BRIEF NOTE

**Non-conjugative R Plasmid with Five Drug Resistance from
Bordetella bronchiseptica of Pig Origin**

Nobuyuki TERAKADO, Seiichi ARAKI*, Yasuyuki MORI,
Tsutomu SEKIZAKI and Kazunori HASHIMOTO

National Institute of Animal Health, 1-1 Kannondai 3-chome,
Yatabe-machi, Tsukuba-gun, Ibaraki 305

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Drug resistance plasmids (R plasmids) have been demonstrated in various species of bacteria, and the genetic and molecular nature of R plasmids has been extensively studied [3]. We have also demonstrated R plasmids from *Bordetella bronchiseptica* which is thought to be a causative agent of infectious atrophic rhinitis in young pigs [5]. Epidemiological surveys have revealed that R plasmids play a major role in the drug resistance of *B. bronchiseptica* [4]. The genetic and biochemical properties of the representative R plasmid (Rte 16) from *B. bronchiseptica* have been described previously [6, 9]. We have recently isolated from young pigs strains of *B. bronchiseptica* which were resistance to streptomycin (Sm), sulfadimethoxine (Su), tetracycline (Tc), chloramphenicol (Cm) and kanamycin (Km). This is the first report on the occurrence of such resistance patterns in this organism. We report here that this resistance detected in *B. bronchiseptica* is also plasmid borne.

In November of 1979, *B. bronchiseptica* strains showing resistance to various antibiotics were isolated from lungs of young pigs with heavy pneumoniae. On the farm,

the pigs had been treated with both tiamulin and levamisole for the prevention of swine enzootic pneumoniae. The minimum inhibitory concentration (MIC) of each drug against isolates was determined by the agar dilution method [5]. In the test for transmission of drug resistance, *Escherichia coli* ML1410 (F⁻ *met* *nal*) was used as the recipient and a mixture of the donor and recipient was cultured at 37°C for 20 hr. Transconjugants were selected by using DHL agar (Eiken) containing both nalidixic acid (Na, 25 µg/ml) and one of the following drugs: Tc, Cm, and Km (12.5 µg/ml), and ampicillin (Ap), mercury chrolide (HgCl₂) and Sm (6.25 µg/ml).

Table 1 shows the MICs of each drug against 8 *B. bronchiseptica* isolates and ATCC4617 as control. Two kinds of drug resistance patterns, (Sm Su Ap Hg) and (Sm Su Tc Cm Km), were found in the isolates. Among them, all strains with (Sm Su Ap Hg)-resistance were able to transfer their resistance as a whole to *E. coli* ML1410. However, no conjugal transfer was observed in the remaining strains with (Sm Su Tc Cm Km)-resistance, suggesting that their

*Present address: Eisai Co., Ltd., 1 Takehaya, Kawashima-cho, Hajima-gun, Gifu, 483.

Table 1. Resistance levels of 8 *Bordetella bronchiseptica* isolates

Name of isolate	Minimum inhibitory concentration (MIC, $\mu\text{g/ml}$)									Resistance pattern
	Sm	Su	Ap	Tc	Cm	Km	Na	Rp	Hg	
ZN-1	≥ 1600	≥ 1600	200	1.56	6.25	6.25	25	12.5	12.5	Sm Su Ap Hg
ZN-2	≥ 1600	≥ 1600	200	1.56	6.25	6.25	25	12.5	12.5	Sm Su Ap Hg
ZN-3	800	≥ 1600	50	100	400	≥ 1600	25	12.5	0.39	Sm Su Tc Cm Km
ZN-4	800	≥ 1600	50	100	400	≥ 1600	25	12.5	0.39	Sm Su Tc Cm Km
ZN-5	800	≥ 1600	50	100	400	≥ 1600	25	12.5	0.39	Sm Su Tc Cm Km
ZN-6	800	≥ 1600	50	100	400	≥ 1600	25	12.5	0.39	Sm Su Tc Cm Km
ZN-7	≥ 1600	≥ 1600	200	1.56	6.25	6.25	25	12.5	12.5	Sm Su Ap Hg
ZN-8	≥ 1600	≥ 1600	200	1.56	6.25	6.25	25	12.5	12.5	Sm Su Ap Hg
ATCC 4617 (control)	100	1.56	25	1.56	6.25	6.25	25	12.5	0.39	

Sm: streptomycin. Su: sulfadimethoxine. Ap: ampicillin. Tc: tetracycline. Cm: chloramphenicol. Km: kanamycin. Na: nalidixic acid. Rp: rifampicin. Hg: mercury chloride.

Table 2. Transformation with plasmid DNA from *Bordetella bronchiseptica* ZN-3*

Donor DNA	Selection	Resistance pattern of transformant
ZN-3	Sm	16/16: Sm Su Tc Cm Km
	Tc	4/ 4: Sm Su Tc Cm Km
	Km	8/ 8: Sm Su Tc Cm Km
ZN-3-1**	Sm	11/16: Sm Su Tc Cm Km
		4/16: Sm Su Km
		1/16: Sm Su
	Tc	16/16: Sm Su Tc Cm Km
	Cm	16/16: Sm Su Tc Cm Km
	Km	6/16: Sm Su Tc Cm Km
		10/16: Sm Su Km

Sm: Streptomycin Su: sulfadimethoxine. Tc: tetracycline. Cm: chloramphenicol. Km: kanamycin.

* *Escherichia coli* C was used as recipient.

** Originated from a transformant with (Sm Su Tc Cm Km)-resistance.

resistances are mediated by non-conjugative R plasmids.

To confirm this hypothesis, we tried to demonstrate R plasmid deoxyribonucleic acid (DNA) from these strains. For this experiment, one strains ZN-3 with (Sm Su Tc Cm Km)-resistance was employed. Plasmid DNA was isolated and purified by the cleared lysate and ethidium bromide-caesium chloride density gradient centrifugation method [8]. Then, transformation experi-

ment was performed by the method of Cohen et al. [1] with an rifampicin-resistant mutant of *E. coli* C (F⁻ restriction deficient) as the recipient. As shown in Table 2, a total of 28 transformants were obtained with different initial selections and all transformants possessed (Sm Su Tc Cm Km)-resistance. However, it was found that these resistances were not transmissible by conjugation. One of such transformants obtained was selected, and isolation of plasmid DNA was again carried out. In the second transformation with this DNA, transformants showing (Sm Su Km)-, or (Sm Su)-resistance, but not (Tc Cm)-resistance, were obtained when Sm and Km were used as the selecting drugs (Table 2). However, the resistances of these transformants were not transmissible by conjugation.

To determine the molecular weights of plasmid DNAs from these transformants, agarose gel electrophoresis was performed by the method of Meyers et al. [2]. For this experiment, plasmid DNAs isolated from transformant *E. coli* χ 490 (*recA*⁻) were supplied, because polymerized DNAs such as dimerous and trimerous were frequently produced when *E. coli* C (*recA*⁺) was used as host bacteria. By comparing their respec-

Fig. 1. Agarose gel electrophoresis of plasmid DNAs from transformant *Escherichia coli* λ 490.

From right to left: *E. coli* λ 490 with (Sm Su Tc Km)-, *E. coli* λ 490 with (Sm Su Km)-, and *E. coli* λ 490 with (Sm Su)-resistance. Numbers indicate sizes (in megadaltons) of bands.

tive migrations rates in 0.7% agarose, the molecular weights of plasmid DNAs from transformants with (Sm Su Tc Cm Km)-, (Sm Su Km)- and (Sm Su)-resistance were estimated to be about 15, 13 and 11 megadaltons, respectively (Fig. 1). From these results, it was concluded that (Sm Su Tc Cm Km)-resistance detected in *B. bronchiseptica* was mediated by a non-conjugative R plasmid of 15 megadaltons, and that R plasmids with (Sm Su Km)- and (Sm Su)-resistance, which were obtained in the second transformation experiments, were segregants from the non-conjugative R plasmid with (Sm Su Tc Cm Km)-resistance.

As examined so far, a total of 6 kinds of drug resistance patterns, (Sm Su Tc Cm Km), (Sm Su Ap Hg), (Sm Su Ap), (Sm Su), (Sm) and (Su), have been detected in naturally occurring *B. bronchiseptica* isolated from pigs. Among them, (Sm Su Ap Hg)- and (Sm Su Ap)-resistance were mediated by

the conjugative R plasmids [4, 5, 7]. In contrast, (Sm Su)-resistance is mediated by non-conjugative R plasmids [7]. It was also reported that at least (Su)-resistance might be mediated by non-conjugative R plasmid, because this resistance can be eliminated at a very high frequency from host bacteria [7]. The results reported here also revealed that (Sm Su Tc Cm Km)-resistance is mediated by non-conjugative R plasmids. It can be concluded from these findings that nearly all drug resistance detected in *B. bronchiseptica* isolated from pigs is plasmid borne. It is indicated that it must be very careful to select a drug for the treatment of *B. bronchiseptica* infection.

References

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要 約

豚の *Bordetella bronchiseptica* 由来非伝達性 5 剤耐性 R プラスミドについて (短報): 寺門誠致・荒木誠一*・森 康之・関崎 勉・橋本和典 (家畜衛生試験場, *エーザイ株式会社)——子豚の肺病巣から 5 剤 (ストレプトマイシン・サルファ剤・テトラサイクリン・クロラムフェニコール・カナマイシン) 耐性の *B. bronchiseptica* が分離された. 混合培養法により該耐性の伝達性を検討したが, 耐性伝達は認められなかった. しかし, プラスミド DNA の直接分離と形質転換実験成績から, 該耐性は分子量 15 メガダルトンの非伝達性 R プラスミドにより支配されていることが明らかにされた.