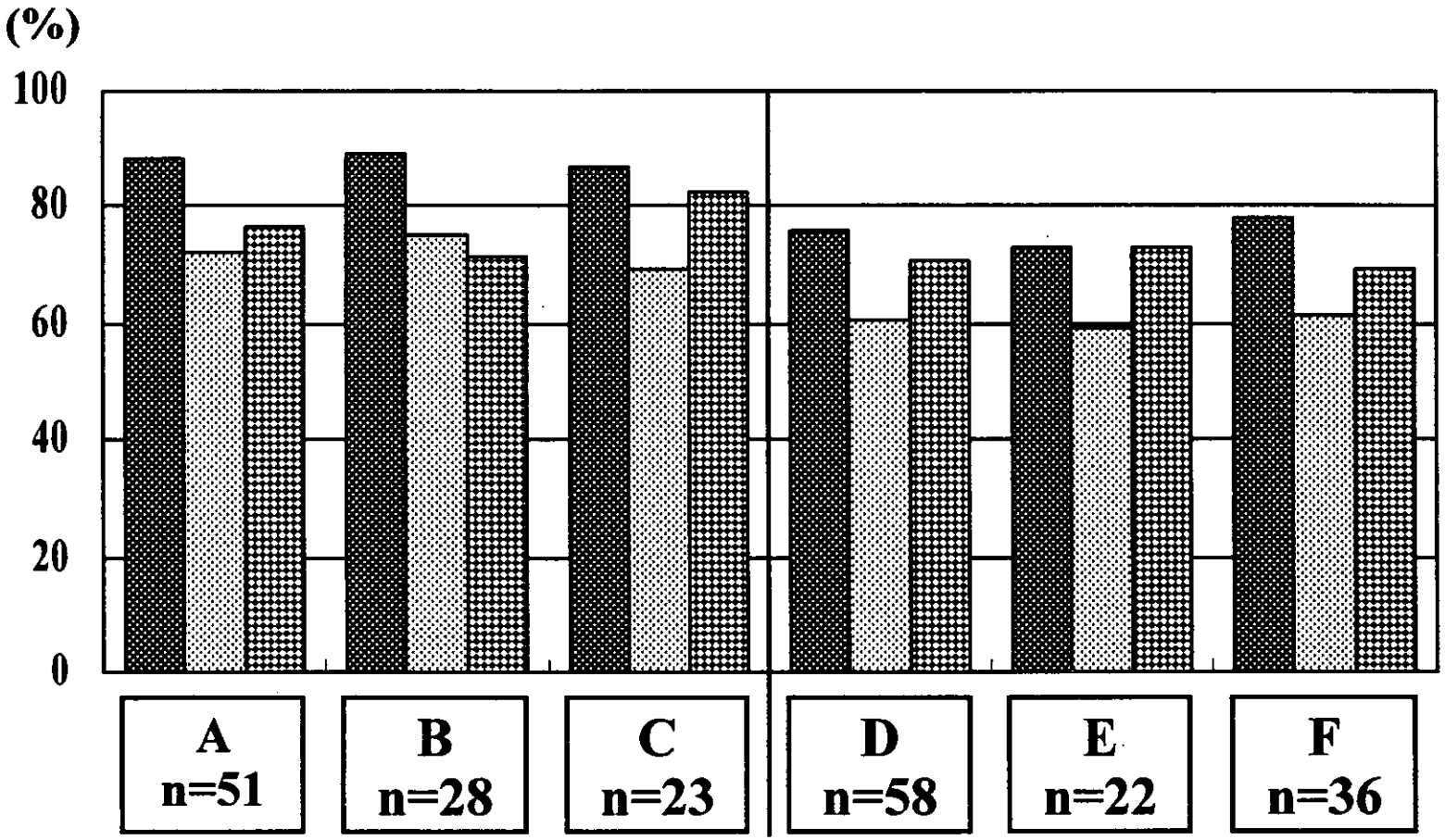


Fig. 4

SPring-8
User Experiment Report
No.11
(2003A)

NOVEMBER 2003

JASRI

Determination of Oxidation States of Pt in mixed-metal Clusters by X-ray Emission Spectroscopy

S. Nanao[a,1103], Y. Watanabe[a,3313], H. Hayashi[b,7277], N. Kawamura[c,1205], Y. Udagawa[b,7366], and Y. Mizobe[a]

[a]IIS, Univ. of Tokyo, [b]Tohoku Univ. [c]JASRI

The molecular mixed-metal clusters have been attracting significant attention in relevance of synthesizing catalysts and metalloenzymes. Sulfido-bridged mixed metal complexes having a $Pt(\mu\text{-}S)_2WS$ core are the basic compounds of them and Pt is the most important metallic element in them. The valence number of Pt, however, is still unknown.

The objective of the present study is to estimate the valence number of Pt in the sulfido-bridged mixed metal complexes by x-ray emission spectroscopy at Pt $L\alpha_1$.

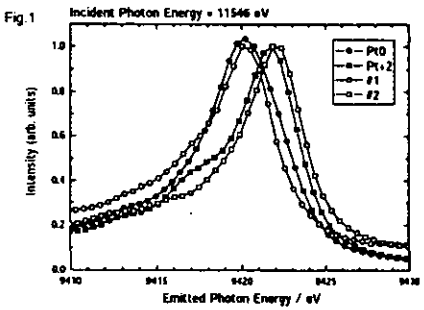
The measurements were performed at BL47U using x-ray emission spectrometer designed by the present authors (Tohoku Univ. group). The samples are $[NEt_4][Tp^*WS_3Pt(PPh_3)]$ and $[NEt_4][Tp^*WS_3PtCl_2]$, which are denoted in this report as #1 and #2, respectively. The structures are shown in ref. 1.

The spectra of $[Pt\,Cl_2(PPh_3)_2]$, $[Pt\,(PPh_3)_3]$ were also measured as references. $[Pt\,Cl_2(PPh_3)_2]$ and $[Pt\,(PPh_3)_3]$ are denoted as Pt+2 and Pt0, respectively. The valence number of them are known as +2 and 0, respectively.

Figure 1 shows a portion of the emission spectra for the incident x-rays of 11.546 keV. These spectra and others indicate the valence numbers of Pt for the samples #1 and #2 are 0 and +2, respectively.

Interesting dependence of the spectra on the incident x-ray energy has been found. The analyses in detail are in progress.

Fig.1



1) Preparation of Mononuclear Tungsten Tris(sulfide) and Molybdenum Sulfido-Tetrasulfido Complexes with Hydridotris(pyrazolyl)borate Coligand and Conversion of the Former into Sulfido-Bridged Bimetallic Complex Having $Pt(\mu\text{-}S)_2WS$ core, H.Seino, Y.Arai, N.Iwata, S.Nagao, Y.Mizobe and M.Hidai, Inorganic Chem. 40, 1677-1682(2001).

Analysis of molecular composition and imaging of *Nanobacteria.*

Kumon* Hiromi (8523)[1], Tomochika Ken-ichi (8531)[2], Uesugi Ken-tarou (1544)[3], and Yagi Naoto (1129)[3]

1. Okayama Univ., 2. Okayama Gakuin Univ. 3. Spring-8 / JASRI

Urinary stone-like precipitates derived from a long culture of several strains of *Nanobacteria*-like organisms (NLO) and packed single cells of NLO were analyzed by a high resolution monochrome X-ray CT. CT images of stone-like precipitates were obtained at resolution power of about 1 mm. Despite the average size of single culture cells (less than 1 mm), the packed cells collected by centrifugation were visible. However, clear CT images of packed cells in a glass capillary tube were not obtained. We need to develop a more suitable method of sample preparation for NLO analysis.

A. 宛名：分担研究者　泉福英信　殿

B. 指定課題名：平成15年度医療技術評価総合研究事業
「高齢者の口腔保健と全身的な健康状態の関係についての総合研究」

C. 研究協力課題名：「前立腺癌患者における手術前後における歯垢中の細菌の同定」

D. 研究協力者：
泉福英信（国立感染症研究所細菌第一部・室長）
公文裕巳（岡山大学大学院医歯学総合研究科泌尿器病態学　教授）
狩山玲子（岡山大学大学院医歯学総合研究科泌尿器病態学　助手）
上原慎也（岡山大学大学院医歯学総合研究科泌尿器病態学　医員）

E. 研究の目的：
近年、口腔内の微生物感染症の新しい概念としてバイオフィルム感染症が提唱された。これは、歯面および口腔内組織の表層に付着した細菌などの微生物が菌体外に産生した多糖体に周囲の無機物や有機物が取り込まれて形成される EPS (Extracellular polymeric substance) なかで微生物が増殖コロニーを維持し、歯や口腔組織の表面をフイルム状に被覆した結果として生じる感染症の一型である。この場合、EPS が微生物の付着を助長するだけでなく、バイオフィルムという増殖様式そのものが生体防御系や抗菌薬などに対する抵抗性を賦与して慢性持続感染が生ずることになる。このような口腔内の持続感染病巣から、歯周組織、口腔粘膜、扁桃、気道、そして食道等を経由して遠隔感染を生じたり、場合によっては血行性に様々な臓器での感染症を生じることとなるだけでなく、局所等で生じる免疫応答が全身性の慢性炎症性疾患の発症とその増悪に関与することとなる。口腔バイオフィルムを形成する細菌として、齲蝕や歯周病の発症に病原性を示すグラム陽性レンサ球菌やグラム陰性桿菌の他に、真菌、腸内細菌、肺炎桿菌、肺炎球菌、黄色ブドウ球菌、緑膿菌、そしてセラチア菌なども関与する。このような多種類の菌が口腔内に多数検出される場合には、日常生活活動度の低下から口腔内清掃が不充分となっている場合だけではなく、宿主側の細菌感染に対する抵抗力の低下などが深く関わっている。過去 2 年間の厚生科学研究において要介護高齢者の口腔微生物叢を検討した結果、*Candida albicans* が歯垢中で 38%と高率に検出され、また *Enterobacter cloacae* も歯垢中で 16%と高率に検出された。*Klebsiella pneumoniae* (9%)、Pseudomonas sp. (12%) も検出された。低率であるが *Staphylococcus aureus* (MRSA; MSSA) も歯垢で検出された。歯垢細菌と歯数との相関性について検討した結果、20 本以上の歯を有する要介護高齢者からは、*C. albicans*, Pseudomonas sp., MSSA が無歯顎の高齢者より高率に検出された。また歯垢中に Pseudomonas sp. が検出された要介護高齢者において、10ー19 本の歯を有する高齢者の心臓疾患を有する割合 (71%) は、無歯顎や 1ー9 本の高齢者 (13%、25%) に比べ有意に高い事が明らかとなった。また、20 本以上の高齢者 (40%) よりも高率であった。
以上のことから、口腔にこれらの細菌が感染しているために歯を喪失しつつある高齢者では、全身疾患へのリスクが高い事が考えられる。しかし、この現象は加齢という基本的背景に偶発的に合併している様々な全身疾患が複合した結果である可能性は否定できず、感染している口腔細菌と個々の疾患を直接結び付けて考えることは困難である。そこで、できるだけ患者個人の全身疾患を限定して口腔