A Contribution of MdfA to Resistance to Fluoroquinolones in Shigella flexneri

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Abstract

In this study, we measured the drug resistance conferred by mdfA mutations in two Shigella flexneri strains. A mutant in mdfA genes was constructed by polymerase chain reaction–based, one-step inactivation of chromosomal genes. The antimicrobial susceptibility of parent and mutant strains to fluoroquinolones was determined by minimal inhibitory concentration (MICs). The ΔmdfA mutants were somewhat more susceptible to fluoroquinolones than the parent strains. The low level changes in MICs of the ΔmdfA mutants suggest that mdfA contributed the fluoroquinolone resistance in S flexneri. This finding found that the increased expression level of an MdfA efflux pump mediated fluoroquinolone resistance, but it is not likely a major effector of higher resistance levels.

1. Introduction

In the recent paper in the resistance to fluoroquinolones by the combination of target site mutations and enhanced expression of genes for efflux pumps in Shigella flexneri [1], we presented that MdfA could be related to fluoroquinolone resistance. The most common example of an major facilitator superfamily (MFS) antibiotic efflux system in gram-negative bacteria is that encoded by the various tet genes associated with tetracycline efflux and resistance [2]. Members of this family of efflux fluoroquinolones are, by contrast, rare in gram-negative bacteria and include only the MdfA transporter of Escherichia coli [3,4]. Therefore, we would like to report the additional evidences that the MdfA is related to fluoroquinolone resistance in S flexneri, even though MdfA does appear to be a more effective pump for nonantibiotics. However, the increased expression level of the MdfA efflux pump mediating fluoroquinolone resistance was first confirmed in the Shigella species strains in this study [1,2]. This study demonstrated that resistance to fluoroquinolone is due to efflux by the MdfA system in the Shigella species. To determine the molecular basis of efflux in the Shigella species, a deletion mutation in mdfA was constructed.
2. Methods

Deletion of the $mfdA$ gene was performed by the method described by Datsenko and Wanner [5]. The kanamycin resistance gene $kan$ flanked by flippase (FLP) recognition target sites was amplified by a standard polymerase chain reaction (PCR) with the templated plasmid pKD4 and hybrid primers. These primers, P1MdfA (AGCTGCGCTTTATTAAACTCTGCGCGATTA TTATTGGCGAAGAAATTGCGTGTA GGCTGGAGCTGCTTC) and P2MdfA (TCACCATT AATTCGAGAATGCCTGATCGCACAAATCAATCA GGCATTTTTATGGGAATTAGCCATGGTCC), were synthesized with 20 nucleotides (nt) of priming sites 1 and 2 of pKD4 and with 50 nt of the 5' and 3' ends of the $mfdA$ gene. The 1.6 kb PCR fragment was purified and electroporated into $S$ flexneri isolates, 021787 and 021895, into which the red recombinase expression plasmid pKD46 was introduced. Transformants were selected at 37°C on Luria–Bertani (LB) agar medium containing kanamycin at 50 ug/ml. Homologous recombination between the genomic DNA and the PCR product resulted in the deletion of the $mfdA$ sequence from nt 50 to 1327 (1,377-bp deletion) and its replacement with the $kan$ gene. This was confirmed by two different PCRs. Deletion of $mfdA$ in the transformants was first shown by PCR with primers MdfA3 (GCTGCGCTTTATTAAACTCTG) and MdfA4 (CCTGATCGCACAAATCATCA GGCATTATTATGGAATTAGCCATGGTCC), which correspond to sequences flanking the $mfdA$ deletion and that resulted in a 1,227-bp fragment for the parental strains and a negative result when $mfdA$ was deleted and replaced by the $kan$ gene flanked by FLP. The third control PCR, with primers k2 (CGGTGCCCTGAATGAACTGC) and k (CGGCACAGTCGATGAAATTCG), was used to detect the 471-bp $kan$ fragment.

3. Results and Discussion

The effect of MdfA was confirmed by inactivating the $mfdA$ gene located at different chromosomal loci in the strains studied. The antimicrobial susceptibilities of the parent and mutant strains are presented in Table 1. Both parent strains were resistant to ciprofloxacin (CIP) at MICs of 16 ug/ml and to NOR at MIC of 32 and 8 ug/ml, respectively. SF021787 and SF021895 were resistant to CIP at MICs of 12 ug/ml and to norprofloxacin (NOR) at MICs of 4 ug/ml regardless of the types of GyrA mutations, which suggests that without a functional MdfA. Compared MICS of the parent with $\Delta mfdA$ mutant strains, $\Delta mfdA$ mutant strain was more susceptible to CIP and NOR.

Interestingly, the resistance level to fluoroquinolone in the mutant strains harboring the $\Delta mfdA$ deletion was the same whether the strains carried three or four mutations in gyrA, it because, other efflux system, acrAB and ndeh contributed to resistance against fluoroquinolone.

The low-level changes in MICs of the $\Delta mfdA$ mutant suggest that $mfdA$ contributed to the fluoroquinolone resistance in $S$ flexneri, but it is not likely to be a major effector for higher resistance levels.

Acknowledgement

This study was supported by an intramural grant from the Korea National Institute of Health (2008-N00392-00).

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| Strains | Substitution in gyrA | MICs (ug/ml) |
|---------|----------------------|--------------|
|         | parC QRDR            | CIP | CIP + CCCP | NOR | NOR + CCCP |
| SF021787 | S83L, S80I, R91Q     | 16  | 0.125      | 32  | 0.25       |
| SF021787ΔmfdA::kan | S83L             | 12  | 4           |     |            |
| SF021895 | S83L, D87G, S80I, R91Q | 16  | 0.125      | 8   | 0.25       |
| SF021895ΔmfdA::kan | S83L, D87G        | 12  | 4           |     |            |