Urol Res (1990) 18:299–303

Urological Research

Original articles

Increased renal scarring by bacteria with mannose-sensitive pili

T. Matsumoto, Y. Mizunoe, N. Sakamoto, M. Tanaka, and J. Kumazawa

Department of Urology, Faculty of Medicine, Kyushu University, Fukuoka, Japan

Accepted: October 1, 1989

Summary. Renal scars are thought to be the end stage of chronic pyelonephritis and one of the most important causes of renal insufficiency and renal hypertension. The role of bacterial pili was examined in scar formation after an infection of newly constructed bacterial strains using the recombinant DNA technique, which possessed either mannose resistant (MR) or mannose sensitive (MS) pili of *Serratia marcescens*. Strains that differed in only a single virulence factor, namely, MR or MS pili, were used in a rat model of chronic pyelonephritis. In this model, MS-piliated bacteria stimulated renal scarring more severely than non-piliated or MR-piliated bacteria.

Key words: MS pili – *Serratia marcescens* – Renal scarring

The role of bacterial pili on the initiation of urinary tract infection has been analyzed by numerous experimental and clinical studies [11]. The fact that uropathogenic *Escherichia coli* has type 1 (common) pili and/or P pili on their surface has been reported [2]. Type 1 pili, which agglutinate guinea pig erythrocytes (GRBC) in a mannose-sensitive (MS) fashion, occur frequently on *E. coli* from many sources [8]. In contrast, P fimbriae, which agglutinate human type 0 erythrocytes in the presence of mannose (MRHA), occur significantly more frequently in strains of *E. coli* involved in pyelonephritis than in cystitis or asymptomatic bacteriuria [8].

Serratia marcescens is a common urinary tract pathogen in patients with predisposing conditions such as urolithiasis, tumors, catheters, etc. Similar to *E. coli, S. marcescens* also possesses MR and/or MS pili [9].

Renal scars may be the end stage of chronic pyelonephritis and may be related to renal insufficiency and renal hypertension. This scarring state is clinically detected by excretory urography, computed tomography or isotopic scintigraphy. However, the mechanism of renal scarring is poorly understood both clinically and experimentally. Some evidence that bacterial growth in the renal medulla does not result in scarring but that the inflammatory process is closely related to the scarring process has been presented in several reports [4, 12]. Using six strains of *E. coli* preincubated in broth, urine or agar, Harber et al [5] have found that MS piliated *E. coli* is related to renal scarring. However, this experimental method could not clarify whether or not piliation was the only determining factor.

Using the recombinant DNA technique, we constructed *E. coli* strains that possessed either MS or MR pili of *S. marcescens* and, through a kidney infection model using the above strains, we found that MS-piliated bacteria stimulated renal scarring, but MR-piliated bacteria did not.

Materials and methods

Bacteria

S. marcescens (US46 strain) was isolated from a patient with a urinary tract infection [10]. This strain showed MRHA to chicken red blood cells (CRBC) and MSHA to CRBC, and two kinds of pili (MR and MS) were observed on the cell surface with electron microscopy. Another clinical isolate of *S. marcescens* (US5 strain) was also used, which only had MS pili and exhibited MSHA to CRBC [13]. Non-piliated *E. coli* (p678-54 strain) was also used. Two derivative strains of p678-54 were constructed as described elsewhere [10]. Briefly, the high-molecular-weight chromosomal DNA of US46 strain was partially digested with *Sau3A* and subjected to 0.5% agarose gel electrophoresis. DNA fragments (35 to 50 kb) were transferred to DE81 paper (Whatman) by electrophoresis. The paper was washed with 800 µl of 0.2 *M* NaCl, and DNA fragments of appropriate sizes were eluted from the paper with 400 µl of 2 *M* NaCl. After ligation of these fragments with cosmid vector (pHC 79), which had been treated with BamHI and bacterial alkaline phosphatase, recombinant molecules were packed in vitro and infected with non-piliated *E. coli* strains (p678-54). Several transformants that showed MRHA or MSHA were selected from 3,000 variants by screening of HA to CRBC. Two recombinant cosmids, pYM7 (for MSHA) and pYM122 (for MRHA), were used and the two recombinant strains were described as either p678-54 (pYM7), meaning p678-54 strain harboring pYM7 cosmid or p678-54 (pYM122) meaning p678-54 strain harboring pYM122 cosmid.

Table. 1. Agglutination properties of bacterial strains

Strain	Hemagglutination[a]		Yeast cell[b] agglutination	Agglutination by antibodies[c]	
	CRBC	GRBC		Anti-MS	Anti-MR
p678-54	—	—	—	—	—
US46	MR	MS	+	+	+
US5	MS	MS	+	+	—
p678-54 (pYM122)	MR	—	—	—	+
p678-54 (pYM7)	MS	MS	+	+	—

[a] Bacteria grown overnight were suspended in PBS and used for hemagglutination (HA) testing to chicken (CRBC) and guinea pig (GRBC) erythrocytes. CRBC and GRBC were suspended in a concentration of 2% in PBS mixed with the bacterial suspension on a glass slide. HA was designated as mannose-resistant (MR) when the same degree of HA occurred with or without 1% mannose, and as mannose-sensitive (MS) when HA was prevented by the presence of mannose

[b] — = agglutination negative; + = agglutination positive

[c] Anti-MS = Anti-MS pili antiserum; anti-MR = anti-MR pili monoclonal antibodies

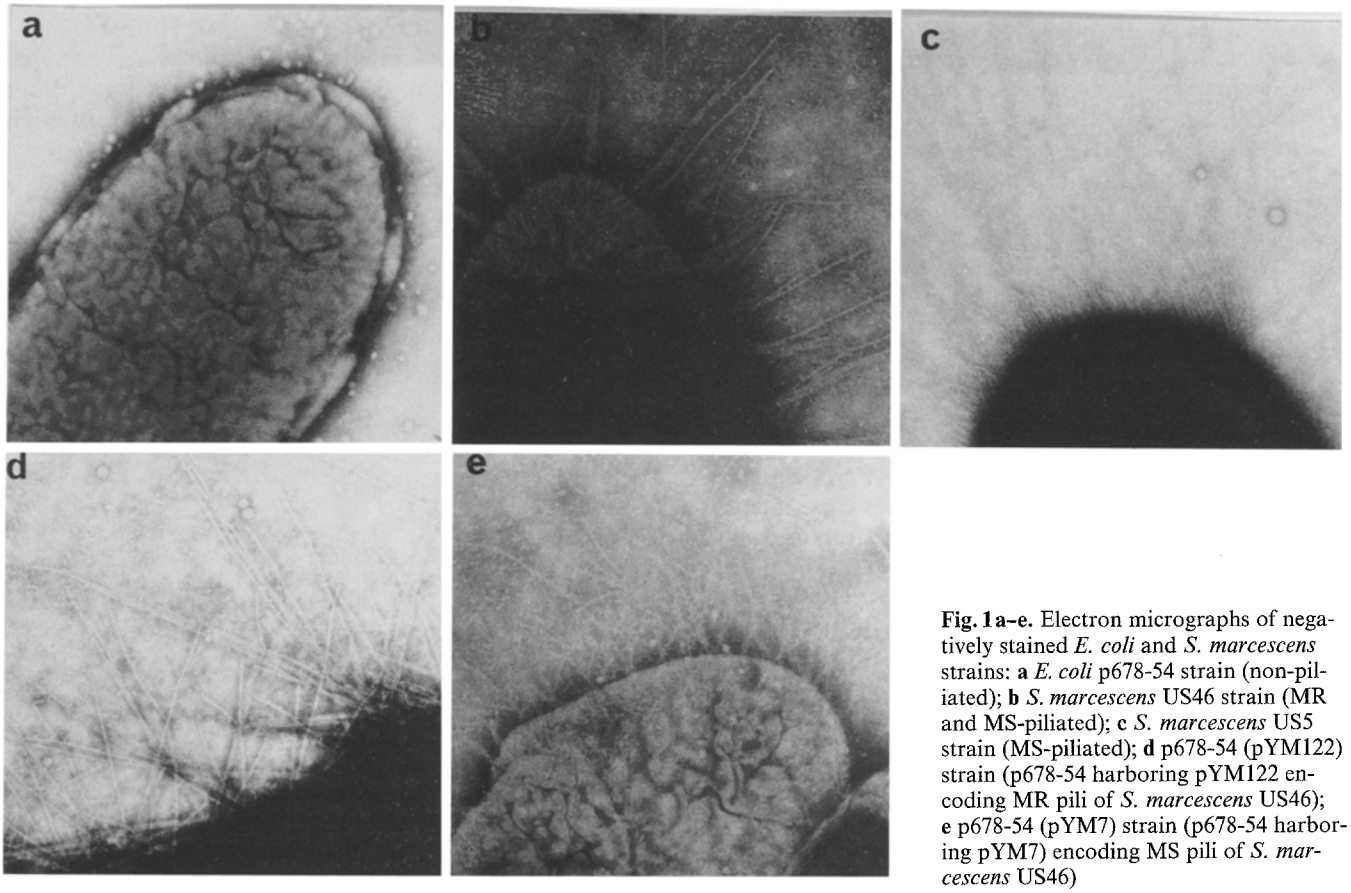

Fig. 1a–e. Electron micrographs of negatively stained *E. coli* and *S. marcescens* strains: **a** *E. coli* p678-54 strain (non-piliated); **b** *S. marcescens* US46 strain (MR and MS-piliated); **c** *S. marcescens* US5 strain (MS-piliated); **d** p678-54 (pYM122) strain (p678-54 harboring pYM122 encoding MR pili of *S. marcescens* US46); **e** p678-54 (pYM7) strain (p678-54 harboring pYM7) encoding MS pili of *S. marcescens* US46)

Determination of agglutination properties

HA properties were determined in phosphate-buffered saline (PBS) with a 2% (vol/vol) suspension of CRBC or GRBC with or without 1% (wt/vol) D-mannose. Yeast cell agglutination was also performed. Yeast cells *(S. cerevisiae)* were cultured on Sabouraud agar and suspended in PBS. The concentration of yeast cells was adjusted to an optical density of 50 Klett units at 660 nm. The yeast cell suspension and bacteria were mixed on a glass slide, and agglutination was read within a few minutes.

Antiserum and antibodies

Ms pili-specific serum was prepared from rabbits with purified MS pili of *S. marcescens* US5, as described elsewhere [7]. MR pili-

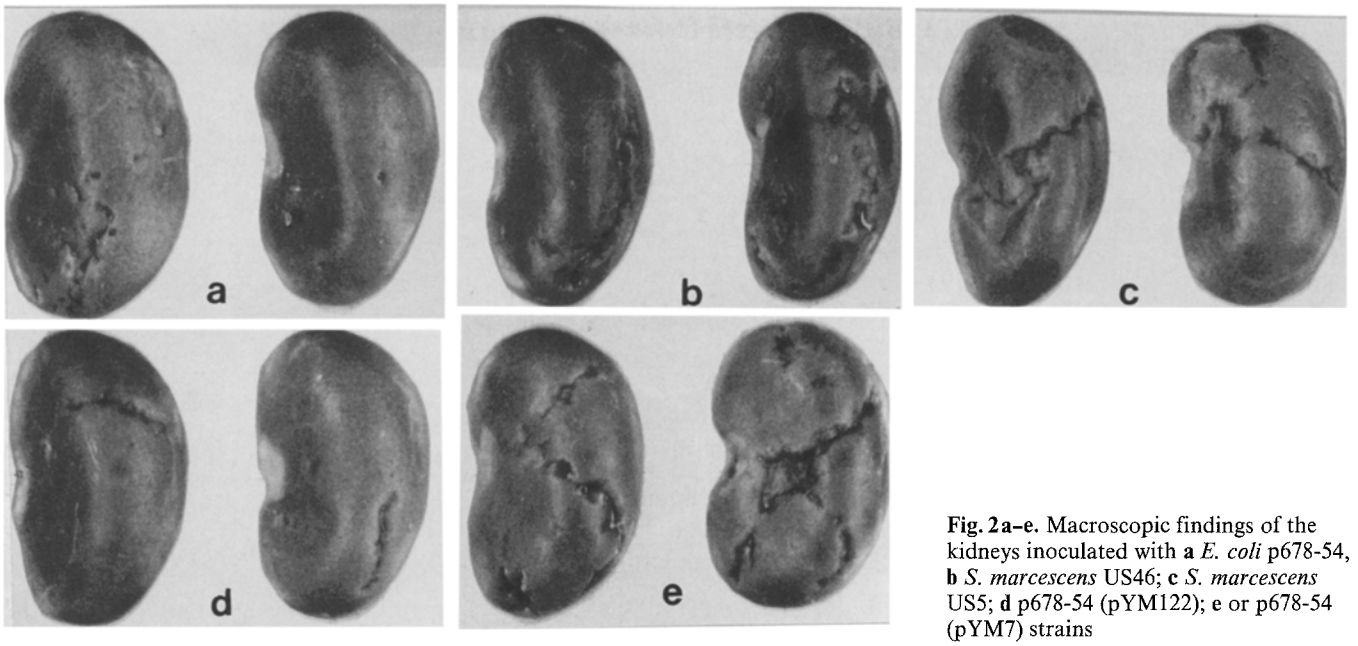

Fig. 2a–e. Macroscopic findings of the kidneys inoculated with **a** *E. coli* p678-54, **b** *S. marcescens* US46; **c** *S. marcescens* US5; **d** p678-54 (pYM122); **e** or p678-54 (pYM7) strains

specific monoclonal antibodies were raised against MR pili of *S. marcescens* US46, as reported elsewhere [6].

Electron microscopic observations

Bacteria were washed twice in 2% ammonium acetate solution, and 1 drop of the suspension was placed on a Formvar-coated grid. These bacteria were stained with 2% sodium phosphotungstate (pH 7.0) and examined under a JEM 100C electron microscope at 80 KV.

Experimental animals

Eight- to ten-week-old female Sprague-Dawley rats were used in all experiments. These rats weighed 200–250 g and were kept in specific pathogen-free conditions at room temperature. They received a mouse diet and tap water ad libitum. All surgical procedures were carried out under ethyl-ether anesthesia.

Bacterial inoculation

Five of the bacterial strains, p678-54, US46, US5, p678-54 (pYM7) and p678-54 (pYM122), were inoculated directly into the renal parenchyma of the rats a dose of 9×10^7 cfu in 0.1 ml of saline with 26-gauge needle. The animals were killed by femoral bleeding and cervical dislocation 6 weeks after bacterial inoculation; the kidneys of inoculated-sides were then removed and subjected to macroscopic and microscopic observations.

Renal scarring

The renal scarring was graded macroscopically as follows; −: no scar, +: linear scar, ++: small scar, +++: large scar with deformity. These grades were given scores of 0, 1, 2 or 3 points, respectively. The total scores were calculated from this scoring. The grade of renal scarring was assessed by another author using the blind method. Histological examinations were also carried out following hematoxylin and eosin staining.

Statistical analysis

This was performed by a χ^2 test on the variation of renal scarring by several species of bacteria.

Results

Agglutination properties of bacteria

Non-piliated *E. coli,* clinical isolates of *S. marcescens* (US46), another clinical isolate (US5) and two recombinant strains, p678-54 (pYM122) and p678-54 (pYM7) were examined for erythrocyte, yeast cell and antibody agglutination. US46 showed MRHA to CRBC, MSHA to GRBC and positive yeast cell agglutination. Furthermore, this strain was agglutinated by both anti-MS and anti-MR antibodies. P678-54 harboring pYM122 plasmid showed MRHA to CRBC and negative agglutination to yeast cells and was agglutinated only by anti-MR antibodies. On the other hand, p678-54 harboring pYM7 plasmid showed MSHA both to CRBC and GRBC, positive yeast-cell agglutination, and was agglutinated only by anti-MS antibodies (Table 1).

Electron microscopic findings

Figure 1 shows electron microscopic features of test strains. A p678-54 strain possessed no pili, and two kinds of pili were observed on the surface of US46 strain, one rigid and the other flexible. The US5 strain demonstrated

Table 2. Scarring of the kidney inoculated with MR or MS piliated bacterial strains

Strain	Piliation	Scarring[a] (Number of rats)				Total score [b]	χ^2 test[c]		
		−	+	++	+++				
p678-54	−	5	3	0	0	3	**	*	NS
US46	MR + MS	0	3	5	0	13			
US5	MS	0	4	3	1	13			
p678-54 (pYM122)	MR	4	4	0	0	4	**		
p678-54 (pYM7)	MS	0	1	6	1	16			

[a] Scarring: renal scarring was observed macroscopically 6 weeks after bacterial inoculation. Grading of the renal scars was done as from − to +++ = as described below. Every group had eight rats. Grade of scarring: − = no scar; + = linear scar; ++ = small scar; +++ = large scar with deformity

[b] Total score was calculated from points given to the grading of scar formation. 0, 1, 2 and 3 points were given to −, +, ++, and +++, respectively

[c] * $P<0.05$; ** $P<0.01$

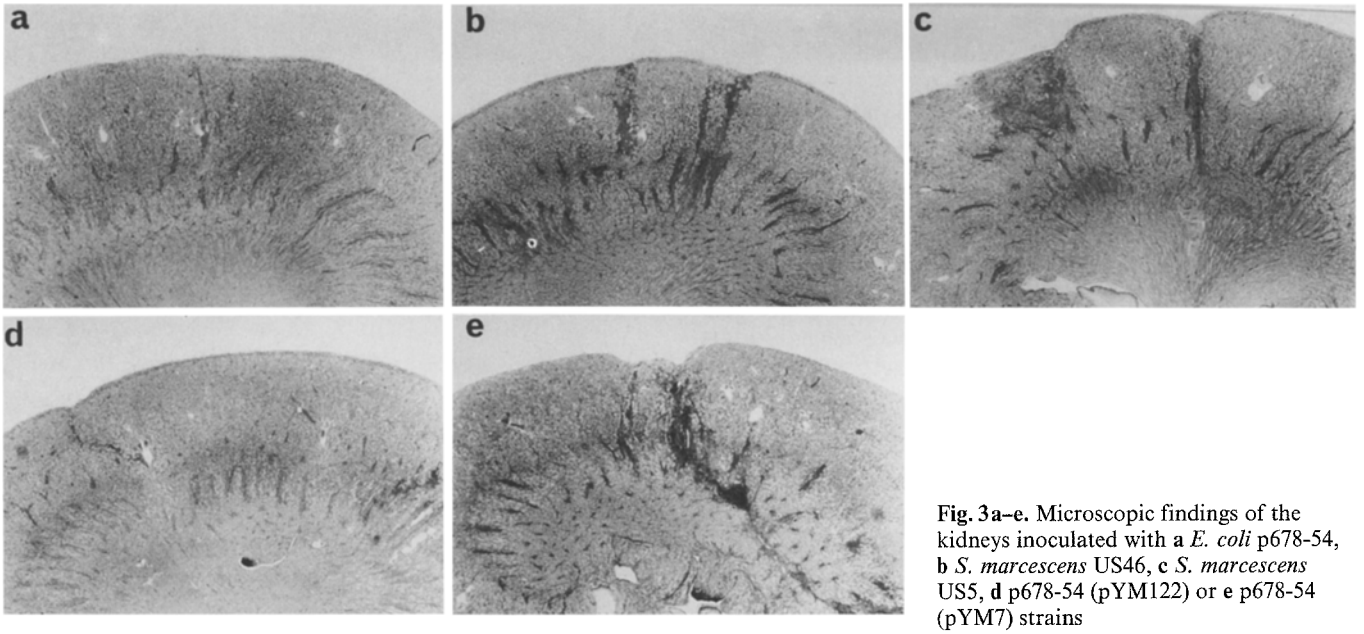

Fig. 3a–e. Microscopic findings of the kidneys inoculated with **a** *E. coli* p678-54, **b** *S. marcescens* US46, **c** *S. marcescens* US5, **d** p678-54 (pYM122) or **e** p678-54 (pYM7) strains

flexible pili on their surface. p678-54 (pYM122) and p678-54 (pYM7) strains possessed one kind of pili on their surface, rigid and flexible pili, respectively.

Macroscopic features of renal scarring

Scar formation experiments were performed by the use of the five strains already described. Bacterial solution (0.1 ml) was inoculated directly into the kidney of eight rats per group, which were killed 6 weeks later, and the scarring of the kidney was observed macroscopically and microscopically. Neither p678-54 nor p678-54 (pYM122) strains stimulated renal scarring; however, US46, US5 and p678-54 (pYM7) provided pronounced stimulation (Fig. 2). These differences were statistically significant, and the results show the close relationship between MS piliation and renal scarring (Table 2).

Microscopic findings of renal scarring

Figure 3 demonstrates the microscopic findings of the kidneys. As in the case of macroscopic findings, inflammatory changes were minimal in the kidneys inoculated with p678-54 or p678-54 (pYM122) strains, except for a small number of chronic inflammatory cells mainly consisting of lymphocytes. Kidneys inoculated with US46, US5 or p678-54 (pYM7) demonstrated a large inflammation site with scattered fibrotic change. Viable bacteria were not detected in all kidneys at this time.

Discussion

Many species of bacteria adhere to the urinary mucosa through bacterial pili. It has been reported that type 1 pili of *E. coli* conjugates to the receptors consisting of mannose residue and P pili react to the P-specific glycolipid receptors. This adherence mechanism has been thought to be the principal factor in the initiation of urinary tract infections (UTI).

S. marcescens is usually isolated from patients with complicated and/or chronic UTI. This bacterium also possesses at least two kinds of pili such as MR and MS pili. In fact, we identified clinical isolates possessing two kinds of pili (US46) and only MS pili (US5). Amako et al. [1] have described the slime agglutination by pili of *S. marcescens,* and Yamamoto et al. [13] have also reported the fimbria-mediated adherence of *S. marcescens* to urinary mucosa. Much evidence has been collected about the kinds of pili, in particular MR and MS pili. Whereas much is known about the role of bacterial pili in the initiation of UTI [12], their significance as virulence factors in chronic UTI is not yet clear.

In order to investigate the significance of MR and MS pili of *S. marcescens* on renal scarring, we constructed two recombinant strains that possessed either MR or MS pili of *S. marcescens* (US 46). These strains were thought to differ only in piliation. From several lines of the scar formation experiments with these strains, renal scarring was found to be related to the piliation of *S. marcescens.* Moreover, we found that MS-piliated bacteria caused more pronounced renal scarring than MR-piliated bacteria.

In the renal parenchyma, bacteria must fight the humoral and cellular defense mechanisms. Polymorphonuclear leukocytes (PMNL) are one of the most important factors here. A few investigators have reported that the renal damage following acute pyelonephritis is not related to bacterial growth but is, in fact, closely related to the inflammatory process and that PMNL has a major role in this process [4, 5, 12]. Harber et al. [5] have reported that MS-fimbriated *E. coli* stimulates PMNL more than non-fimbriated or MR-fimbriated *E. coli,* as detected by the chemiluminescence method. In conclusion, MS-piliated bacteria stimulated renal scarring far more than non-piliated or MR-piliated bacteria. It is conceivable that this increased scarring is due to the increased stimulation of PMNL by MS pili [5].

References

1. Amako K, Ohshima H, Yasunaka K (1981) Pili mediated agglutination of *Serratia marcescens* in human urine. Microbiol Immunol 25:981
2. Dominique GJ, Roberts JA, Laucirica R (1985) Pathogenic significance of P-fimbriated *Eschericia coli* in urinary tract infections. J Urol 133:983
3. Evans DG (1979) Purification and characterization of the CFA/I antigen of enterotosigenic *Escherichia coli.* Infect Immunol 25:738
4. Glauser MP, Lyons JM, Braude AI (1978) Prevention of chronic experimental pyelonephritis by suppression of acute suppuration. J Clin Invest 61:403
5. Harber MJ, (1986) Virulence factors of urnary pathogens in relation to kidney scarring. In: Asscher AW, Brumfitt W (eds) Microbial disease in Nephrology. Wiley, Chichester, p 69
6. Jingushi S, Mitsuyama M, Morya T, Amako K (1987) Antigenic analysis of *Serratia marcescens* fimbriae with monoclonal antibodies. Infect Immun 55:1600
7. Kohno K, Yamamoto T, Kuroiwa A, Amako K (1984) Purification and characterization of *Serratia marcescens* US5 pili. Infect Immun 46:295
8. Latham RH, Stamm WE (1984) Role of fimbriated *Escherichia coli* in urinary tract infections in adult women: correlation with localization studies. J Infect Dis 149:835
9. Matsumoto T, Tanaka M, Kumazawa J (1987) Hemagglutination by various species of bacteria isolated from the urine of patients with urinary tract infections. Nishinihon J Urol 49:95
10. Mizunoe Y, (1988) Cloning and sequence of the gene encoding the major structural component of mannose-resistant fimbriae of *Serratia marcescens.* J Bacteriol 170:3567
11. Reid G, Sobel JD (1987) Bacterial adherence in the pathogenesis of urinary tract infection: a review. Rev Infect Dis 9:470
12. Slotki IN, Asscher AW (1982) Prevention of scarring in experimental pyelonephritis in the rat by early antibiotic therapy. Nephron 30:262
13. Yamamoto T, Ariyoshi A, Amako K (1985) Fimbria-mediated adherence of *Serratia marcescens* strain US5 to human urinary bladder surface. Microbiol Immunol 29:677

T. Matsumoto, MD
Department of Urology
Faculty of Medicine
Kyushu University
Fukuoka
812, Japan