Amino Acid Substitutions of CrrB Responsible for Resistance to Colistin through CrrC in *Klebsiella pneumoniae*

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Colistin is a last-resort antibiotic for treatment of carbapenem-resistant *Klebsiella pneumoniae*. A recent study indicated that missense mutations in the CrrB protein contribute to colistin resistance. In our previous study, mechanisms of colistin resistance were defined in 17 of 26 colistin-resistant *K. pneumoniae* clinical isolates. Of the remaining nine strains, eight were highly resistant to colistin. In the present study, crrAB sequences were determined for these eight strains. Six separate amino acid substitutions in CrrB (Q10L, Y31H, W140R, N141I, P151S, and S195N) were detected. Site-directed mutagenesis was used to generate crrB loci harboring individual missense mutations; introduction of the mutated genes into a susceptible strain, A4528, resulted in 64- to 1,024-fold increases in colistin MICs. These crrB mutants showed increased accumulation of H239_3062, H239_3059, pmrA, pmrC, and pmrH transcripts by quantitative reverse transcription (qRT)-PCR. Deletion of H239_3062 (but not that of H239_3059) in the A4528 crrB(N141I) strain attenuated resistance to colistin, and H239_3062 was accordingly named crrC. Similarly, accumulation of pmrA, pmrC, and pmrH transcripts induced by crrB(N141I) was significantly attenuated upon deletion of crrC. Complementation of crrC restored resistance to colistin and accumulation of pmrA, pmrC, and pmrH transcripts in a crrB(N141I) ΔcrrC strain. In conclusion, novel individual CrrB amino acid substitutions (Y31H, W140R, N141I, P151S, and S195N) were shown to be responsible for colistin resistance. We hypothesize that CrrB mutations induce CrrC expression, thereby inducing elevated expression of the pmrHFIJKLM operon and pmrC (an effect mediated via the PmrAB two-component system) and yielding increased colistin resistance.

*Klebsiella pneumoniae* causes several nosocomial and community-acquired diseases (1). Carbapenem is used to treat infections caused by *K. pneumoniae* isolates harboring extended-spectrum β-lactamases (ESBLs) (2). However, the emergence of carbapenem-resistant *K. pneumoniae* (CRKP) has become a significant problem worldwide (3, 4). Colistin is one of the few remaining last-resort antibiotics that can be used to treat CRKP infection (3). However, recent reports indicate increasing colistin resistance among clinical isolates of *K. pneumoniae* (5), with 27% of Greek *K. pneumoniae* isolates (6) and 17% of Taiwanese CRKP isolates (7) exhibiting resistance to colistin. Furthermore, our recent study reported that 31% (8/26) of Taiwanese colistin-resistant *K. pneumoniae* isolates are highly resistant to the agent, exhibiting MICs ranging from 512 to >2,048 μg/ml (8).

Colistin (also referred to as polymyxin E) is a cationic peptide that binds bacterial lipopolysaccharide (LPS) and causes cell membrane leakage (9, 10). In *K. pneumoniae*, resistance to colistin is mediated by neutralization of negatively charged LPS by modification with 4-amino-4-deoxy-L-arabinose (Ara4N) and phosphoethanolamine (PeN) (11, 12). Modifications of LPS with Ara4N and PeN are achieved by the activities of PmrHFIJKLM and PmrC, respectively (13, 14). Previous studies showed that PmrAB, PmrD, PhoPQ, and MgrB regulate (directly or indirectly) expression of the pmrHFIJKLM operon (15–20). MgrB is a transmembrane peptide that inhibits the PhoQ signaling cascade (21), and disruption of mgrB results in increased expression of the pmrHFIJKLM operon and consequent colistin resistance (18, 22, 23).

In our recent study, mutation of mgrB was the most frequently observed (14/26; 54%) mechanism of resistance to colistin in *K. pneumoniae* (8). Amino acid substitutions in PhoQ and PmrB are also responsible for colistin resistance (3/26; 12%). However, approximately one-third (8/26; 31%) of the colistin-resistant *K. pneumoniae* clinical isolates in our survey did not exhibit mutations in mgrB, phoPQ, or pmrAB (known regulators of pmrHFIJKLM) despite increased accumulation of pmrH mRNA in these strains. These data implied that a novel mechanism(s) might cause elevated resistance to colistin in these eight isolates.

A recent report speculated that a newly identified two-component system, CrrAB, may affect colistin resistance through regulation of a crrAB-adjacent gene, H239_3059, that encodes a glycosyltransferase involved in LPS modification (24). To investigate the potential role of CrrAB in resistance to colistin, we determined the sequences of crrAB in the eight clinical isolates for which a resistance mechanism had not been identified. Our results suggest a mechanism for CrrAB-mediated resistance to colistin in *K. pneumoniae*.

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TABLE 1 Capsular type, CrrB amino acid substitutions, MICs, and β-lactamases of colistin-resistant strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Capsular type</th>
<th>Amino acid at CrrB position</th>
<th>Colistin MIC (µg/ml)</th>
<th>Presence in strain</th>
<th>TEM-1</th>
<th>TEM-116</th>
<th>SHV-1a</th>
<th>SHV-31</th>
<th>CTX-M Group 2</th>
<th>CTX-M Group 9</th>
<th>DHA</th>
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<tbody>
<tr>
<td>Col4</td>
<td>K64</td>
<td>Ile</td>
<td>&gt;2.048</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
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<td></td>
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</tr>
<tr>
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<td>Ile</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
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<td>Leu</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
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<td>NA</td>
<td>NA</td>
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</table>

Notes:
- Capsular type and MICs of colistin were determined in our recent study (8).
- Positions of amino acid substitutions in CrrB.
- +, present. According to previous studies (8, 34), β-lactamase was amplified by PCR. The PCR products were sequenced, and β-lactamases were classified by NCBI Blast.
- NA, the β-lactamase(s) of strain Col28 was not available.

MATERIALS AND METHODS

Bacterial isolates and culture conditions. A total of 26 colistin-resistant K. pneumoniae isolates were collected in Taiwan, and the mechanisms responsible for the colistin resistance of 17 of the isolates were identified in our previous study (8). However, resistance mechanism(s) were not defined for the nine remaining strains (Col4, Col7, Col20, Col21, Col22, Col28, Col36, and Col44). The eight strains were isolated from different patients and distinguished by different characterisations (i.e., MICs, capsular types, and β-lactamases) (Table 1). Therefore, the eight strains were not identical. We intended to compare the transcript accumulations and crrAB sequences in these nine strains to those in eight colistin-susceptible isolates described in our previous study (8). However, one of these colistin-resistant isolates (Col5) and four of the control strains lacked crrA and crrB sequences that could be amplified using our PCR amplification technique and detected by Southern blotting (see Fig. S1 in the supplemental material). Therefore, only the eight crrAB-containing colistin-resistant isolates (Col5, Col7, Col20, Col21, Col22, Col28, Col36, and Col44) and the four crrAB-containing colistin-resistant strains (A4528, reference strain 64, N4252, and N5906) were used in the present study. Escherichia coli DH10B was used for cloning and molecular manipulations. Both K. pneumoniae and E. coli were grown in Luria-Bertani broth supplemented (as appropriate) with 50 µg/ml kanamycin, 100 µg/ml ampicillin, or 100 µg/ml chloramphenicol.

Determination of susceptibility to antibiotics. The MICs of colistin were determined by agar dilution according to the CLSI procedures. Specifically, aliquots of K. pneumoniae (1 × 10^5 CFU) were spotted on Mueller-Hinton agar plates (Difco) containing different concentrations of colistin. The MICs were determined after overnight incubation of the plates at 37°C. The MIC for E. coli ATCC 25922 was determined in parallel to provide a quality control.

Sequence analysis of amino acid substitutions in CrrAB. The crrA and crrB genes of each strain were amplified by PCR using the primers Tupa-F and H236-2575-R; the resulting genes were subjected to nucleotide sequencing using primers CrrAB-seqF1, Tupa-F, and H236-2575-R. ( Primer sequences are provided in Table S1 in the supplemental material.) The crrAB sequences from the colistin-resistant strains were compared to those from our four colistin-susceptible isolates and 46 colistin-susceptible strains deposited in GenBank (see Table S2 in the supplemental material) to identify CrrAB amino acid substitutions, excluding polymorphisms observed in the colistin-susceptible strains.

Site-directed mutagenesis of crrB. crrB DNA fragments incorporating the relevant individual missense mutations were created by fusion PCR. For instance, distinct halves of crrB (Q101L) (encoding CrrB carrying the Q101L substitution) were PCR amplified (from A4528 genomic DNA) using primer pairs CrrB-ST-F plus Q101L-R and CrrB-ST-F plus Q101L-R. The two resulting fragments were then merged by overlap PCR using the flanking primers (CrrB-ST-F and CrrB-ST-R). An equivalent procedure was repeated with allele-specific primers to generate crrB loci encoding proteins carrying (separately) the Q101L, Y31H, W140R, N141I, P151S, or S195N substitution. All the primer sequences for PCR are listed in Table S1 in the supplemental material. Each of the resulting crrB loci was cloned into a blunt NotI-digested pKO3-km plasmid (25). The resulting pKO3-km-derived plasmids were transformed (separately) into the A4528 strain by electroporation. The point mutants were generated after replacement of crrB using a previously described method (26). The presence of the mutated locus in each of the resulting strains was confirmed by amplification and sequencing from genomic DNA.

Genetic manipulations for gene deletion and complementation. The resulting fragments were then removed by inverse PCR with primer pair H239_3095-inverse-F and H239_3095-inverse-R for H239_3095, H239_3062-flank-F and H239_3062-flank-R for H239_3062, and pmrAB-flank-F and pmrAB-flank-R for pmrAB. The resulting products were cloned (separately) into the pJET1.2 plasmid (Thermo Scientific). The coding regions of the respective open reading frames (ORFs) were then removed by inverse PCR with primer pair H239_3095-inverse-F and H239_3095-inverse-R for H239_3095, H239_3062-inverse-F and H239_3062-inverse-R for H239_3062, and pmrAB-inverse-F and pmrAB-inverse-R for pmrAB. The fragments with the ORF deleted were amplified by PCR with the flanking primer pairs indicated above and subcloned (separately) into a blunt NotI-digested pKO3-km plasmid. The primer sequences for genetic manipulations are listed in Table S1 in the supplemental material. The resulting pKO3-km-derived plasmids were transformed (separately) into the A4528 strain by electroporation to generate the deletion mutants, using a previously described method (26). The H239_3062 locus (including both the ORF and flanking sequences) was amplified from A4528 by PCR with primer pair H239_3062-com-F and H239_3062-com-R, and the resulting fragment was cloned into EcoRV-digested pACYC184 (27). The resulting plasmid (pACYC184-3062) was transformed into the A4528 crrB(N141I) strain by electroporation to generate the deletion mutants, using a previously described method (26). The H239_3062 locus (including both the ORF and flanking sequences) was amplified from A4528 by PCR with primer pair H239_3062-com-F and H239_3062-com-R, and the resulting fragment was cloned into EcoRV-digested pACYC184-3062. The subsequent plasmid (pACYC184-3062) was transformed into the A4528 crrB(N141I) strain by electroporation to generate the deletion mutants, using a previously described method (26). The H239_3062 locus (including both the ORF and flanking sequences) was amplified from A4528 by PCR with primer pair H239_3062-com-F and H239_3062-com-R, and the resulting fragment was cloned into EcoRV-digested pACYC184. The subsequent plasmid (pACYC184) was transformed into the A4528 crrB(N141I) strain by electroporation to generate the deletion mutants, using a previously described method (26).

Determination of mRNA expression levels by qRT-PCR. Total RNA was isolated from the K. pneumoniae strains using the RNeasy minikit (Qiagen). An aliquot (400 ng) of total RNA from each strain was subjected to cDNA synthesis using SuperScript II reverse transcriptase (Invitrogen). The cDNAs of pmrD, phoP, pmrA, pmrC, H239_3059, H239_3062, and 23S rRNA (used as an internal control) were quantified using Power SYBR green master mix (Thermo Scientific) and an ABI 7900 real-time PCR system according to the manufacturers’ instructions. The sequences of the transcript-specific primers used for quantitative reverse transcription are listed in Table S1.
Amino acid substitutions of CrrB are detected in colistin-resistant isolates. A previous report indicated that mutations resulting in amino acid substitutions in CrrB cause decreased susceptibility to colistin (24). To identify possible crrAB mutations in our strains, the predicted CrrA and CrrB amino acid sequences of eight highly colistin-resistant isolates were compared with those of colistin-susceptible isolates. The results showed that the predicted CrrA amino acid sequences in all 58 strains (both colistin-resistant and colistin-susceptible isolates) were identical, whereas each of the colistin-resistant strains encoded CrrB harboring a single amino acid substitution (Table 1). A total of six separate amino acid substitutions in CrrB (Q10L, Y31H, W140R, N141I, P151S, and S195N) were detected among the eight colistin-resistant isolates examined in the present work (DDBJ accession no. LC121085 to LC121092). A previous study demonstrated that the CrrB(Q10L) substitution contributes to colistin resistance (24); the other five CrrB amino acid substitutions were novel. We therefore tested the potential contributions of these alleles to colistin resistance.

According to the predictions of the SMART tool (28, 29; http://smart.embl-heidelberg.de/), the 6 amino acid substitutions identified here were not localized to a single domain of CrrB. Amino acid positions 12 to 34 and 54 to 76 of CrrB are predicted to lie within transmembrane domains, positions 81 to 135 are predicted to constitute a HAMP (histidine kinases, adenyl cyclases, methyl-binding proteins, and phosphatases) domain, positions 136 to 200 are predicted to form a histidine kinase domain, and positions 244 to 353 are predicted to form a histidine kinase-like ATPase domain. Four of the amino acid substitutions (W140R, N141I, P151S, and S195N) detected in the present study would fall within the putative histidine kinase domain, Y31H would fall within a putative transmembrane domain, and Q10L does not appear to fall within a conserved domain.

Amino acid substitutions in CrrB contribute to reduced susceptibility to colistin. To verify whether these substitutions contributed to colistin resistance, crrB loci harboring each of the six missense mutations were constructed (by site-directed mutagenesis) and then introduced (separately) into a colistin-susceptible strain, A4528 (MIC = 1 μg/ml). Each of the six amino acid substitutions resulted in decreased susceptibility to colistin in the A4528 background (MICs = 64 to 1,024 μg/ml) (Table 2). Notably, five of these amino acid substitutions (Q10L, W140R, N141I, P151S, and S195N) resulted in high resistance to colistin (MICs = 512 to 1,024 μg/ml) in the A4528 background.

CrrB-mediated resistance to colistin is mediated through H239_3059 and H239_3062 expression. A previous study showed that amino acid substitutions in CrrB might influence the expression of the crrAB-proximal loci H239_3059 (encoding a putative glycosyltransferase) and H239_3062 (encoding a protein of unknown function) (24). We observed that mRNA levels of H239_3059 and H239_3062 were significantly increased in our eight clinical isolates compared with those in the four colistin-susceptible strains (Fig. 1A and B). Similarly, A4528-derived strains engineered to encode each of the six CrrB proteins with amino acid substitutions displayed significantly enhanced expression of H239_3059 and H239_3062 compared with the A4528 parental strain (Fig. 1C). These data demonstrated that amino acid substitutions in CrrB resulted in increased mRNA expression of H239_3059 and H239_3062, suggesting that the CrrAB two-component system is a regulator of H239_3059 and H239_3062 transcription.

CrrB mediates colistin resistance through H239_3062. To examine whether H239_3059 and H239_3062 are involved in resistance to colistin, the H239_3059 and H239_3062 loci were (separately) deleted in the A4528 crrB(N141I) background. Susceptibility to colistin of the A4528 crrB(N141I) ΔH239_3059 strain was unchanged compared to the parent. In contrast, the colistin MIC for A4528 crrB(N141I) ΔH239_3062 was decreased 512-fold compared to that for the parent, with susceptibility approaching that of A4528 itself (Table 3). H239_3062 complementation in the A4528 crrB(N141I) ΔH239_3062 strain (by introduction of an H239_3062-bearing plasmid) restored resistance to colistin (MIC > 2,048 μg/ml) (Table 3). These data indicated that CrrB(N141I)-induced resistance to colistin is mediated through H239_3062 but not through H239_3059. H239_3062 was accordingly named crrC.

CrrB-regulated expression of the pmrHFIJKL operon is mediated through crrC. Our previous study (8) showed that the eight highly colistin-resistant isolates characterized in the present work have significantly increased pmrH mRNA expression, suggesting that CrrAB regulates expression of the pmrHFIJKL operon (24). Using qRT-PCR analysis, we confirmed that pmrH transcript accumulation was elevated (compared to that in A4528) in each of the six A4528 crrB missense mutants constructed here (Fig. 2A). To verify whether the regulation of pmrHFIJKL operon expression by CrrB is mediated through CrrC, the pmrH transcript levels in A4528 crrB(N141I) with and without crrC were compared. The pmrH mRNA expression in A4528 crrB(N141I) was significantly elevated compared to that in A4528 crrC(N141I)A4528 crrB(N141I).
was significantly decreased upon deletion of crrC, and complementation of A4528 crrB(N141I) ΔcrrC with crrC restored pmrH expression to levels within 2-fold of those seen in A4528 crrB(N141I) (Fig. 2B). These data suggested that CrrB regulation of pmrHFIJKLM operon expression is mediated through crrC.

CrrC regulates pmrHFIJKLM operon and crrC expression through PmrAB. Previous work (15, 20, 30) showed that the pmrHFIJKLM operon is directly regulated by PmrAB and PhoPQ. However, a strain encoding CrrB(N141I) exhibited significantly increased mRNA levels of pmrA, but not of phoP (Fig. 3A). Therefore, we speculated that CrrC regulates pmrHFIJKLM expression via PmrAB. Consistent with this hypothesis, pmrA mRNA expression in the A4528 crrB(N141I) ΔcrrC strain was significantly decreased compared with that in A4528 crrB(N141I), and complementation of the former strain with plasmid-borne crrC restored expression of pmrA (Fig. 3B). In addition to the pmrHFIJKLM operon, PmrAB also regulated expression of pmrC (30). Consistent with expression of pmrH, pmrC mRNA expression in the A4528 crrB(N141I) ΔcrrC strain was significantly decreased compared with that in A4528 crrB(N141I), and complementation with crrC in the A4528 crrB(N141I) ΔcrrC strain could restore the expression of pmrC (Fig. 3C).

To confirm whether CrrC regulated the pmrHFIJKLM operon and pmrC directly through PmrAB, strain A4528 crrB(N141I) ΔpmrAB was constructed. The induced expression of crrC in strain A4528 crrB(N141I) was not significantly influenced after deletion of pmrAB (Fig. 3D), whereas the expression of pmrH and

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**TABLE 3** MICs of deletion and complementation of the crrC locus in strain A4528 crrB(N141I)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Colistin MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A4528 wild type</td>
<td>1</td>
</tr>
<tr>
<td>A4528 crrB(N141I)</td>
<td>1.024</td>
</tr>
<tr>
<td>A4528 crrB(N141I) ΔH239_3059</td>
<td>1.024</td>
</tr>
<tr>
<td>A4528 crrB(N141I) ΔcrrC</td>
<td>2</td>
</tr>
<tr>
<td>A4528 crrB(N141I) ΔpmrAB</td>
<td>1</td>
</tr>
<tr>
<td>A4528 crrB(N141I) ΔcrrC/pACYC184a</td>
<td>1</td>
</tr>
<tr>
<td>A4528 crrB(N141I) ΔcrrC/pACYC184-3062b</td>
<td>2.048</td>
</tr>
</tbody>
</table>

*Strain A4528 crrB(N141I) ΔcrrC was transformed with a pACYC184 plasmid.

*Strain A4528 crrB(N141I) ΔcrrC was transformed with a pACYC184-3062 plasmid, a pACYC184 plasmid harbored crrC loci.
PhoP, and PhoQ) have been reported to lead to increased resistance mechanisms that disrupt the pmrHFIJKLM operon and the pmrC locus, which are regulated by PhoPQ and PmrAB (15, 17, 30). Previous studies showed that disruption of mgrB is a major mechanism contributing to colistin resistance (18, 19, 22, 23). Additionally, mutations leading to disruptants (14/26; 54%) (8) and amino acid alterations in CrrB are the two major mechanisms involved in the histidine kinase domain. These findings suggest that amino acid substitutions located in the putative histidine kinase domain might affect the kinase activity of CrrB and therefore might increase autophosphorylation of CrrB. Moreover, the amino acid substitution in the transmembrane domain might cause conformational changes that also would result in altered CrrB activity.

A previous study (24) proposed that CrrAB contributes to colistin resistance in K. pneumoniae via changes to the LPS, with modifications effected by the H239_3059 protein (predicted to constitute a TupA-like glycosyltransferase) and by PmrHFIJKLM. However, our results demonstrated that deletion of H239_3059 in A4528 crrB(N141I1) did not attenuate colistin resistance, indicating that the H239_3059 locus is not involved in CrrB-mediated resistance to colistin in K. pneumoniae. In contrast, deletion of crrC, a proximal locus also regulated by CrrB, attenuated the colistin resistance of the A4528 crrB(N141I) strain, indicating that crrC plays an important role in CrrB-mediated resistance. Furthermore, we demonstrated that CrrC modulates the expression of PmrHFIJKLM and Pmrc, an effect mediated through the PmrAB two-component system.

Homologs of CrrC were found in many Gram-negative bacteria (i.e., E. coli, Klebsiella oxytoca, Klebsiella variicola, Enterobacter cloacae, and Shigella flexneri) and shared at least 45% identity of amino acid sequences. However, the function of CrrC remained unknown. The crrC locus was also predicted to encode a possible transporter protein, whereas no specific substrate of a transporter could be predicted by the TrSSP tool (http://bioinfo.noble.org/TrSSP/) (33). According to the structural prediction, CrrC contains four putative transmembrane regions, so we suggest that CrrC might provide a connection (direct or indirect) between the CrrAB and PmrAB systems. The molecular function(s) of CrrC in the context of colistin resistance will require further investigation.

Clinical strains with crrB mutations are highly resistant to colistin, and introduction into A4528 of crrB loci encoding pro-

**FIG 2** mRNA expression of pmrH in A4528-derived strains. qRT-PCR analysis revealed the relative mRNA expression of pmrH in A4528-derived strains that harbored 6 different amino acid substitutions compared with that in the A4528 wild-type strain (A) and in the A4528 wild-type strain and strains A4528 crrB(N141I1), A4528 crrB(N141I) ΔcrrAB, A4528 crrB(N141I) ΔcrrC, A4528 crrB(N141I) ΔcrrC carrying a pACYC184 plasmid, and A4528 crrB(N141I) ΔcrrC carrying a pACYC184-3062 plasmid (B). The mRNA expression of each strain was calculated by qRT-PCR independently in quadruplicate, and statistical analysis was performed using Student’s t test (**, P < 0.01; ***, P < 0.001; ns, P > 0.05). The error bars represent SD.
proteins with amino acid substitutions also resulted in high resistance to colistin (MICs = 512 to >2,048 μg/ml). Moreover, we showed that CrrB mutations resulted in colistin resistance through CrrC. However, the mRNA levels of pmrHFIJKLM in crrB mutant strains were not significantly higher than those observed in strains rendered resistant by other mechanisms (8). These results imply that CrrC not only regulated PmrHFIJKLM through PmrAB but might also regulate another factor(s) that results in high resistance to colistin.

In conclusion, we showed that novel amino acid substitutions in the K. pneumoniae CrrB protein (Y31H, W140R, N141I, P151S, or S195N) contribute to resistance to colistin. Although crrB nonsense mutants exhibited increased transcription of H239_3059 and crrC, the former locus did not contribute to resistance to colistin under laboratory conditions. In contrast, crrC was necessary for CrrB-mediated resistance to colistin; we hypothesize that the crrC gene product regulates expression of the pmrHFIJKLM operon and pmrC through effects on the PmrAB two-component system (Fig. 4).

FIG 3 mRNA expression levels of pmrA, phoP, pmrC, and crrC in A4528-derived strains. (A) The relative mRNA expression levels of pmrA and phoP in A4528 crrB(N141I) were determined and compared with that in the A4528 wild type. (B and C) mRNA expression levels of pmrA (B) and pmrC (C) in the A4528 wild-type strain and strains A4528 crrB(N141I), A4528 crrB(N141I) ΔpmrAB, A4528 crrB(N141I) ΔcrrC, A4528 crrB(N141I) ΔcrrC carrying a pACYC184 plasmid, and A4528 crrB(N141I) ΔcrrC carrying a pACYC184-3062 plasmid were determined and compared. (D) Relative fold expression of crrC in strains A4528 crrB(N141I), A4528 crrB(N141I) ΔpmrAB, and A4528 crrB(N141I) ΔcrrC compared with that in the A4528 wild-type strain. The mRNA expression of each strain was calculated by qRT-PCR independently in quadruplicate, and statistical analysis was performed using Student’s t test (**, P < 0.01; ***, P < 0.001; ns, P > 0.05; ND, not detected). The error bars represent SD.

FIG 4 Model of CrrC-mediated colistin resistance in K. pneumoniae. Two-component systems (CrrAB, PmrAB, and PhoPQ) are regulated by a negative regulator (Mgrp) and connectors (CrrC and PmrD). A mutation(s) or interruption(s) in these components could abnormally regulate the downstream locus and result in increasing LPS modification-associated loci (pmrHFIJKLM operon and pmrC).
REFERENCES


