〈Brief Report〉

Properties of *Achromobacter xylosoxidans* highly resistant to aminoglycoside antibiotics

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We herein discovered a highly resistant clinical isolate of *Pseudomonas aeruginosa* with MICs to amikacin, gentamicin, and arbekacin of 128 μg/mL or higher in a drug sensitivity survey of 92 strains isolated from the specimens of Yoka hospital patients between January 2009 and October 2010, and *Achromobacter xylosoxidans* was separated from this *P. aeruginosa* isolate. The sensitivity of this bacterium to 29 antibiotics was investigated. The MICs of this *A. xylosoxidans* strain to 9 aminoglycoside antibiotics were: amikacin, gentamicin, arbekacin, streptomycin, kanamycin, neomycin, and spectinomycin, 1,024 μg/mL or $\geqq$1,024 μg/mL; netilmicin, 512 μg/mL; and tobramycin, 256 μg/mL. This strain was also resistant to dibekacin. This aminoglycoside antibiotic resistant phenotype is very rare, and we are the first report the emergence of *A. xylosoxidans* with this characteristic.

In the present study, we investigated the antibiotic sensitivities of 92 clinical isolates of *Pseudomonas aeruginosa* collected from Yoka hospital between January 2009 and October 2010. The agar plate dilution and disc diffusion methods specified by the Clinical and Laboratory Standards Institute were employed for the antibiotic sensitivity test. Ninety-two strains were tested, and the agents used were 16 antibiotics to treat *P. aeruginosa* infections: piperacillin, piperacillin-

tazobactam, ceftazidime, cefepime, cefozopran, aztreonam, imipenem, meropenem, biapenem, doripenem, ciprofloxacin, levofloxacin, pazufloxacin, amikacin, gentamicin, and arbekacin. The MIC distribution of these 16 antibiotics is shown in Table 1. Their antimicrobial activities were retained at relatively high levels. Of the aminoglycoside antibiotics tested, there were only 4 strains for which the MIC values of gentamicin and amikacin were 128 μg/mL or higher, and only one strain for which the MIC values of all 3 antibiotics including arbekacin were $>$128 μg/mL. We focused on the *P. aeruginosa* strain that showed high-level resistance to the 3 antibiotics with the high MIC value of arbekacin not indicated for treatment against *P. aeruginosa*. When this strain was subjected to disc diffusion tests with aminoglycoside antibiotics, bacteria grew in the growth inhibition ring. At that time, we detected contaminating *P. aeruginosa*. Hence, we employed the disk diffusion method to check for other *P. aeruginosa* (bacterial) strains. However, no other strains were identified. Thus, the *P. aeruginosa* strain was subjected to re-isolation on Drigalski medium, which resulted in the formation of large and pin-point colonies. Based on biochemical tests, the ID test, NF-18 simple identification kit 'Nissui', and the 16S rRNA base sequence[1)], the large colony was identified as *P. aeruginosa*, and the pin-point colony as *Achromobacter xylosoxidans*. The clinical isolate of *P. aeruginosa* and separated strains were designated as the *P. aeruginosa* 60 and 600L strains and *A. xylosoxidans* 600S strain, respectively.

The sensitivities of these 3 strains to streptomycin, kanamycin, netilmicin, spectinomycin, neomycin, tobramycin, dibekacin, chloramphenicol, tetracycline, erythromycin, vancomycin, oxacillin, and sulfamethoxazole-trimethoprim were investigated, in addition to the 16 antibiotics described above. The MIC values for and antibiotic sensitivity phenotypes of the 3 strains on the disc diffusion tests are shown in Table 2. The high MIC values of cefepime, cefozopran, aztreonam, and the 3 aminoglycoside antibiotics for the *P. aeruginosa* 60 strain were derived from the *A. xylosoxidans* 600S strain. This multidrug resistance pattern was consistent with the previously reported pattern for *A. xylosoxidans*[2,3)]. The MIC values of the aminoglycoside antibiotics were: tobramycin, 256 μg/mL; netilmicin, 512 μg/mL; and the 7 other antibiotics, 1,024 μg/mL or higher. The aminoglycoside resistance of *A. xylosoxidans* is well-known, whereas the MIC value varies and was previously reported to be approximately 128 μg/mL[4)]. Non-fermentative Gram-negative rods have RND-type efflux pumps[5,6,7)]. In 2013, Bador *et al.* reported that the drug efflux pump, AxyXY-OprZ, is involved in the aminoglycoside resistance of *A. xylosoxidans*, for which the MIC values of amikacin and gentamicin are greater than 256 μg/mL[8)]. The MIC values of streptomycin and spectinomycin were greater (1,024 μg/mL or higher) for the related bacterium, *Burkholderia pseudomallei*[9)]. The MIC value (1,024 μg/mL) for the *A. xylosoxidans* 600S strain was high and equivalent to those for related bacteria. Based on the phylogenetic tree of bacteria with drug efflux pumps[10)] and previous findings by Bador *et al.*[8)], *A. xylosoxidans*, *Pseudomonas*, and *Burkholderia* were found to be very closely related. Therefore, the high MIC value for the *A. xylosoxidans* 600S strain suggests the involvement of drug efflux pumps.

Table 1. MIC distribution of 16 antibiotics for 92 clinical isolates of *Pseudomonas aeruginosa*

Antibiotics	MIC (μg/mL)													MIC_{50}	MIC_{90}
	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128		
piperacillin	0	0	0	3	2	4	26	22	13	12	0	5	5	8	128
tazobactam/ piperacillin	0	0	0	3	3	0	35	17	17	8	3	2	4	8	32
ceftazidime	0	0	0	6	27	21	14	13	4	1	5	0	1	2	16
cefepime	0	0	1	4	22	15	25	18	5	0	1	0	1	4	8
cefozopran	0	0	3	14	22	26	13	6	5	1	0	0	2	2	8
imipenem	0	0	2	5	47	26	1	3	5	2	1	0	0	1	8
meropenem	10	11	21	17	16	8	2	3	2	1	0	0	1	0.5	2
biapenem	0	3	12	28	23	14	2	3	5	1	0	0	1	1	8
doripenem	4	14	20	10	18	9	10	2	2	0	0	0	3	0.5	4
aztreonam	0	0	4	2	0	10	36	12	11	12	5	0	0	4	32
amikacin	0	0	1	2	6	37	19	20	5	0	0	1	1	2	8
gentamicin	0	1	1	2	10	50	13	11	2	0	0	1	1	2	8
arbekacin	0	1	3	4	38	24	14	6	0	0	1	0	1	1	4
pazufloxacin	5	21	23	9	11	4	6	8	2	1	0	2	0	0.25	8
levofloxacin	2	11	13	22	13	8	5	3	10	3	1	0	1	0.5	16
ciprofloxacin	8	27	11	21	3	3	4	5	6	2	1	0	1	0.25	16

Table 2. MICs and sensitivity phenotypes of 29 antibiotics for *Pseudomonas aeruginosa* 60 strain, *Pseudomonas aeruginosa* 600 L strain and *Achromobacter xylosoxidans* 600S strain

Antibiotics	MIC (μg/mL)					
	Pseudomonas aeruginosa 60 strain		*Pseudomonas aeruginosa* 600L strain		*Achromobacter xylosoxidans* 600S strain	
piperacillin	4		4		0.25	
tazobactam/ piperacillin	4		4		0.5	
ceftazidime	8		2		8	
cefozopran	≧128		0.5		512	
cefepime	64		1		64	
imipenem	1		1		1	
meropenem	0.125		0.25		0.25	
doripenem	4		0.0625		2	
biapenem	1		0.5		1	
aztreonam	64		8		64	
ciprofloxacin	8		0.125		4	
pazufloxacin	2		0.25		1	
levofloxacin	4		0.5		4	
amikacin	≧128	R	1	S	1024	R
gentamicin	≧128	R	2	S	1024	R
arbekacin	≧128	R	0.5	S	≧1024	R
spectinomycin			256	I	≧1024	R
streptomycin			8	S	≧1024	R
kanamycin			64	R	1024	R
neomycin			4	S	≧1024	R
tobramycin			0.5	S	256	R
netilmicin			2	S	512	R
dibekacin				S		R
tetracycline			32	R	256	R
erythromycin			256	R	256	R
chloramphenicol			128	R	32	I
oxacillin				R		R
vancomycin				R		R
sulfamethoxazole/ trimethoprim				I		S

R: resistant, I: intermediate, S: susceptible

However, no strain with high MIC values for many types of aminoglycoside antibiotics has previously been reported. This resistance pattern of the *A. xylosoxidans* 600S stain may be rare, and several resistance mechanisms, such as acquired 16S rRNA methylase genes[11] and *aac*A4 gene cassettes[12], including drug efflux pumps may be involved.

A. xylosoxidans is an aerobic, Gram-negative, non-fermentative rod that is indigenous[13,14]. It is rarely detected in routine tests, and issues associated with misidentification as *P. aeruginosa* on

microbial identification and drug resistance information are not widely shared. The characteristics of aminoglycoside antibiotics and the disc diffusion method allowed us to identify mixed bacteria. The prevention of misjudgments in routine tests is important, for which it may be necessary to investigate simple methods in order to isolate/differentiate mixed bacteria from related bacteria. Moreover, it is unclear whether the *A. xylosoxidans* 600S strain is involved in the route of mixing and infection. However, it is important to note that the *A. xylosoxidans* 600S strain was mixed with a clinical isolate of *P. aeruginosa*. The bacterium *A. xylosoxidans* has been isolated from dialysates, incubators, and antiseptic solutions in hospitals at familiar sites[15,16,17]. It is a new aerobic, Gram-negative rod that requires attention, particularly in infection-sensitive individuals.

Conflicts of interest

None to declare.

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