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Amycolamicin: A Novel Antibiotic from *Amycolatopsis* sp.

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ABSTRACT

Background: In the course of our screening for antibacterial agents, a novel antibiotic, amycolamicin (AM) was found in a culture broth of an actinomycete strain MK575-fF5 identified as *Amycolatopsis* sp.

Methods: AM was isolated by use of the Diaion HP20 and silicagel column chromatographies and HPLC. The structure of AM was determined by the spectroscopic and degradation studies. The antibacterial activities of AM were measured by the agar-dilution method.

Results: AM ($C_{44}H_{80}Cl_2N_2O_{14}$, MW 938) showed the strong antibacterial activity against not only Gram-positive bacteria such as MRSA, VRE and PRSP but also some Gram-negative bacteria such as BLNAR *H. influenzae*. The MIC_{90}s (range) of AM were 0.39 (0.1-0.39) μg/ml for MSSA (n=12), 0.39 (0.1-0.39) μg/ml for MRSA (n=10), 0.2 and 0.78 μg/ml for VSE (n=2), 0.2-0.78 μg/ml for VRE (n=4), 0.39 (0.1-0.39) μg/ml for PSSP/PRSP/MRSP (n=15), and 0.78 (0.2-0.78) μg/ml for BLNAS/BLNAR *H. influenzae* (n=11), respectively. The macromolecular synthesis experiment and the enzyme assay showed that AM inhibits the DNA biosynthesis. Moreover, the cross-resistance experiments showed that the mode of action of AM was different from those of novobiocin and levofloxacin. In the septic model mice of *S. aureus* infection (n=5), AM showed a good therapeutic efficacy at 50 mg/kg by subcutaneous administration. AM showed no acute toxicity in mice even at 250 mg/kg by subcutaneous injection and also no genotoxicity in Ames test.

Conclusion: AM was isolated from the culture broth of *Amycolatopsis* sp. AM was found to possess the strong antibacterial activity against a wide range of Gram-positive bacteria such as MRSA, VRE and PRSP and some Gram-negative bacteria such as BLNAR *H. influenzae*. AM showed the good antibacterial efficacy and safety profiles in mice. The PK/PD study and the detailed mode of action on AM are now in progress.

Isolation and Structure of Amycolamicin

***Amycolatopsis* sp. MK575-fF5**
production med. 30 L

Myc.

ext. with MeOH concd.

Hexane

ppt

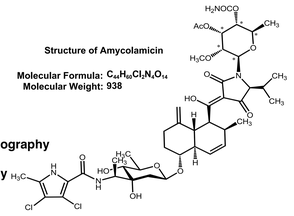
Sephadex LH20 column chromatography (MeOH)

Silicagel column chromatography ($CHCl_3$-MeOH)

Amycolamicin (2 g)

Structure of Amycolamicin

Molecular Formula: $C_{44}H_{80}Cl_2N_2O_{14}$
Molecular Weight: 938



MICs of Amycolamicin against Gram-positive and Negative Bacteria

Gram-positive bacteria	MIC (mg/L)	Gram-negaitive bacteria	MIC (mg/L)
Staphylococcus aureus FDA209P	0.39	*Haemophilus infulenzae* IID938(BLNAS)	0.78
S. aureus Smith	0.39	*H. influenzae* ME587(BLNAR)	0.78
S. aureus MS9610	0.39	*H. influenzae* ME870(BLNAR)	0.78
S. aureus MRSA No.5	0.39	*H. influenzae* ARD476(BLPAR)	0.78
S. aureus MRSA No.17	0.39	*Escherichia coli* K-12	12.5
S. aureus MS16526(MRSA)	0.39	*E. coli* NIHJ	12.5
S. aureus TY-04282(MRSA)	0.39	*E. coli* BE1211	12.5
Enterococcus faecalis JCM 5803	0.2	*Shigella dysenteriae* J S11910	25
Ent. faecalis NCTC12201(VRE)	0.1	*Sh. flexneri* 4b J S11811	12.5
Ent. faecalis NCTC12203(VRE)	0.2	*Sh. sonnei* J S11746	100
Ent. faecium JCM 5804	0.78	*Salmonella enteritidis* 1891	25
Ent. faecium NCTC12202(VRE)	0.78	*Serratia marcescens*	50
Ent. faecium NCTC12204(VRE)	0.78	*Proteus vulgaris* OX-9	25
Streptococcus pneumoniae S-223	0.39	*Pseudomonas aeruginosa* A3	25
S. pneumoniae CR-2 (PRSP)	0.39	*Ps. aeruginosa* GN315 (MDR)	25
S. pneumoniae T037 (PRSP)	0.39	*Klebsiella pneumoniae* PCI602	6.25
Mycobacterium smegmatis ATCC607*	> 100	*Candida albicans* 3147	> 100

MATERIALS AND METHODS

MIC : MICs were determined by the standard agar dilution method recommended by Clinical and Laboratory Standards Institute. *S. aureus, E. faecalis, E. faecium* and *E. coli* were incubated in Mueller Hinton agar at 37°C for 18 hours. *M. smegmatis* ATCC607 was incubated in Mueller Hinton agar supplemented with 1% glycerol at 37°C for 42 hours. *S. pneumoniae* was incubated in Mueller Hinton agar supplemented with 5% horse blood at 37°C for 18 hours under 5% CO_2 atmospheric condition. *H. influenzae* was incubated in Mueller Hinton agar supplemented with 5% Fildes Enrichment at 37°C for 18 hours under 5% CO_2 atmospheric condition.

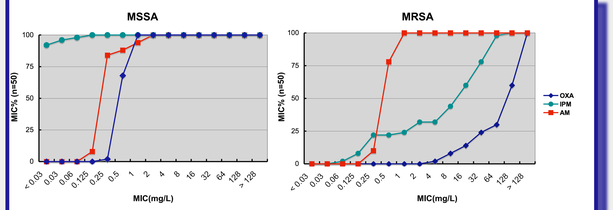
MICs of Amycolamicin against *S. pneumoniae* and *H. influenzae*

species	strain	type	MIC(mg/L) AM	OXA	AZM
Streptococcus pneumoniae	S-223	PSSP	**0.5**	<0.03	0.25
S. pneumoniae	TY-5834	PSSP	**0.25**	<0.03	0.25
S. pneumoniae	TY-5708	PRSP -	**0.25**	8	8
S. pneumoniae	TY-5745	PRSP -	**0.25**	8	8
S. pneumoniae	TY-5840	PRSP -	**0.25**	8	4
S. pneumoniae	CR-2	PRSP *ermB*	**0.5**	16	>64
S. pneumoniae	T037	PRSP -	**0.5**	8	4
S. pneumoniae	H69	PRSP *ermB*	**0.5**	1	>64
S. pneumoniae	KM99	PRSP *ermB*	**0.5**	1	>64
S. pneumoniae	JPS326	PRSP *ermB*	**0.5**	8	>64
S. pneumoniae	JPS344	PRSP *ermB*	**0.5**	16	>64
S. pneumoniae	JPS380	PRSP *ermB*	**0.5**	8	>64
S. pneumoniae	JPS399	PRSP *ermB*	**0.5**	8	>64
S. pneumoniae	JPS408	PRSP *ermB*	**0.5**	4	>64
S. pneumoniae	JPS317	PRSP *metA*	**0.5**	8	2
S. pneumoniae	JPS390	PRSP *metA*	**0.5**	8	4
S. pneumoniae	JPS401	PRSP *metA*	**0.5**	8	2
S. pneumoniae	JPS402	PRSP *metA*	**0.25**	4	1
S. pneumoniae	JPS403	PRSP *metA*	**0.5**	4	2
S. pneumoniae	ATCC700904	PRSP *erm*	**0.5**	16	>64
S. pneumoniae	ATCC700906	PRSP *erm*	**0.5**	4	>64

species	strain	type	MIC(mg/L) AM	OXA	AZM
Haemophilus influenzae	IID938		**1**	16	2
H. influenzae	IID985		**0.5**	8	2
H. influenzae	IID986		**1**	4	2
H. influenzae	IID987		**2**	32	4
H. influenzae	IID988		**1**	16	2
H. influenzae	T196		**0.5**	8	2
H. influenzae	ME570	BLNAR	**0.5**	>128	2
H. influenzae	ME587	BLNAR	**0.5**	16	0.5
H. influenzae	ME870	BLNAR	**0.5**	128	2
H. influenzae	MT066	BLNAR	**1**	>128	2
H. influenzae	ARD476	BLPAR	**1**	>128	2
Escherichia coli	*K-12*		**32**	128	16
Klebsiella pneumoniae	PCI 602		**4**	16	2
K. pneumoniae	-22#3038		**16**	>128	64

AM: amycolamicin, OXA: oxacillin, AZM: azithromycin -: not determined

MIC Distribution Curves of Amycolamicin against *S. aureus*



	MIC (mg/L) Amycolamicin Range	MIC_{50}	MIC_{90}	Imipenem Range	MIC_{50}	MIC_{90}	Oxacillin Range	MIC_{50}	MIC_{90}
MSSA (n=52)	**0.125 -2**	**0.5**	**1**	<0.03-0.125	<0.03	<0.03	0.25-1	0.5	1
MRSA (n=50)	**0.125 -1**	**0.5**	**1**	0.06->128	16	64	4-128	128	>128

The *in vitro* bactericidal activities of AM are compared with those of IMP and OXA against MRSA (50 clinical isolates).
MICs of MRSA were determined by using Muller-Hinton agar at 37°C for 18 hrs.
AM: amycolamicin, IPM: imipenen, OXA: oxacillin

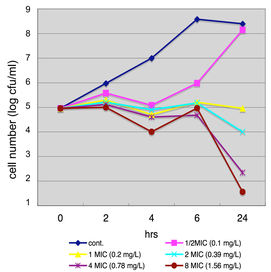
MICs of Amycolamicin against Enteric Bacteria

test organism	MIC (mg/L) AM	AZM	TOB
Bacteroides distasonis JCM 5825	**1.56**	3.13	>100
B. fragilis JCM 11019	**12.5**	3.13	>100
B. merdae JCM 9497	**0.20**	12.5	>100
B. ovatus JCM 5824	**25**	3.13	>100
B. thetaiotaomicron JCM 5827	**25**	6.25	>100
B. uniformis JCM 5828	**0.78**	3.13	>100
B. vulgatus JCM5826	**1.56**	6.25	>100
Bifidobacterium adolescentis JCM 1251	**6.25**	0.78	>100
Bif. angulatum JCM 7096	**6.25**	0.10	>100
Bif. bifidum JCM 1209	**25**	0.10	>100
Bif. breve JCM 1192	**>100**	0.20	100
Bif. infantis JCM 1222	**6.25**	1.56	100
Lactobacillus acidophilus JCM 1132	**12.5**	0.39	12.5
L. fermentum JCM 1173	**3.13**	0.39	3.13
L. gasseri JCM 1131	**6.25**	0.39	6.25
L. plantarum JCM 1149	**6.25**	0.78	6.25
Porphyromonas gingivalis JCM8525	**0.78**	0.39	>100
Escherichia coli NIHJ	**6.25**	3.13	3.13
E. coli K-12	**25**	6.25	12.5

AM: amycolamicin, AZM: azithromycin, TOB: tobramycin

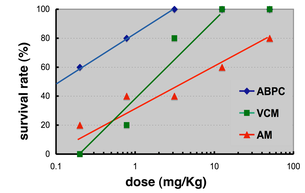
GAM agar (Nissui Seiyaku Co., Ltd, Tokyo, Japan) for anaerobic bacteria incubated at 37°C for 18 h. Anaerobic bacteria was incubated in an anaerobic condition by AnaeroPak chamber (Mitsubishi Gas Chemical Co., Japan).

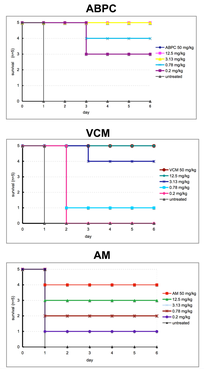
Bactericidal Activity against *S. aureus* Smith



Therapeutic Efficacy against *S. aureus* in the Murine Septicemia Model

	ED_{50} (mg/kg) Amycolamicin (AM)	Ampicillin (ABPC)	Vancomycin (VCM)
S. aureus Smith	**3.82**	0.10	1.58





Animal model: Mice (4 weeks old female ICR Swiss mice, n=5) were inoculated intraperitoneally (i.p.) with approximately 5 x 10^5 CFU of *S. aureus* Smith suspended in 5% hog gastric mucin. After 2 hours, groups of five mice were treated with a dose of compound by subcutaneous injection (s.c.). The lethal effects of infection were checked every 24 hours for 7 days. The 50% effective dose (ED_{50}) was estimated graphically by plotting survival versus concentrations.
ABPC: ampicillin, VCM: vancomycin, AM: amycolamicin

Toxicological Profile

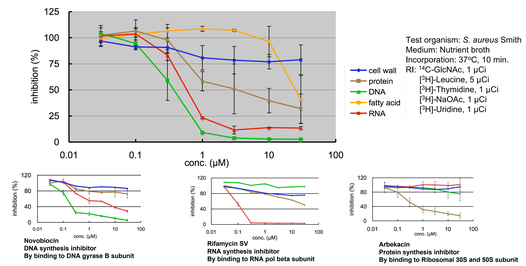
1. Single dose toxicity
The lethal dose was estimated to be more than 250 mg/kg in mice by subcutaneous injection.

2. Genotoxicity
The Ames test revealed that Amycolamicin has no genetic-mutation.

3. Cytotoxicity
Amycolamicin showed no cytotoxicity at 10 μg/ml in cultured tumour cell lines.

Mode of Action

Inhibition of macromolecular biosynthesis of Amycolamicin against *S. aureus*



Inhibitory activities of amycolamicin against DNA topoisomerases

	IC_{50} (ng/ml) DNA Gyrase	bacterial Topo IV	human Topo II
Amycolamicin	**12.2**	**6250**	**>25000**
Levofloxacin	97.6	1562	>25000
Novobiocin	97.6	>12500	NT
Coumermycin	6.1	6250	NT
Etoposide	>8000	NT	3130

Antibacterial activities of amycolamicin, novobiocin, evofloxacin and coumermycin against their *in vitro* acquired resistants

organisms	strain	MIC (mg/L) AM	NB	LVFX	CM
Staphylococcus aureus	**Smith**	**0.1**	**0.2**	**0.025**	**0.003**
S. aureus	**Smith AM^r**	**3.12**	**6.25**	**0.2**	**6.25**
S. aureus	**FDA209P**	**0.39**	**0.2**	**0.1**	**0.006**
S. aureus	**FDA209P NB^r**	**0.78**	**100**	**0.2**	**6.25**
S. aureus	**MRSA1 LVFX^r**	**0.2**	**0.2**	**50**	**0.003**

Broth dilution assay: MH broth, 37°C, 18 hrs
AM: Amycolamicin, NB: Novobiocin, LVFX: Levofloxacin, CM: coumermycin
AM^r: Amycolamicin resistant, NB^r: Novobiocin resistant, LVFX^r: Levofloxacin resistant

CONCLUSIONS

1. **Amycolamicin is a novel antibiotic with a unique structure isolated from Actinomycete.**
2. **Amycolamicin has excellent anti-bacterial activities against Gram-positive bacteria including MRSA, VRE and PRSP. Amycolamicin also has potent anti-bacterial activities against some Gram-negative bacteria including BLNAR.**
3. **Amycolamicin shows a good therapeutic efficacy against *S. aureus* in the murine septicemia model.**
4. **Amycolamicin has no acute toxicity in mice and also no genotoxicity by Ames test.**
5. **Amycolamicin seems to inhibit DNA biosynthesis of *S. aureus*. The mode of action of Amycolamicin is different from those of novobiocin and levofloxacin.**