Involvement of MarR and YedS in Carbapenem Resistance in a Clinical Isolate of Escherichia coli from China

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A carbapenem-resistant clinical isolate of Escherichia coli, which lacked OmpF and OmpC porins, carried a marR mutation and expressed a functional yedS, a normally nontranslated gene. MarR and YedS are described here as having effects on the ability of this strain to resist carbapenems. Additionally, expression of YedS was regulated by the small RNA MicF in a MarA-dependent way. These findings illustrate how broadly bacteria can mutate within a selective clinical setting, in this case, resistance to carbapenems, by altering three porin genes and one regulatory gene.


The marRAB operon of E. coli encodes the MarR repressor, the transcriptional regulator MarA, and a putative small protein, MarB (9). MarR represses transcription of marRAB by binding to marO and negatively controlling MarA-dependent expression of other genes in the regulon (10, 11). Upon induction by a variety of compounds (12) or by mutation of marO or marR, the repressor is rendered inactive (10). The resulting overexpression of MarA produces antibiotic resistance by increasing the expression of the major multidrug efflux pump AcrAB-TolC (13, 14) and downregulating the outer membrane protein OmpF via the small RNA (sRNA) MicF (15, 16). In this study, a carbapenem-resistant, non-carbapenemase-producing clinical isolate of E. coli from China (CH4) was investigated to determine the genetic basis for the carbapenem resistance phenotype.

PCR amplification and sequencing using the primers marR-for (5’-ATTAGCGGCGCATCGGTCAATTCAT) and marR-rev (5’-ATAGGATCCCTACGGCGTCCGATGTA) revealed numerous mutations in the marR open reading frame (ORF) of strain CH4 and other clinical isolates from China (Table 1). We cloned ORFs containing the various marR mutations, using the primers marR-clone-For and marR-clone-Rev (17), into expression vector pET-13a (18), for which expression was controlled by the T7 promoter. Expression of T7 polymerase was induced from plasmid pACT7-Spc (19) via isopropyl-β-D-thiogalactopyranoside (IPTG) in the reporter strain SPC-106, a marO-lacZ fusion that contains a ΔmarR mutation (12). Analysis of LacZ activity (11, 20) showed that the Gly42Arg mutation in the CH4 marR gene did not complement the ΔmarR mutation in this reporter strain (Fig. 1), indicating that this mutation affected the activity of MarR.

We then complemented the marR mutation in CH4 by transforming the strain with pET-marRwildtype and pACT7-Spc or pAC-MarRwt (17). MICs were determined. The data showed that the two expression vectors produced similar decreases in resistance in strain CH4 that were not seen with an empty vector con-
molecular weight marker. Each lane was loaded with 5 μg of total outer membrane protein. The order in which these mutations occurred is not known; however, the accumulation of so many mutations in a single isolate is evidence of the genetic fluidity of the bacterial cell that seeks to survive in response to the lack of functional OmpF and OmpC. Additionally, maintenance of a functional YedS in strain CH4 is an evolutionary adaptation to the presence of this carbapenem portal presents a selective pressure for the expression of the yedSCH4 gene encoding the YedSCH4 protein, which is in strain CH4 enabled a pseudogene to be expressed, and derepressed the yedS gene in strain CH4. Our findings implicate the outer membrane protein YedSCH4 in carbapenem sensitivity/resistance. We hypothesize that the maintenance of a functional YedS in strain CH4 is an evolutionary response to the lack of functional OmpF and OmpC. Additionally, the expression of this carbapenem portal presents a selective pressure for this strain to maintain its novel micF regulon. The absence of carbapenemase, selection may occur for strains containing functional micF genes. Our findings suggest how this uniquely selective environment may affect genetic fluidity of the bacterial cell that seeks to survive in response to different insults. The isolate described here has mutated two of its porins, enabling a pseudogene to be expressed, and derepressed the marRA operon, sufficient to produce a drug-resistant strain. The order in which these mutations occurred is not known; however, the accumulation of so many mutations in a single isolate is a clear display of bacterial adaptation.

**Nucleotide sequence accession number.** The sequence of the yedS gene in strain CH4 was deposited in GenBank under accession number JX392406.

**ACKNOWLEDGMENT**

We thank bioMérieux for providing the Etests used in this work.

**REFERENCES**

