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The Clinical Aspects of β -Lactam-Resistant *Stenotrophomonas maltophilia*

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Recent papers argue that major increases in the isolation of multidrug-resistant Gram-negative bacteria have been of some concern in clinical practice. Among these bacteria, *Stenotrophomonas maltophilia* is an intrinsic producer of β -lactamases, and has been recognized as a nosocomial pathogen. But few reports are available on the impact of the potential risk of mixed infections. The goal of this review is to explore that impact. *S. maltophilia* is often isolated from the respiratory tract together with other Gram-negative species, and yields at least two β -lactamases. The enzymes show the capacity to hydrolyze a large amount of imipenem and ceftazidime, and exhibit a susceptibility to aztreonam in combination with ceftazopran. The last section elaborates on the idea that *S. maltophilia* can assist in the survival of other imipenem-susceptible bacteria such as *Serratia marcescens* and *Pseudomonas aeruginosa*. This theory is also valid for ceftazidime-susceptible *P. aeruginosa*. The present review confirms the potential threat of *S. maltophilia* as an indirect pathogen, and brings an often ignored fact to light: even originally fragile bacteria can live through a strong antimicrobial attack in the presence of a helper bacteria such as *S. maltophilia*. Although it is sometimes difficult to attribute a causative role to this bacterium, in fact, the existence of *S. maltophilia* is worthy of attention.

Key words: indirect pathogenicity; β -lactamase; *Stenotrophomonas maltophilia*

Gram-negative bacilli as a cause of hospital-acquired infection has recently been receiving increasing attention, and its resistance to multidrug therapy baffles medical workers (Avison et al., 2001; Livermore, 2002). Hanberger et al. (2001) reported that immunodeficiency is the most important factor in infections by multidrug-resistant strains, and in addition, improper usage of broad-spectrum antimicrobial agents accompanied with prolonged hospital stays may contribute to this phenomenon (Denton and Kerr, 1998; Geiger et al., 2001). Among Gram-negative bacilli, multidrug-resistant *Pseudomonas aeruginosa* has been solely

designated as a notifiable organism in Japan since 1999. Nonetheless, this step is apparently incomplete because the resistant mechanism differs from species to species (Kataoka et al., 2001). Clinicians need to recognize *Stenotrophomonas maltophilia* as an important nosocomial pathogen because this bacterium retains a noticeable resistance to multidrug treatment and disinfectants. This review focuses on studies about the clinical aspects of β -lactam-resistant *S. maltophilia*.

The plan of the review is as follows: Section I outlines the significance of *S. maltophilia* among Gram-negative bacilli; Section II considers clinical

Abbreviations: BTB, bromothymol blue; CFU, colony forming unit; MIC, minimum inhibitory concentration; SMA, sodium mercaptoacetic acid

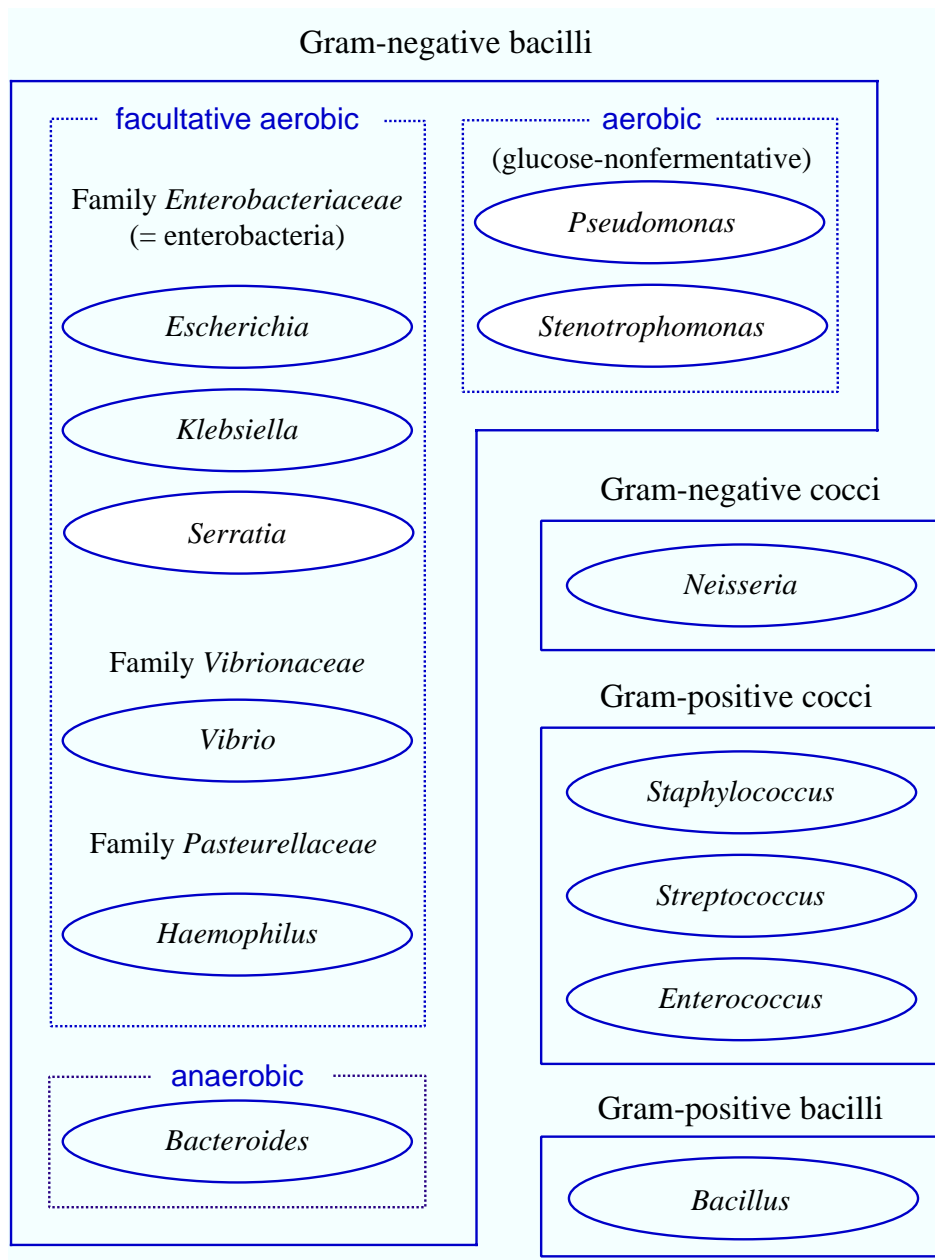


Fig. 1. Schema of bacteriological classification. Solid lines, grouping by Gram stain. Dotted lines, grouping by metabolism. Ellipse, typical genus.

properties including habitat and antimicrobial resistance. In Section III, we present differential activities of β -lactamases, such as hydrolytic activity and susceptibility to aztreonam as an inhibitor. Section IV describes indirect pathogenicity, that is, hidden risks in mixed infections.

I. Significance of *S. maltophilia* among Gram-Negative Bacilli

The emergence of β -lactam-resistant Gram-negative bacilli has increasingly been recognized on the grounds that β -lactam agents came to be used ex-

tensively in wards (Livermore, 1995). β -Lactam agents are classified into several groups according to chemical structure. In general, penicillins are still effective against Gram-positive cocci, while they are weak in fighting Gram-negative bacilli. The newer the cephalosporins and cephamycins, the wider the antimicrobial spectrum. On one hand, the antimicrobial spectrum of monobactams is limited to Gram-negative bacilli in contrast to penicillins. On the other hand, the spectrum of carbapenems covers almost all Gram-negative bacteria (Kataoka et al., 2002a).

The antimicrobial susceptibilities of Gram-negative bacilli were surveyed at Tottori University Hospital in 2001. As a matter of course, Gram-negative cocci and Gram-positive bacteria including *Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp. and *Bacillus* spp. are not intended infections (Fig. 1). The survey showed that 176 strains (6.3%) out of 2785 were resistant to all 3 broad-spectrum β -lactam agents, that is, imipenem, ceftazidime and aztreonam. Most of the 176 strains are not enterobacteria but glucose-nonfermentative and aerobic bacteria such as *P. aeruginosa*, *Chryseobacterium* spp. and *S. maltophilia* in particular

Table 1. Number (%) of broad-spectrum β -lactam-resistant* strains for bacterial species

Number of strains	176 (100.0)
Bacterial species	
<i>Stenotrophomonas maltophilia</i> †	96 (54.5)
<i>Pseudomonas aeruginosa</i> †	34 (19.3)
<i>Chryseobacterium indologenes</i> †	10 (5.7)
<i>Chryseobacterium meningosepticum</i> †	7 (4.0)
<i>Serratia marcescens</i> ‡	6 (3.4)
<i>Acinetobacter</i> spp.†	4 (2.3)
Other species	19 (10.8)

* Resistant to imipenem, ceftazidime and aztreonam.

† Glucose-nonfermentative bacteria.

‡ Enterobacteria.

(Table 1). These bacteria occupy ecological niches both inside and outside hospitals, and cause opportunistic infection or iatrogenic transmission. The growth of these bacteria is slower than that of enterobacteria; however, glucose-nonfermentative bacteria are often of resistant to multidrug treatment and disinfectants (Mori, 2000). Classical resistance to β -lactam agents in these bacterial species is usually due to an alternation of penicillin-binding proteins, changes in outer membrane permeability to β -lactams and most frequently, intrinsic β -lactamase production (Gál et al., 2000; Fang et al., 2002). There is a likelihood of invalidity with standard chemotherapy because of hydrolysis by the β -lactamases.

The exact opposite of glucose-nonfermentative bacteria, most enterobacteria are naturally susceptible to β -lactams (Kataoka et al., 2002a). However, an overdose of β -lactams during hospitalization causes an emergence of AmpC β -lactamase, whose gene is commonly found on the chromosomes of several enterobacteria species and is typically induced by ceftazidime or imi-

Table 2. Classification of β -lactamases

	Molecular class	Main bacterial species
Serine- β -lactamase	A (penicillinase)	<i>Haemophilus influenzae</i> <i>Klebsiella oxytoca</i> <i>Klebsiella pneumoniae</i> <i>Proteus mirabilis</i> <i>Stenotrophomonas maltophilia</i>
	C (cephalosporinase)	<i>Citrobacter freundii</i> <i>Enterobacter cloacae</i> <i>Morganella morganii</i> <i>Pseudomonas aeruginosa</i> <i>Serratia marcescens</i>
	D (oxacillinase)	<i>Pseudomonas aeruginosa</i>
Metallo- β -lactamase	B (carbapenemase)	<i>Aeromonas hydrophila</i> <i>Bacillus cereus</i> <i>Bacteroides fragilis</i> <i>Burkholderia cepacia</i> <i>Stenotrophomonas maltophilia</i>

From Kuga and Inoue, 2002 with some modifications.

penem (Coudron et al., 2000; Kataoka et al., 2002b). Therefore, the treatment against enterobacteria should be considered carefully. In passing, AmpC is grouped into class C (cephalosporinase) of Ambler's prestigious classification (Table 2) (Ambler, 1980). AmpC β -lactamase of *Serratia marcescens* is presented in Section IV once again.

II. Clinical Properties

Although *S. maltophilia* is a little-known microbe, it was the 5th Gram-negative species according to the number of isolates found at Tottori University Hospital in 2001, after *P. aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *S. marcescens* (Kataoka et al., 2002a). *S. maltophilia* is a Gram-negative aerobic bacillus (Fig. 1), having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor (Park, 1967; Snell and Lapage, 1971). As Hugh and Ryschenkow (1961) noted, a key distinguishing feature of *S. maltophilia* is acid production not from glucose but from maltose (Fig. 2 and Table 3). The term "*maltophilia*" seems to come from the bacterium's preference for maltose. It goes without saying that all enterobacteria including *S. marcescens* ferment glucose, and produce some acid and/or gas (Sakazaki, 1992).

Table 3. Acid production from sugars

Sugar	Bacterial species		
	<i>S. maltophilia</i>	<i>S. marcescens</i>	<i>P. aeruginosa</i>
Glucose	–	F	O
Lactose	–	–	–
Maltose	O	F	–

F, fermentation; O, oxidation; –, negative of acid production. From Kataoka et al., 2003b with some modifications.

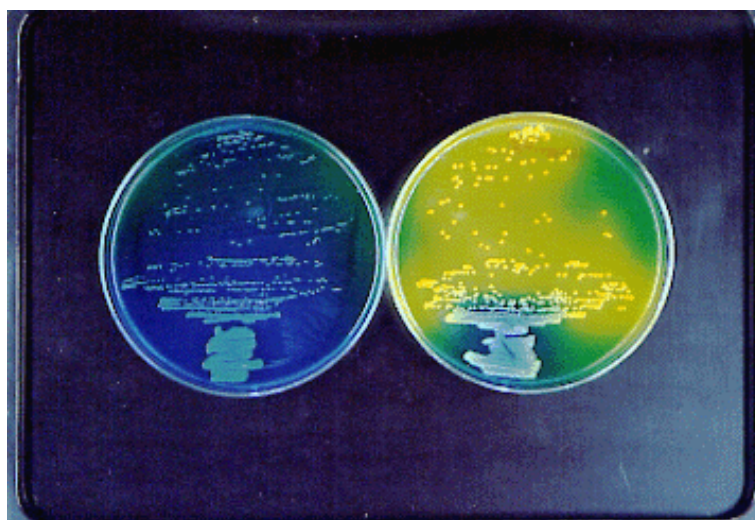


Fig. 2. Acidity of bacterial colony. Both media contain glucose and bromothymol blue (BTB), which is a pH-indicator. On the left plate, colorless colonies (*S. maltophilia*) are observed because it does not produce any acid from glucose. *S. maltophilia* needed to metabolize peptone for growth, and some alkalis made from peptone have turned the appearance of colonies bluish. On the right plate, yellow colonies (*S. marcescens*) are grown with a fair amount of acidity by fermentation of glucose.

S. maltophilia is found in a wide variety of environments, and has been recovered from nosocomial water sources including ice-making machines, sink traps and nebulizers (Sattler, 2000; Rogues et al., 2001). Despite the fact that almost all strains of *S. maltophilia* are isolated from inpatients, Denton et al. (1998) maintain that person-to-person transmission is an infrequent occurrence in the nosocomial setting. Epidemiological research of *S. maltophilia* also highlights that babies and elderly people are more likely to get this bacterium than those in the generations in between. As to the sources, they are also usually isolated from the respiratory tract such as sputum and the broncho-alveolar lavage.

The respiratory tract is the most common site of *S. maltophilia*'s isolation in hospitalized patients; however, most patients are colonized rather than infected at this site (Geiger et al., 2001). As Rogues et al. (2001) have stated: A ventilator can be colonized with the patient's endogenous flora and becomes a hotbed of secondary infections. In our investigation, more than 10 strains of *S. maltophilia* were actually separated from superinfections of

pneumonia, bacteremia or cholangitis (Kataoka et al., 2003b). *S. maltophilia* has been associated with an expansive spectrum of clinical syndromes: endocarditis, conjunctivitis, keratitis, urethritis, ulcerative colitis and Crohn's disease (Denton and Kerr, 1998).

S. maltophilia has been reported to be resistant to extended-spectrum cephalosporins, carbapenems and aminoglycosides (Denton et al., 1999; Barbier-Frebourg et al., 2000). The medical community has increasingly become dependent on carbapenems, because of their high affinity for penicillin-binding proteins, excellent penetration across bacterial outer membranes, and resistance to hydrolysis by the majority of serine-based β -lactamases (Payne et al., 1997; Rasmussen and Bush, 1997; Yin et al., 2003). Therefore, carbapenems are so-called the final trumps. But all strains really revealed resistance to imipenem grouped into carbapenems (Table 4), and this peculiarity coincides with former documents (Hanberger et al., 2001; Simm et al., 2002). Ceftazidime appeared to possess reasonable activity against some strains (Table 4), but as resistance is highly variable, ceftazidime cannot be used in empiric chemotherapy (Geiger et al., 2001). β -Lactamase inhibitor such as sulbactam was exceptionally effective, so inhibition of β -lactamases could be a key to the problem. This hypothesis leads to the Discussion in the next Section.

Table 4. Antimicrobial susceptibility (%) of *S. maltophilia*

Antimicrobial agent	<i>S. maltophilia</i> [n = 155]		
	Susceptible*	Intermediate*	Resistant*
Piperacillin†	11 (7.1)	0 (0.0)	143 (92.9)
Cefsulodin†	3 (1.9)	9 (5.8)	142 (92.2)
Ceftazidime	35 (22.6)	24 (15.5)	96 (61.9)
Sulbactam/ Cefoperazone	105 (67.7)	41 (26.5)	9 (5.8)
Aztreonam	4 (2.6)	0 (0.0)	151 (97.4)
Imipenem	0 (0.0)	0 (0.0)	155 (100.0)

* Categories are recommended by the National Committee for Clinical Laboratory Standards (2000).

† No data with 1 isolate.

This Section argues that *S. maltophilia* can live through the selective pressure of standard chemotherapy using β -lactams, and get its chance in the limelight. For this reason, clinicians should attach more importance on information from the clinical laboratory in order not to overlook this so-called "reserved" organism.

III. Differential Activities of β -Lactamases

The most common theory is that *S. maltophilia* chromosomally yields at least 2 inducible β -lactamases, a class B metallo- β -lactamase (carbapenemase) L1 and a class A active-site serine- β -lactamase (penicillinase) L2 (Table 2) (Ambler, 1980). Both enzymes mediate an essential resistance to β -lactam agents as a result of cutting the carbon-nitrogen bond of β -lactam rings off (Ullah et al., 1998; Avison et al., 2001; Spencer et al., 2001). There are still no clinically useful inhibitors of metallo- β -lactamases in contrast with serine- β -lactamases (Simm et al., 2002). The expression of L1 and L2 has been shown by coinducibility under the existence of a single β -lactam agent as an inducer, and also by some single site mutations resulting in over-expression of both genes (Avison et al., 2002).

In recent years, the sodium mercaptoacetic acid (SMA) disk was developed for the screening of metallo- β -lactamase-producing Gram-negative bacilli (Arakawa et al., 2000). Though the main target of this thiol compound is originally IMP-1-producing *P. aeruginosa* or *S. marcescens*, this convenient test can be applied to L1-producing *S. maltophilia*. Even the imipenem-resistant *P. aeruginosa* strain indicated no reaction, whereas *S. maltophilia* showed good susceptibility to SMA (Fig. 3). The resistance to imipenem of that *P. aeruginosa* strain is not due to production of carbapenemase, but to mutational impermeability of up-regulated MexEF-OprN and reduced OprD (Livermore, 2002).

The hydrolytic activities of L1 and L2 β -lactamases have already been measured (Kataoka et al., 2003a). Decrease in UV absorption at an appro-

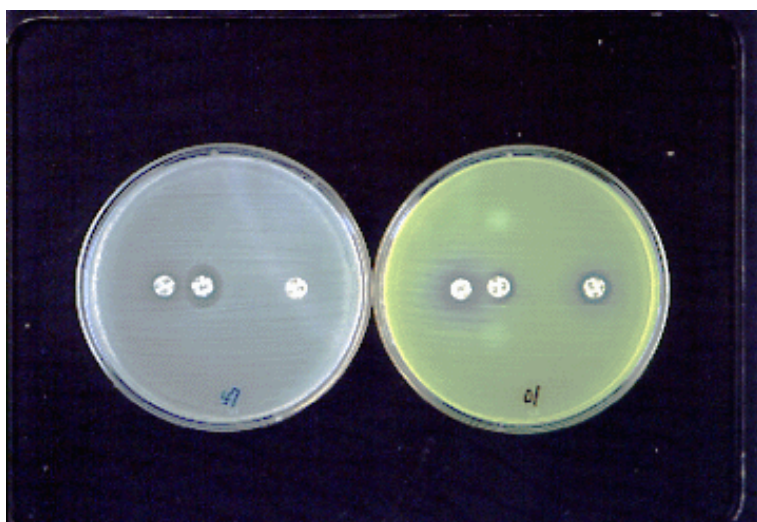


Fig. 3. Inhibition by sodium mercaptoacetic acid (SMA). On the left plate (*S. maltophilia*), a considerable expansion in zone size around an imipenem (IPM) disk close to SMA is revealed, in contrast to IPM without SMA. That means positive of carbapenemase. On the right plate (*P. aeruginosa*, which is yellow-green due to the fluorescent pigment), no expansions between the two IPM disks are shown. That naturally means negative of carbapenemase despite resistance to IPM. From Kataoka et al., 2003b.

priate wavelength means hydrolysis of the β -lactam ring (Samuni, 1975). By inactivation for 4 h, an average reduction of approximately 20% was shown in both absorbances of imipenem and ceftazidime (Fig. 4). Taking into consideration the affinity, the hydrolysis of imipenem is performed by L1, and that of ceftazidime is largely related to L2. It appears that the hydrolytic function of L2 is equivalent to that of an extended-spectrum β -lactamase.

The notion of coordinated expression allowed Krueger et al. (2001) to point out that the bacterial primary resistance to β -lactams is not overcome until both β -lactamases are inhibited. Although β -lactam agents are thought to be unrealistic tools against infections with *S. maltophilia*, this bacterium is useful as an extreme model of a β -lactamase producer. Our inhibition test notes that imipenem and ceftazidime are predominantly hydrolyzed by L1, whereas ceftazidime is predominantly hydrolyzed by L2 (Kataoka et al., 2003a). Incidentally, aztreonam grouped into monobactams has been reported as an inhibitor of extracellular β -lactamase

(Lister et al., 1998; Song et al., 2003). These reports are in harmony with the proposal of Nishida et al. (1999) that a monobactam derivative can indicate considerable synergy with ceftazidime. Upon further examination, no synergy of aztreonam with imipenem is exhibited. These results also explain that aztreonam tends not to inhibit carbapenemases, because imipenem should be broken down only by carbapenemase L1. Therefore, the synergy of aztreonam with ceftazidime was probably due to the aztreonam's inhibition of penicillinase L2.

The inhibitory activity of aztreonam against the penicillinases of *S. maltophilia* has been explored (Kataoka and Tanaka, in press). All 5 strains of *S. maltophilia* had already been confirmed susceptible to clavulanic acid so they were identified possessors of L2 β -lactamase (Fang et al., 2002;

Coudron et al., 2003). In spite of little synergy of imipenem to all 5 strains, a considerable synergy of aztreonam with ceftazidime was found in 4 strains out of 5 (Table 5). Sulbactam is definitely known as a potent inhibitor of penicillinases (Mahgoub and Aly, 1998), and actually showed an excellent

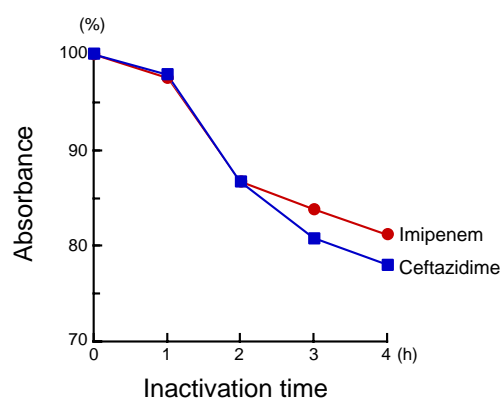


Fig. 4. Hydrolysis of β -lactam agents by *S. maltophilia*. The absorbances of the β -lactam rings were measured by UV spectrophotometry. Closed circle, imipenem; closed square, ceftazidime.

Table 5. MICs against *S. maltophilia*

Strain	MIC ($\mu\text{g/mL}$)				
	Aztreonam	Imipenem	Aztreonam/ Imipenem	Cefozopran	Aztreonam/ Cefozopran
<i>S. maltophilia</i> TOT8	256	512	512/512	256	256/256
<i>S. maltophilia</i> TOT16	1024	512	512/512	256	128/128
<i>S. maltophilia</i> TOT19	1024	256	256/256	256	128/128
<i>S. maltophilia</i> TOT43	256	256	256/256	128	64/64
<i>S. maltophilia</i> TOT57	512	256	256/256	128	64/64

MIC, minimum inhibitory concentration. From Kataoka and Tanaka, in press with some modifications.

synergy with cefoperazone (not cefozopran) against *S. maltophilia* (Table 4). These findings support the idea that aztreonam as well as sulbactam inhibits penicillinases instead of performing aztreonam's own antimicrobial activity. This account is defended by both the high resistant frequency of aztreonam and the high MICs of aztreonam alone against *S. maltophilia* (Tables 4 and 5). Figure 5

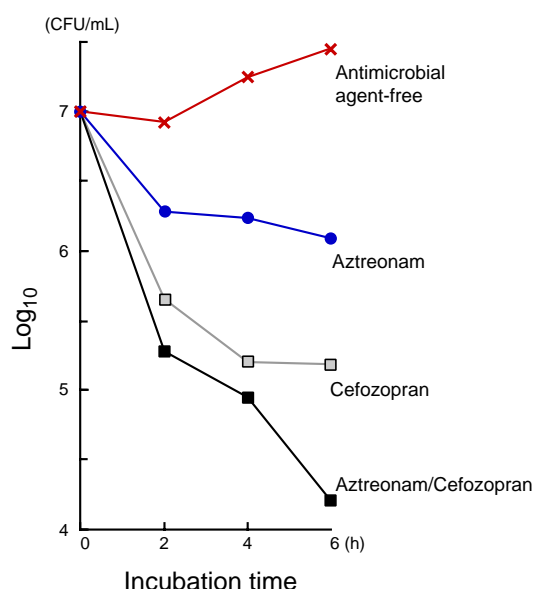


Fig. 5. Colony forming unit (CFU) count of *S. maltophilia* in culture treated with antimicrobial agents as follows: Cross, antimicrobial-agent free; closed circle, aztreonam; open square, cefozopran; closed square, aztreonam and cefozopran. From Kataoka and Tanaka, in press with some modifications.

presents the antimicrobial effect of the combination of aztreonam and cefozopran. The colony forming unit (CFU) counts at 6 h in cultures treated with aztreonam-cefzopran were approximately 1 log lower than those in cultures treated with cefozopran alone, and 2 logs lower than those treated with aztreonam alone.

Aztreonam is additionally suitable in combination chemotherapy for Gram-negative infections. One reason is that monobactams have no antimicrobial activity against Gram-positive bacteria: The risk of superinfection by Gram-positive bacteria, for instance enteritis caused by *Staphylococcus aureus*, is estimated to be low. Improper usage of broad-spectrum antimicrobial agents might prepare a hotbed of multidrug-resistant strains; therefore, the minimum usage of narrow-spectrum ones has recently been recommended for the control of hospital-acquired infections. We hope that a reduction in the dosage of cefozopran by employing aztreonam comes to not only obtain antimicrobial synergy and minimize toxicity but also to prevent the emergence of penicillinase producers.

IV. Indirect Pathogenicity

A popular idea exists nowadays that *S. maltophilia* is of strictly limited pathogenicity (Denton and Kerr, 1998), while this organism has the capacity to hydrolyze a variety of β -lactam antimicrobial agents and the possibility of assisting the survival of other

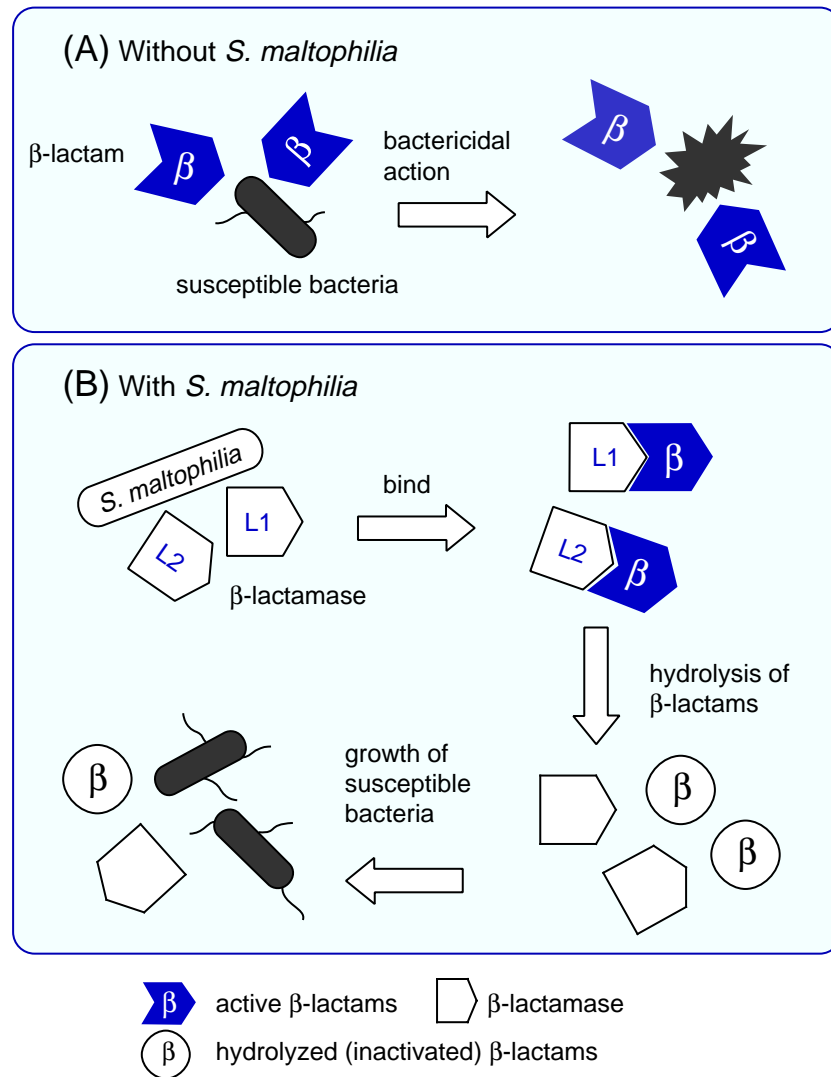


Fig. 6. Schema of indirect pathogenicity.

A: Without *S. maltophilia*, susceptible bacteria are killed by β -lactam agents.

B: With *S. maltophilia*, β -lactamases leaking from *S. maltophilia* hydrolyze β -lactam agents, so even susceptible bacteria can survive.

susceptible pathogens. *S. maltophilia* is apparently a β -lactamase supplier rather than a direct cause of disease where it is confronted with β -lactam agents. It is generally isolated from severely immunosuppressed hosts because of its weak virulence, and has frequently been isolated together with other Gram-negative species in our observations (Kataoka et al., 2003b). It looks like broad-spectrum antimicrobial therapy takes aim at the major opportunistic pathogens such as *P. aeruginosa* and *S. mar-*

cescens in most cases of Gram-negative infection. As it turns out, the existence of *S. maltophilia* is apt to be less reflected in the choice of antimicrobial agents. However, broad-spectrum β -lactam agents could act as a sieve of multidrug-resistant strains such as *S. maltophilia* and serve to induce their own β -lactamases (Kataoka et al., 2001, 2002b). That is why we propose the concept of indirect pathogenicity (Fig. 6): A chain of the preceding processes can result in the encouragement of mixed

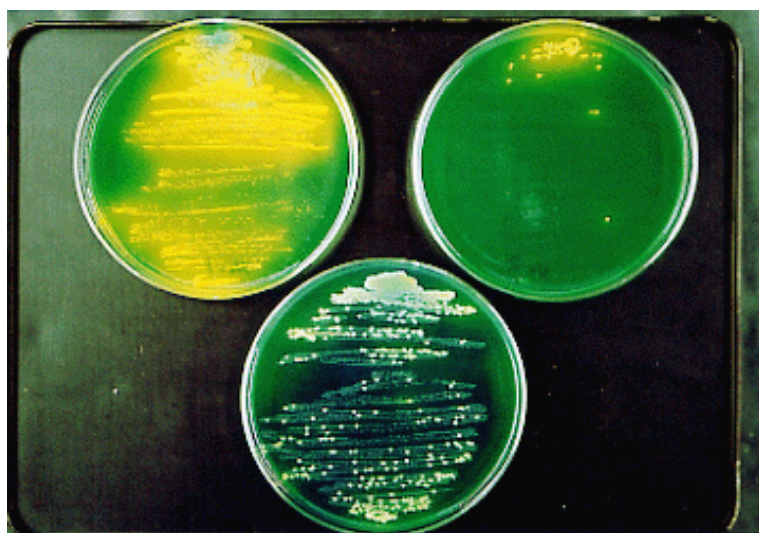


Fig. 7. Mixed culture of *S. marcescens* and *S. maltophilia*. On the top left plate, an extremely large number of yellow colonies (*S. marcescens*) are seen (none for imipenem was supplemented). On the top right plate, the number of yellow colonies is obviously decreased because of 16 µg/mL of imipenem in the medium. On the bottom plate, however, a fairly large number of yellow colonies and colorless colonies (*S. maltophilia*) are indicated even under exposure to 16 µg/mL imipenem (from Kataoka et al., 2003b).

infections caused by another pathogen, even if *S. maltophilia* originally colonizes in the patient's respiratory tract.

The indirect pathogenicity of *S. maltophilia* has already been confirmed (Kataoka et al., 2003b). The CFU of the yellow colonies (*S. marcescens* and *P. aeruginosa*) was counted for each plate, because both *S. marcescens* and *P. aeruginosa* form yellow colonies by acid production from glucose in contrast with colorless colonies of *S. maltophilia* (Figs. 2 and 7).

Figure 8 demonstrates that *S. marcescens* exposed to imipenem increased in the existence of *S. maltophilia*. This concept is also applicable to a mixed culture of *P. aeruginosa* with *S. maltophilia* (Kataoka et al., 2003b). On the other hand, the CFU count of *S. marcescens* seemed not to be influenced in cases when it was faced to ceftazidime instead of imipenem (Kataoka et al., 2003b), because *S. marcescens* is a potential owner of AmpC β-lactamase (Coudron et al., 2000). An induction of AmpC makes the strain highly resistant to

ceftazidime (Raimondi et al., 2001). We consider that both β-lactamases of L2 and AmpC were yielded during exposure to ceftazidime in mixed culture, and have assumed that L2 leaking from *S. maltophilia* hid itself behind *S. marcescens*' own AmpC. As proof of the output of L2, *P. aeruginosa* confronted with a large quantity of ceftazidime could grow under the support of *S. maltophilia* (Kataoka et al., 2003b). As Livermore (2002) has demonstrated, *P. aeruginosa* also harbors AmpC β-lactamase, but unlike *S. marcescens* the level of resistance depends on the degree of depression. Following this thesis, the *P. aeruginosa* strain does not seem to have produced enough β-lactamases to protect itself from ceftazidime.

Though a multidrug-resistant strain is not always relevant to indirect pathogenicity, at least our positive findings can be thought of as a formal explanation for some potential threat: Even an ordinary bacterium can endure antimicrobial attack

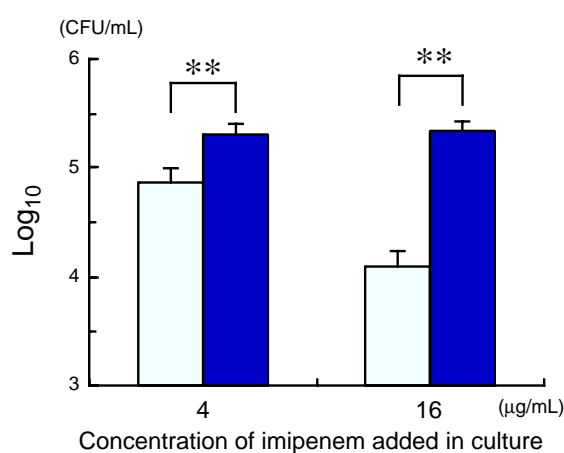


Fig. 8. Colony forming unit (CFU) count of *S. marcescens* in culture which contains imipenem. Pale bar, pure culture of *S. marcescens*; dark bar, mixed culture of *S. marcescens* with *S. maltophilia*. ** $P < 0.01$ with *t*-test. The bars represent means plus SD. From Kataoka et al. (2003b) with some modifications.

by the assistance of β -lactamases from an indirect pathogen, namely *S. maltophilia*.

Conclusion

Carbapenems and cephalosporins are among the most frequently administered antimicrobial agents due to their excellent antimicrobial activity and low toxicity; therefore, these compounds are often prescribed for severe Gram-negative infections (Coudron et al., 2003; Yin et al., 2003). The present study upholds the theory that overuse of these antimicrobial trumps has a profound effect on a selection pressure on account of the induction of β -lactamases, and results in the appearance of an antibiotic-free area around *S. maltophilia*. In other words, sulbactam, minocycline and trimethoprim-sulfamethoxazole, relatively unpopular but effective drugs, should be selected more in a case where mixed infection occurs (Kataoka et al., 2002a). Although it is sometimes difficult to attribute a causative role to *S. maltophilia* when the organism is isolated from respiratory secretions alone (Sattler, 2000), throughout the course of this essay we establish that *S. maltophilia* is worthy of attention. Thus, a full understanding of the indirect pathogenicity in vivo awaits future study.

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