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Original article

Characteristics of antibiotic resistance and sequence type of *Acinetobacter baumannii* clinical isolates in Japan and the antibacterial activity of DS-8587

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ABSTRACT

DS-8587 is a novel broad-spectrum fluoroquinolone with extended antimicrobial activity against both Gram-positive and Gram-negative pathogens. In this study, we evaluated the antibacterial activity and mechanism of DS-8587 in 31 quinolone-resistant *Acinetobacter baumannii* clinical isolates. Efflux pump and *qnr* genes, mutations in quinolone resistance-determining regions of target enzymes, and sequence types determined by multilocus sequence typing were analyzed. Forty-two quinolone-susceptible clinical isolates were analyzed for comparison. For susceptibility testing, DS-8587 exhibited more effective antibacterial activity when compared with ciprofloxacin and levofloxacin. When combined with the efflux pump inhibitor 1-(1-napthylmethyl)-piperazine, the MIC of DS-8587 was less affected when compared with the MIC exhibited by combined ciprofloxacin and 1-(1-napthylmethyl)-piperazine. The efflux pump genes *adeA*/*adeB*/*adeC* and regulatory elements *adeR*/*adeS* were detected in 23 of 31 quinolone-resistant isolates. The *qnrA*/*qnrB*/*qnrS* genes were not detected in any *A. baumannii* isolates analyzed. Mutations in quinolone resistance-determining regions were observed in all 31 quinolone-resistant isolates. Multilocus sequence typing analyses revealed that 22 of 31 quinolone-resistant isolates belonged to ST-2, corresponding to international clonal lineage II. In conclusion, DS-8587 exhibits potent antibacterial activity against quinolone-resistant *A. baumannii* isolates that harbor mutations in quinolone resistance-determining regions. In the presence of the efflux pump inhibitor 1-(1-napthylmethyl)-piperazine, no significant changes were observed in the MIC for DS-8587. DS-8587 should be considered as a treatment option for *A. baumannii* including ST-2 strains that are predominant among the quinolone-resistant *A. baumannii* isolates found in Japan.

1. Introduction

Acinetobacter baumannii, a Gram-negative coccobacillus, is an increasingly important nosocomial pathogen that causes bloodstream, urinary tract, skin and soft tissue infections, and pneumonia [1]. Recently, *A. baumannii* infections have caused serious clinical problems worldwide, mainly because of emerging *A. baumannii* strains that are resistant to commonly prescribed antibiotics [2].

Quinolones, especially ciprofloxacin and levofloxacin, have previously demonstrated efficacy against *A. baumannii* in clinical settings. However, a steady increase in quinolone resistance has been observed over the last decade, and quinolone resistance reached 54.8% in 2009 [3]. Quinolone resistance mechanisms reported in *A. baumannii* isolates include mutations in target bacterial enzymes, efflux pumps, and plasmid-mediated quinolone resistance (Qnr) proteins [4,5]. The most commonly identified quinolone resistance mechanisms are mutations in the target bacterial enzymes DNA gyrase and topoisomerase IV. Efflux pumps such as AdeABC, AdeIJK, AdeFGH, and AbeM also increase the quinolone MIC in *A. baumannii* [6]. Although isolates that harbor plasmid-mediated expression of *qnr* genes are still rare, *qnrA*-producing *A. baumannii* has been reported in Algerian hospitals [7].

A. baumannii clones responsible for major outbreaks belong to three lineages designated international clonal lineage I, II, and III [8]. Sequence type 2 (ST-2), corresponding to international clonal lineage II, was recently found responsible for outbreaks in China, Italy,

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Table 1
Primers used in this study.

Target gene	Primer	Sequence (5′–3′)
For detection of *adeS*/*adeR*/adeA/*adeB*/*adeC* and *adeL*/*adeF*/*adeG*/*adeH* genes		
adeS	Ab_AdeS_F	TAGTCACGGCGACCTCTCTGCT
	Ab_AdeS_R	AATGCCGGGGCCTTCATCCT
adeR	Ab_AdeR_F	CGCATAGGTGCAGATGACTTTGTGGTGA
	Ab_AdeR_R	CGCTCTAGTGCATCGCTATCATTCATGCAG
adeA	Ab_AdeA_F	TACGGCGGAAATCCGTCCGCAAGT
	Ab_AdeA_R	CCAATACGCCCAGAAATAGGCGCTCGAA
adeB	Ab_AdeB_F	GCGACAACAGATACCTCCGGTACA
	Ab_AdeB_R	TCGGGTCGACCCAAATACGCATAG
adeC	Ab_AdeC_F	CCGTGATTTACGGACTGCTACGCT
	Ab_AdeC_R	CGCTGAGCGTCTAATCGTTCACCA
adeL	Ab_adeL-F	CCACAAAGTCCCAATCGAAGTTGCGT
	Ab_adeL-R	GCCAAGAGGTCAGCTTCGTATTGATGTG
adeF	Ab_adeF-F	CCTCGTCCTTTTGAAGCAGAACTGAACC
	Ab_adeF-R	TGTAACAGGGTCACCCGGACGAA
adeG	Ab_adeG-F	CAGGTGTCTGGTTGACAGCTGGAGA
	Ab_adeG-R	GAACGTAAAAGGCCGGGGTGAGGA
adeH	Ab_adeH-F	GCAGCTCAAGCTGAACGCTTACC
	Ab_adeH-R	TGTTCTGCACTGGCTCTGTTACGAG
For sequencing analysis of *gyrA*, *parC* and *parE* genes		
gyrA	Ab GyrA-1	AGGAGTACATATGAGCGTATCGGAAATCCG
	Ab GyrA-4	GCAATACCCGCAGCACCGTTAATTAACA
parC	Ab ParC-1	TAAGCTGCATATGACCAGCCTTGCGC
	Ab ParC-4	CCATCAAAGTTATCTTGCCATTCGC
parE	Ab ParE-F1	CTCTTTATTGTTGAGGGTGACTCTG
	Ab ParE-R1	ACGTAGTTGAATTGCGTTCATCTC

Spain, Germany, and Latvia [9–12]. International clonal lineage II is also the common lineage among carbapenem-non-susceptible *A. baumannii* identified in Japan [13,14]. However, the predominant lineage of quinolone-resistant *A. baumannii* remains unclear.

DS-8587 is a novel broad-spectrum fluoroquinolone that was designed and synthesized by Daiichi Sankyo Co., Ltd. to potentiate antibacterial activity against Gram-positive and Gram-negative pathogens [15]. In this study, we measured the antibacterial activity of DS-8587 against quinolone-resistant clinical isolates with or without the efflux inhibitor 1-(1-napthylmethyl)-piperazine (NMP). In addition, we investigated the distribution of efflux pump genes, *qnr* genes, mutations in quinolone resistance-determining regions (QRDRs) of target enzymes, and the sequence types (STs) of clinical isolates tested to better understand the factors underlying the potent antibacterial activity exhibited by DS-8587 on quinolone-resistant *A. baumannii* isolates.

2. Materials and methods

2.1. Bacterial strains

A total of 73 non-duplicate *A. baumannii* clinical isolates, including 42 quinolone-susceptible and 31 quinolone-resistant (MIC for ciprofloxacin: $\geq$ 4 mg/l) strains, were collected by the LVFX Surveillance Group in Japan in 2010 [16]. Isolates were confirmed as *A. baumannii* by detection of the $bla_{\text{oxa-51-like}}$ gene [17] and sequencing of the 16S-23S ribosomal RNA intergenic spacer region [18].

2.2. Antimicrobial agents and susceptibility testing

DS-8587 and levofloxacin were synthesized at Daiichi Sankyo Co., Ltd (Tokyo, Japan). Ciprofloxacin, tigecycline, and imipenem were purchased from LKT Laboratories, Inc. (St. Paul, MN, USA). Amikacin and NMP were purchased from Sigma–Aldrich (St. Louis,

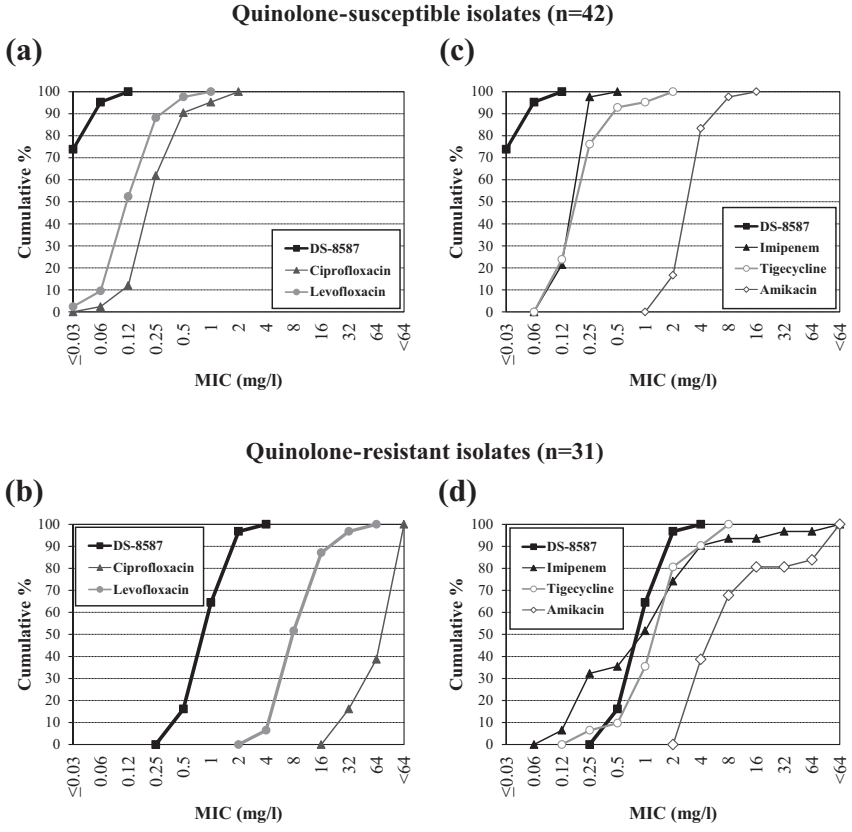

Fig. 1. Cumulative distribution of MICs against 42 quinolone-susceptible [(a) and (c)] or 31 quinolone-resistant [(b) and (d)] *A. baumannii* clinical isolates from Japan. (a) and (b) Closed square: DS-8587; closed triangle: ciprofloxacin; closed circle: levofloxacin. (c) and (d) Closed square: DS-8587; closed triangle: imipenem; open circle: tigecycline; open diamond: amikacin.