Environmental Distribution and Drug Susceptibility of *Achromobacter Xylosoxidans* Isolated from Outdoor and Indoor Environments

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ABSTRACT

Achromobacter xylosoxidans is an environmental bacterium with multi-drug resistance. We isolated *Achromobacter xylosoxidans* and investigated its susceptibility to 13 drugs. Seventy-eight water samples were collected from rivers and ponds, and 11 samples were swabbed from residential sinks and baths. Nine strains of *Achromobacter xylosoxidans* were isolated from the 89 samples. Five strains, including 2 that were sampled from residential homes, showed high resistance to multiple aminoglycosides. This indicated that *Achromobacter xylosoxidans* is widely distributed in various outdoor and indoor environments. Moreover, since these highly resistant bacteria were present in indoor environments, caution should be taken for elderly people living at home. Furthermore, a careful assessment should be made for diagnosing and treating compromised hosts.

Key words *Achromobacter xylosoxidans*; environmental distribution; environmental resident bacterium; multi-drug resistance; opportunistic infection

Achromobacter xylosoxidans (*A. xylosoxidans)* is a catalase-positive, oxidase-positive, and glucose-non-fermenting Gram-negative rod. Non-fermenting Gram-negative rods have similar properties and are difficult to differentiate.[1] Furthermore, since *A. xylosoxidans* is an environmental pathogen, it is usually overlooked in routine clinical examinations. *A. xylosoxidans* is an opportunistic bacterium with multi-drug resistance and infections are known to cause clinical difficulties since it becomes chronic and refractory.[2]

We previously reported a rare case whereby a strain classified as multidrug-resistant *Pseudomonas aeruginosa* (*P. aeruginosa*) was further identified as drug-susceptible *P. aeruginosa* and multidrug-resistant *A. xylosoxidans*.[3] Based on this experience, we became aware of the importance of surveying the environmental distribution and drug-susceptibility of *A. xylosoxidans* existing in nature. We devised an isolation procedure suited for the properties of *A. xylosoxidans*, isolated it from various environmental samples, and investigated the environmental distribution and drug susceptibility of the isolates. The results suggest that more attention to this species is necessary.

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Abbreviations: *A. xylosoxidans, Achromobacter xylosoxidans*; AG, aminoglycoside; AMK, amikacin; AZT, aztreonam; CFPM, cefepime; CPFX, ciprofloxacin; GM, gentamicin; HIVA, Heart Infusion broth including Vancomycin and Aztreonam; IPM, imipenem; KM, kanamycin; MIC, minimal inhibition concentration; NEO, neomycin; NET, netilmicin; PIPC, piperacillin; *P. aeruginosa, Pseudomonas aeruginosa*; SM, streptomycin; SPCT, spectinomycin; TOB, tobramycin; X-MacVA, Xylose-Macconkey agar including Vancomycin and Aztreonam

MATERIALS AND METHODS

Culture media

Heart Infusion broth including Vancomycin and Aztreonam (HIVA) broth was prepared by adding 32 μg/mL of vancomycin and aztreonam to heart infusion broth and was used as an enrichment medium. Xylose-Macconkey agar including Vancomycin and Aztreonam (X-MacVA) agar plate was prepared by adding 1% xylose (X) and 20 μg/mL of vancomycin and aztreonam to MacConkey agar and was used as an isolation medium.[2]

Method for isolation from the environment

Samples were collected from rivers, ponds, irrigation ditches, and residential homes within an area of 20 km of the Yumigahama Peninsula in the west area of Tottori Prefecture, Japan. The samples were either collected in volumes of 15 mL from outdoor water sources or obtained from drains of residential sinks and baths using sterilized swabs, which was subsequently suspended with 15 mL of distilled water. These samples were centrifuged at 4,000 × g for 15 minutes, and the sediment was cultured in HIVA broth 72 h at 35 °C. Cultured bacterial fluid was cultured on X-MacVA agar 72 h at 35 °C. The colonies that formed on the agar were typed, and the number of colony types was regarded as the number of isolated colonies. A typical colony in each colony type was subjected to differentiation tests. Of the bacteria determined to be Gram-negative rods, only

those that were positive for catalase and oxidase tests were further tested for their non-fermentability, growth ability in stab culture, citrate utilization ability, and nitrate-reducing ability. These differential test-positive bacteria were identified using ID test NF-18 (Nissui Pharmaceutical, Tokyo, Japan), a simple identification kit for glucose-non-fermenting Gram-negative rods. A final identification was made according to a 16S rDNA base sequence.[4]

Bacterial strains

Nine environmentally isolated *A. xylosoxidans* and *A. xylosoxidans* 600S strains[3] were used.

Drug susceptibility tests

The minimal inhibition concentration (MIC) was determined by the agar plate dilution method using the Clinical and Laboratory Standard Institute procedure. The following drugs were tested: piperacillin (PIPC), cefepime (CFPM), aztreonam (AZT), imipenem (IPM), ciprofloxacin (CPFX), amikacin (AMK), gentamicin (GM), streptomycin (SM), kanamycin (KM), netilmicin (NET), spectinomycin (SPCT), neomycin (NEO) and tobramycin (TOB).

RESULTS

Table 1 shows the isolation state of *A. xylosoxidans* from various samples and the MICs of 13 antibacterial drugs

Table 1. The state of isolation of *Achromobacter xylosoxidans* from environmental materials and the MICs of 13 antibacterial drugs against environmentally isolated *Achromobacter xylosoxidans*

Samples *n*	Number of colonies				Antibiotics MIC (μg/mL)					AGs							
	Isolated *n*	Differentiated *n*	Identified *n*	Identified name	PIPC	CFPM	AZT	IMP	CPFX	AMK	GM	SPCT	SM	KM	TOB	NET	NEO
Water 78	57	45	6	W1	0.5	8	128	1	1	8	4	128	≥ 128	8	4	4	16
				W2	0.25	8	128	1	2	128	32	128	16	128	8	4	128
				W3	0.5	4	128	1	1	4	2	≥ 128	64	8	2	2	8
				W4	1	32	64	4	2	≥ 128	≥ 128	≥ 128	≥ 128	128	64	128	≥ 128
				W5	0.25	8	128	1	2	4	2	128	32	8	2	2	8
				W6	16	32	128	1	4	64	64	≥ 128	≥ 128	128	16	32	128
Swabs 11	8	8	3	S1	0.5	32	128	2	8	≥ 128	128	≥ 128	≥ 128	≥ 128	64	128	≥ 128
				S2	0.5	32	64	2	2	≥ 128	≥ 128	≥ 128	128	64	64	128	≥ 128
				S3	0.25	8	128	1	1	4	2	128	64	8	2	4	8
Achromobacter xylosoxidans 600S					0.5	128	128	1	16	≥ 128	≥ 128	≥ 128	≥ 128	≥ 128	≥ 128	≥ 128	≥ 128

Samples: water; rivers, ponds, irrigation ditches, swabs; the drains of residential sinks and baths.
Drugs:AG; aminoglycoside; AMK, amikacin; AZT, aztreonam; CFPM, cefepime; CPFX, ciprofloxacin; GM, gentamicin; IMP, imipenem; KM, kanamycin; NEO, neomycin; NET, netilmicin; PIPC, piperacillin; SPCT, spectinomycin; SM, streptomycin; TOB, tobramycin.
MIC, minimal inhibition concentration.

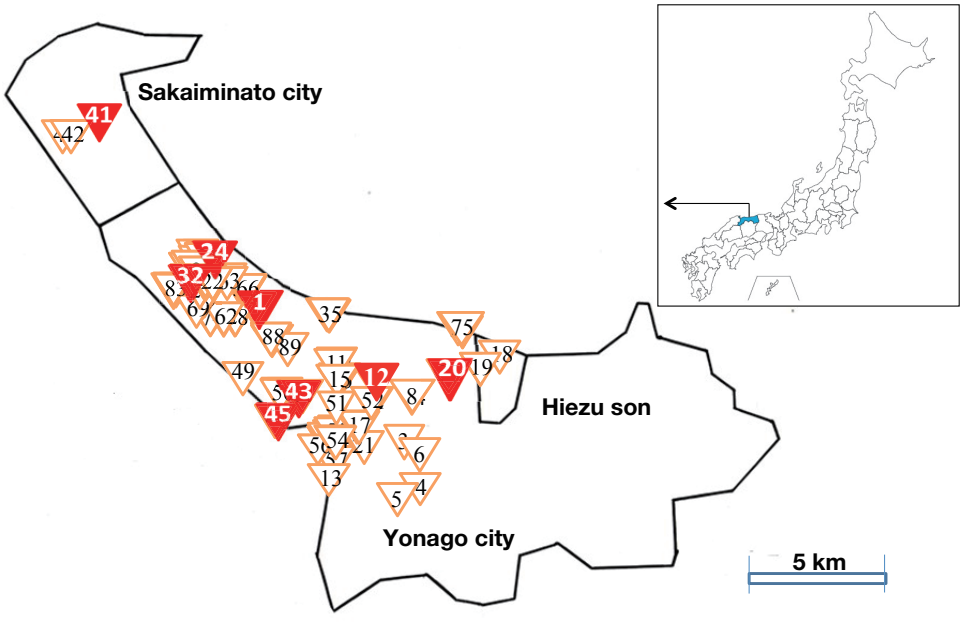

Fig. 1. Map of the sampling points and *A. xylosoxidans* isolation. Map of the Yumigahama Peninsula extending from Yonago City to Sakaiminato City, Tottori Prefecture, Japan. The sampling points of *A. xylosoxidans*-positive and -negative materials are indicated as ▼ and ▽, respectively. Sample numbers are shown in the symbols. *A. xylosoxidans*, *Achromobacter xylosoxidans*.

for the isolated *A. xylosoxidans*. Figure 1 shows the map of the sampling points and *A. xylosoxidans* isolation.

State of isolation

Of the 89 samples, 9 strains of *A. xylosoxidans* were identified. The identification rate was 10.1% (6 strains (7.7%) in outdoor water samples and 3 strains (27.3%) in swab samples from residential homes) (Table 1). The species was identified in a wide sampling area (Fig. 1).

Evaluation of the isolation and identification methods

The number of isolated colonies that formed on X-MacVA agar was low, at around 70% per sample. The differentiation tests showed that 81.5% of the isolated colonies were positive for oxidase and catalase. Based on the bacterial identification tests, 10.1% of the colonies were identified as *A. xylosoxidans*. Furthermore, according to the isolation and identification protocol, of the red colonies that formed on X-MacVA agar, the large colonies were identified as *Pseudomonas fluorescens* and the pinpoint colonies as *A. xylosoxidans*. Finally, *A. xylosoxidans* was confirmed using the 16 rDNA base sequence.

Drug susceptibility tests

The strains were susceptible to β-lactams (PIPC, IMP, CFPM) except for monobactam (AZT), and new quinolone (CPFX). Of the 8 aminoglycosides (AGs; AMK, GM, SPCT, SM, KM, TOB, NET, NEO) that were tested, the MIC of SPCT was 128~ ≥ 128 μg/mL for all strains. However, drugs other than SPCT and SM had low MICs for 4 strains (W2, W3, W5, S3) and high MICs for 5 strains (W1, W4, W6, S1, S2), which closely resembled the resistance pattern of the *A. xylosoxidans* 600S strain. Of the 5 multidrug-resistant strains (W2, W4, W6, S1, S2) in all, 2 were isolated from residential homes (S1, S2) (Table 1).

DISCUSSION

Since *A. xylosoxidans* is an environmental bacterium, it is rarely detected in routine clinical examinations, and, if it is detected, its differentiation and identification is difficult as it is often mistaken for *P. aeruginosa*. *A. xylosoxidans* has multidrug resistance and erroneous identification of the bacteria will lead to inappropriate selection of drugs. Therefore, information concerning the distribution of this species and the drug susceptibility of bacteria distributed in the environment is important.

The enrichment and isolation media used in this study, which included AZT resistance and xylose degradability, showed high selectivity. Red colonies that formed on X-MacVA agar were likely glucose-non-fermenting Gram-negative *Pseudomonas* and *A. xylosoxidans*. Pin-point colonies, in particular, were considered to be an important criterion for detecting *A. xylosoxidans*.[2] Although the differential tests are effective for confirming major properties of bacteria, they are not able to precisely determine the species. Therefore, a definitive diagnosis based on the 16S rDNA base sequence is important. Since *Pseudomonas* and *A. xylosoxidans* have similar properties, they are often misidentified or overlooked due to differences in their growth ability.[3] Proper knowledge regarding the properties of *A. xylosoxidans* is necessary to avoid misdiagnoses in routine examinations.

Amoureux et al.[2] isolated *A. xylosoxidans* in a wide area and reported identification rates of 28% and 12% from samples collected from the outdoor and indoor environments, respectively. In our study, the identification rate for samples collected from indoor residences was high at approximately 30% for bath drains. In contrast, the identification rate was relatively low for outdoor water sources, which may have been due to the small sample volume of 15 mL. In addition, *A. xylosoxidans* has a biofilm-forming ability.[5] Biofilm can form in the drains of sinks and baths, which may have led to the high bacterial concentrations in the swab samples. Considering these points, we deem our bacterial isolation procedure appropriate. Furthermore, the wide distribution of this species in indoor environments was demonstrated.

The 9 strains of *A. xylosoxidans* isolated from the various environments resembled the common multidrug resistance pattern,[6] and some drugs showed for high MICs, AGs in particular. The MICs of the 8 AGs for the *A. xylosoxidans* 600S strain[3] were ≥ 128 μg/mL, and 5 (W2, W4, W6, S1, S2) strains were isolated from both the outdoor and indoor samples. Since *A. xylosoxidans* can be mistaken for *P. aeruginosa*, care is necessary when selecting drugs for Gram-negative rod infections. In addition, this species is drug-resistant and is distributed in wet areas. The latter feature may cause problems since the bacteria may accumulate in the sinks of residential kitchens and baths. Currently, residences of elderly people in need of medical and nursing care are increasingly transitioning from medical facilities to residential homes, especially care homes for the elderly. Sufficient attention is necessary for vulnerable elderly people and compromised hosts who live at home.

The authors declare no conflict of interest.

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