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OmpC regulation differs between ST131 and non-ST131 *Escherichia coli* clinical isolates and involves differential expression of the small RNA MicC

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Background: Virulence genes and the expression of resistance mechanisms undoubtedly play a role in the successful spread of the pandemic clone *Escherichia coli* ST131. Porin down-regulation is a chromosomal mechanism associated with antibiotic resistance. Translation of porin proteins can be impacted by modifications in mRNA half-life and the interaction among small RNAs (sRNAs), the porin transcript and the sRNA chaperone Hfq. Modifications in the translatability of porin proteins could impact the fitness and therefore the success of *E. coli* ST131 isolates in the presence of antibiotic.

Objectives: To identify differences in the translatability of OmpC and OmpF porins for different STs of *E. coli* by comparing steady-state RNA levels, mRNA half-life, regulatory sRNA expression and protein production.

Methods: RNA expression was evaluated using real-time RT–PCR and OmpC mRNA half-life by northern blotting. OmpC, OmpF and Hfq protein levels were evaluated by immunoblotting.

Results: Differences between ST131 and non-ST131 isolates included: (i) the level of OmpC RNA and protein produced with mRNA expression higher for ST131 but OmpC protein levels lower compared with non-ST131 isolates; (ii) OmpC mRNA half-life (21–30 min for ST131 isolates compared with <2–23 min for non-ST131 isolates); and (iii) levels of the sRNA MicC (2- to 120-fold for ST131 isolates compared with −4- to 70-fold for non-ST131 isolates).

Conclusions: Mechanisms involved in the translatability of porin proteins differed among different STs of *E. coli*. These differences could provide a selective advantage to ST131 *E. coli* when confronted with an antibiotic-rich environment.

Introduction

Escherichia coli ST131 is a successful pandemic clone associated with the spread of β-lactam, fluoroquinolone and aminoglycoside resistance and is associated with urinary tract infections in both community- and hospital-acquired infections.[1–3] The newer β-lactam/β-lactamase inhibitor combinations or carbapenems are the β-lactam therapy of choice when treating cases of urosepsis caused by CTX-M-producing ST131 *E. coli*.[4] ST131 *E. coli* can be further characterized based on ancestral lineage or clade.[5] CTX-M-producing ST131 *E. coli* are most commonly associated with clade C, which includes the subclades C1, C1-M27 and C2. To date, the success of ST131 *E. coli* has largely been attributed to the resistance and virulence genes it possesses.[6] The lack of porin production can contribute to β-lactam resistance and yet no studies have evaluated physiological differences in porin regulation between ST131 *E. coli* and non-ST131 *E. coli*.

E. coli, like other members of the Enterobacteriaceae, can alter the permeability of its outer membrane, contributing to antibiotic resistance.[7,8] A decrease in permeability is typically associated with a defect in porin structure or production. The primary outer membrane proteins implicated in decreased permeability in *E. coli* are the porins OmpC and OmpF. Both of these porins are non-specific and allow the diffusion of hydrophilic molecules including β-lactams.[9] The presence of OmpC and OmpF in the outer membrane is controlled at the transcriptional level by the EnvZ-OmpR two-component system.[10] In addition, regulation of OmpC and OmpF at the post-transcriptional level is controlled by several small, regulatory RNAs (sRNAs).[11] The mechanism of sRNA

regulation can affect the translatability of the transcript or mRNA half-life through targeted RNase E degradation.[12] The sRNAs MicC, RybB, RseX and IpeX have been shown to post-transcriptionally regulate OmpC, while MicF and IpeX post-transcriptionally regulate OmpF.[13–17] The sRNAs involved in post-transcriptional regulation of OmpC and OmpF require the RNA chaperone protein Hfq to facilitate the sRNA/transcript interaction.[18] The result of this interaction is the inhibition of OmpC and OmpF translation through blockage of the ribosomal binding site.

Aberrations in permeability are correlated with decreased carbapenem susceptibility when the organism produces an ESBL or plasmid-encoded AmpC in the absence of a carbapenem-hydrolysing enzyme.[19] Altering the production of one or both porins could provide ST131 *E. coli* with an advantage over non-ST131 *E. coli* during antibiotic treatment. Likewise, alterations in ST131 *E. coli* porin production may increase its environmental adaptability compared with non-ST131 *E. coli*. Therefore, the goal of this study was to evaluate the variability of OmpC and OmpF production in CTX-M-14- and CTX-M-15-producing *E. coli* clinical isolates among different *E. coli* STs. We sought to identify correlations among the level of porin production, porin mRNA half-life and sRNA expression that could explain the variability observed in the production of OmpC and OmpF proteins.

Methods

Bacterial isolates, sequencing, sequence typing and ST131 clade determination

Ten CTX-M-14-producing and 10 CTX-M-15-producing *E. coli* clinical isolates of various STs were collected from urine.[20] These isolates were collected from varying geographical regions to ensure that the data represented a wide distribution of CTX-M-producing isolates and not a local clonal outbreak (Table 1). The K-12 derivative WT strain BW25113 (BW) and its single-gene knockouts JW2203-1 (Δ*ompC*), JW0912-1 (Δ*ompF*) and JW4130-1 (Δ*hfq*) were obtained from the Keio collection.[21] For isolate K15, PCR and sequencing analysis of the seven MLST loci was performed according to the Achtman system (https://enterobase.warwick.ac.uk/species/ecoli/allele_st_search). To determine which clade the evaluated ST131 isolates belonged to, multiplex PCR was done according to Matsumura *et al.*[5] (Figure S1, available as Supplementary data at *JAC* Online). PCR amplicons were sequenced by Functional Biosciences™ (Madison, WI, USA).

RNA isolation and expression analysis

RNA isolation and expression analyses were carried out as previously described.[20] Relative mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method using the clinical isolate XQ13 as the comparator. Because the level of OmpC and OmpF protein production was equivalent to the laboratory strain BW25113, we chose to use strain XQ13 as our comparator as this strain represents WT levels of protein production within a clinical isolate. Expression data were normalized using the single-copy gene *frr*. The data represent the mean of three independent RNA isolations and three independent real-time RT–PCR assays with a coefficient of variance of <10%.

Evaluation of OmpC mRNA half-life

Cultures were grown to mid-logarithmic growth (OD_{600} = ~0.5) in Mueller–Hinton broth at 37°C. Total RNA was isolated using TRIzol® Max™ (Invitrogen™) at 0, 2, 4, 6, 8, 10, 15, 20, 25 and 30 min post-addition of 0.2 g/L rifampicin. mRNA half-life was evaluated by northern-blot analysis using digoxigenin-labelled probes specific for *ompC* and the 16S rRNA gene, which served as a loading control. Densitometry was used to calculate the amount of transcript remaining from T_0 at each timepoint and were plotted on a semi-logarithmic plot. Half-life was calculated using the equation $t_{1/2} = \ln(2)/\lambda$, where λ is the slope of the line of best fit from the plot.

Protein isolation and western blot analysis

Cells from cultures grown to mid-logarithmic growth (OD_{600} = ~0.5) in Mueller–Hinton broth at 37°C were lysed using the SoniBeast™ (Biospec). Custom, polyclonal peptide antibodies specific for OmpC, OmpF and Hfq were generated by GenScript (Piscataway, NJ, USA). The anti-OmpC antibody was directed toward the peptide sequence SKGKNLGRGYDDED, the anti-OmpF antibody was directed toward the peptide sequence GKNERDTARRSNGD and the anti-Hfq antibody was directed toward the peptide sequence SAQNTSAQQDSEETE.[22] The linear range of all three antibodies was 5 to 80 μg/mL. The antibody dilution factors for the anti-ompC and anti-OmpF antibodies were 1:50 000 and 1:40 000, respectively (Figure S2 and Figure S3). The anti-Hfq antibody was diluted 1:40 000. The secondary antibody (horseradish peroxidase-goat anti-rabbit IgG) was used with a dilution factor of 1:30 000 for all antibodies. Total protein (30 μg) was normalized among isolates using Stain-Free™ technology and the ChemiDoc™ MP imaging system (Bio-Rad). Relative fold change was calculated using densitometry with XQ13 as the comparator. The data represent the mean of three independent isolations and three separate western blots.

Results

Relative mRNA expression and protein production of OmpC and OmpF

Changes in membrane permeability may have the potential to provide ST131 *E. coli* with selective and/or environmental advantages compared with non-ST131 *E. coli*. In addition, the presence or absence of an ESBL may be important for the physiological differences in porin production. Therefore, we wanted to determine whether there were differences in the mRNA expression and protein production of OmpC and OmpF between ST131 and non-ST131 *E. coli* clinical isolates. The other parameter we investigated was whether the isolates produced a CTX-M-14 or CTX-M-15 β-lactamase. Previous data from our laboratory showed that ST did not impact CTX-M protein levels, but perhaps the presence of a particular CTX-M could impact porin production.[20] The overall trend for *ompC* expression was highest for ST131 isolates (Figure 1) with a range of expression from 457- to 6483-fold compared with XQ13 (ST68) regardless of whether CTX-M-14 or CTX-M-15 was produced. The trend for OmpC mRNA levels in non-ST131 isolates was lower and ranged from no difference compared with XQ13 to 638-fold. However, three isolates [JJ2235S (ST167), FS-ESBL014 (ST10) and JJ2131 (ST167)] had levels of *ompC* expression that were 106 374-, 171 962- and 1 136 762-fold higher than XQ13, respectively. These high levels of OmpC mRNA did not correlate with the CTX-M β-lactamase produced. When whole-cell lysates were evaluated for OmpC protein, the level of protein produced was not indicative of the RNA levels observed. ST131 isolates produced the lowest levels of OmpC protein compared with XQ13, with decreased levels from 2- to 13-fold (Table 1). In contrast, 8/12 non-ST131 isolates had comparable levels of total OmpC protein compared with XQ13 even though the level of mRNA varied among those isolates.

In contrast to the OmpC data, analyses of the relative expression of *ompF* showed no difference compared with XQ13 in 17/20

Table 1. Characteristics, mRNA expression and protein production, and mRNA half-life for the *E. coli* clinical isolates used in this study

Strain[a]	Location of isolation[a]	$bla_{CTX\text{-}M}$ allele[a]	ST[a]	Relative fold change					*ompC* half-life (min)	Relative fold change				
				ompC	OmpC	*micC*	*rybB*	*ipeX*		*ompF*	OmpF	*micF*	*hfq*	Hfq
XQ13 (comparator)	Seattle, WA, USA	CTX-M-14	68	1	1	1	1	1	17	1	1	1	1	1
D14	Omaha, NE, USA	CTX-M-14	405	10.95	−1.20 ± 0.32	8.08	−4.27	3.28	14	−1.34	−1.52 ± 0.35	−1.23	1.03	−1.09
Lo14	Omaha, NE, USA	CTX-M-14	405	7.08	−2.03 ± 0.13	16.91	−2.29	−1.75	<2	1.07	−10.71 ± 0.02	−1.01	−2.58	1.49
C14	Omaha, NE, USA	CTX-M-14	648	2.08	1.41 ± 0.42	−3.53	−1.68	−1.20	11	−2.18	−1.34 ± 0.37	−2.53	1.34	1.20
La14	Omaha, NE, USA	CTX-M-14	648	1.51	−1.44 ± 0.24	1.93	−1.61	1.74	10	420.12	6.66 ± 0.35	−1.34	−2.57	1.16
FS-ESBL013	Denmark	CTX-M-14	38	−1.32	−1.62 ± 0.31	69.79	−1.62	4.74	15	−1.16	−1.33 ± 0.13	−1.60	−1.57	−1.11
N14	Omaha, NE, USA	CTX-M-14	3856	7.44	−1.11 ± 0.25	30.38	1.69	1.54	<2	2.98	1.49 ± 0.30	−1.28	−3.05	1.19
FS-ESBL014	Denmark	CTX-M-14	10	171 962.19	−2.11 ± 0.33	6.00	1.31	6.68	8	−1.18	−2.52 ± 0.15	−1.46	−1.27	−1.05
NL217	UK	CTX-M-14	131	456.51	−1.68 ± 0.55	74.54	−1.12	1.76	30	−1.83	1.52 ± 0.78	1.17	1.23	−1.55
FO44	UK	CTX-M-14	131	5029.72	−6.00 ± 0.01	1.61	4.38	4.21	12	2.26	2.13 ± 0.27	−3.08	2.22	1.12
CUMC247	Omaha, NE, USA	CTX-M-15	131	3342.53	−3.33 ± 0.27	120.26	−1.70	4.16	29	−1.23	3.18 ± 1.64	−4.22	2.59	−1.52
FHM6	India	CTX-M-15	131	1089.02	−5.26 ± 0.89	46.21	−1.43	3.89	28	1.44	4.77 ± 0.21	−9.26	−1.20	1.66
RS059	UK	CTX-M-15	131	6141.25	−12.50 ± 0.02	50.39	−2.49	1.65	21	1.12	3.22 ± 1.42	−1.81	4.82	1.33
RS135	UK	CTX-M-15	131	2628.46	−8.33 ± 0.04	40.22	−3.13	−1.65	29	−2.63	1.62 ± 0.34	−3.39	3.53	−1.06
XQ12	Seattle, WA, USA	CTX-M-15	131	6483.31	−3.30 ± 0.09	21.33	−1.17	−1.32	29	−2.92	4.06 ± 1.54	−2.17	4.35	1.29
JJ2131	Minneapolis, MN, USA	CTX-M-15	167	1 136 762.11	−4.65 ± 0.50	21.04	−1.00	−1.92	<2	3.33	2.74 ± 0.90	−2.56	−1.57	1.98
JJ2235S	Houston, TX, USA	CTX-M-15	167	106 374.04	−5.02 ± 0.11	4.69	−1.60	1.83	<2	−1.30	3.49 ± 0.72	−6.58	2.12	2.01
H15	Omaha, NE, USA	CTX-M-15	205	291.37	−6.38 ± 0.06	9.13	−2.08	1.02	23	1.55	2.52 ± 0.40	−6.99	−1.45	1.57
K15	Myanmar	CTX-M-15	127	637.81	−5.78 ± 1.23	22.09	−3.48	3.88	4	16.12	5.26 ± 0.47	−3.95	2.39	1.28
C15	Omaha, NE, USA	CTX-M-15	405	2.24	−2.38 ± 0.08	1.13	−5.78	−1.18	8	25.29	1.04 ± 0.24	−2.63	−1.87	−1.01

[a]Data obtained from Geyer *et al.*[20]

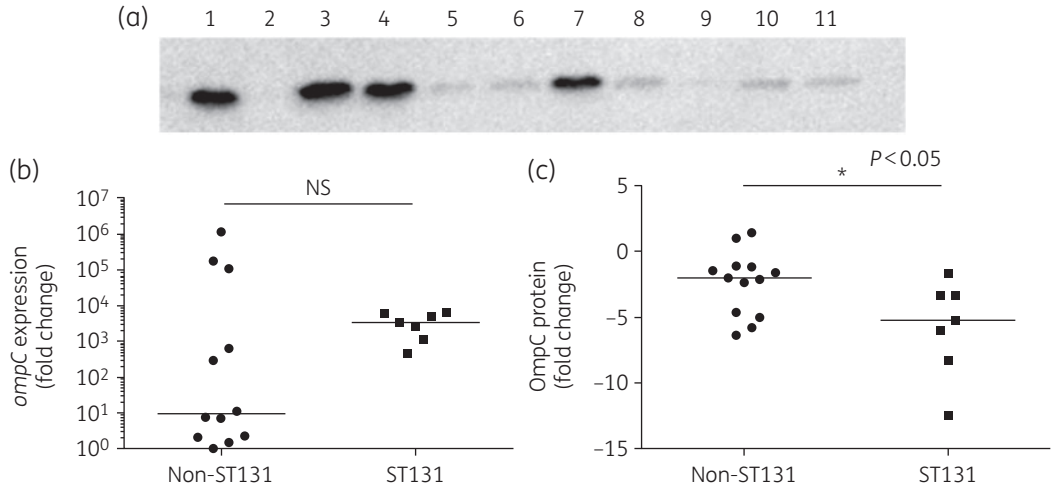

Figure 1. OmpC mRNA expression and protein production in clinical isolates. (a) Western blot for OmpC in representative clinical isolates. Protein levels were compared with isolate XQ13. Lane 1, BW25113; Lane 2, Δ*ompC*; Lane 3, XQ13; Lane 4, C14; Lane 5, RS135; Lane 6, FHM6; Lane 7, D14; Lane 8, H15; Lane 9, RS059; Lane 10, K15; Lane 11, FO44. (b) Comparison of *ompC* expression between ST131 and non-ST131 clinical isolates. (c) Comparison of OmpC protein between ST131 and non-ST131 clinical isolates. Bars indicate median fold change. Statistical significance was evaluated using a *t*-test (two-tailed, unpaired). NS, not significant.

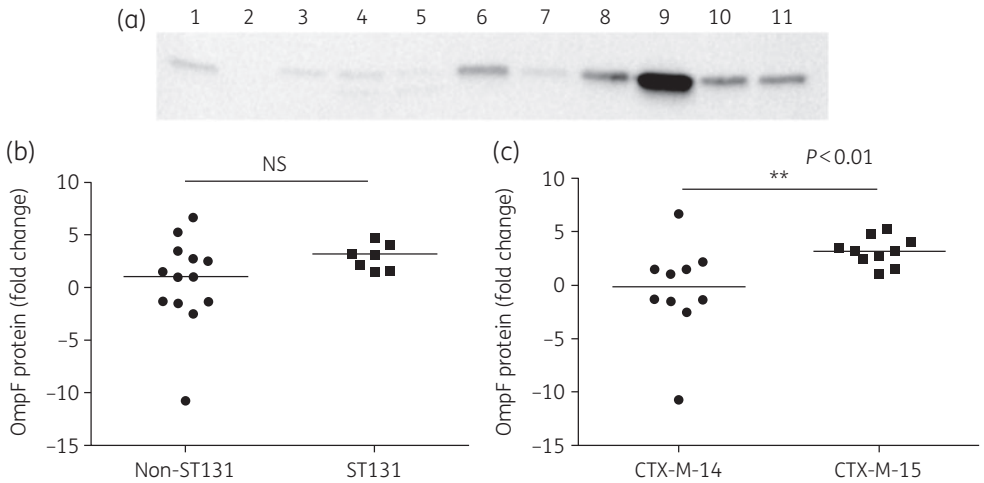

Figure 2. OmpF protein production in clinical isolates. (a) Western blot for OmpF in representative clinical isolates. Protein levels were compared with isolate XQ13. Lane 1, BW25113; Lane 2, Δ*ompF*; Lane 3, XQ13; Lane 4, N14; Lane 5, D14; Lane 6, JJ2235S; Lane 7, RS135; Lane 8, CUMC247; Lane 9, La14; Lane 10, RS059; Lane 11, XQ12. (b) Comparison of OmpF protein between ST131 and non-ST131 clinical isolates. (c) Comparison of OmpF protein between CTX-M-14- and CTX-M-15-producing clinical isolates. Bars indicate median fold change. Statistical significance was evaluated using a *t*-test (two-tailed, unpaired). NS, not significant.

of the isolates regardless of ST (Table 1). However, the remaining non-ST131 isolates had *ompF* transcript levels that were 16-, 25- and 420-fold higher. Isolates K15 and La14, with 16- and 420-fold higher *ompF* transcript levels, showed concomitant levels of OmpF protein that were 5- and 7-fold higher, respectively. Interestingly, 8/10 CTX-M-15-producing isolates showed higher OmpF levels ranging from 3- to 5-fold compared with just 1/10 CTX-M-14-producing isolates, which was 7-fold higher (Figure 2).

Transcriptional regulation of OmpC and OmpF

One explanation for the difference in the levels of OmpC and OmpF mRNA expression could be differences in the promoter sequences driving expression or modifications in the EnvZ-OmpR two-component transduction system involved in regulating the OmpC and OmpF promoter. Sequence analysis of the promoter region and the two-component system in six isolates representing large ranges of mRNA expression revealed no mutations compared with XQ13. Taken together, these data suggest transcription initiation was not responsible for the observed differential expression of *ompC* or *ompF*.

OmpC mRNA half-life

The degradation rate of a transcript, or its half-life, can influence steady-state mRNA levels as well as the amount of protein

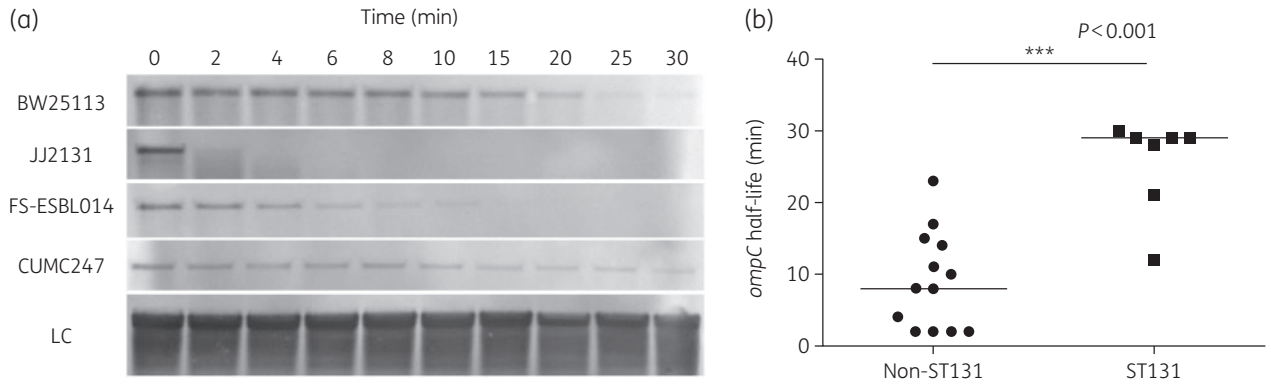

Figure 3. OmpC mRNA half-life in clinical isolates. (a) Northern blots for OmpC mRNA half-life from representative clinical isolates: BW25113 (ST10); JJ2131 (ST167); FS-ESBL014 (ST38); and CUMC247 (ST131). LC, loading control. (b) Comparison of OmpC mRNA half-life between ST131 and non-ST131 clinical isolates. Bars indicate median half-life. Statistical significance of the difference between ST131 and non-ST131 clinical isolates was evaluated using a *t*-test (two-tailed and unpaired).

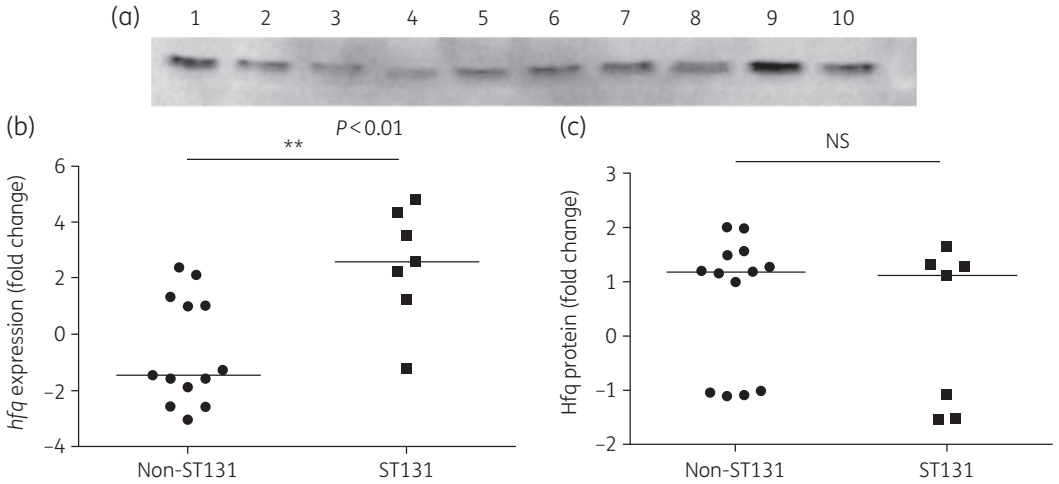

Figure 4. Hfq mRNA expression and protein production in clinical isolates. (a) Western blot for Hfq in representative clinical isolates. Protein levels were compared with isolate XQ13. Lane 1, BW25113; Lane 2, XQ13; Lane 3, D14; Lane 4, N14; Lane 5, C14; Lane 6, NL217; Lane 7, RS135; Lane 8, CUMC247; Lane 9, JJ2235S; Lane 10, RS059. (b) Comparison of *hfq* expression between ST131 and non-ST131 clinical isolates. (c) Comparison of Hfq protein between ST131 and non-ST131 clinical isolates. Bars indicate median fold change. Statistical significance was evaluated using a *t*-test (two-tailed, unpaired). NS, not significant.

produced. Therefore, to determine whether the observed differences in mRNA expression and protein production could be explained by a post-transcriptional mechanism, OmpC mRNA half-life was evaluated (Table 1). Differences between ST131 isolates and non-ST131 *E. coli* were observed (Figure 3). Five of seven ST131 isolates had an extended half-life of 28–30 min compared with non-ST131 isolates, which had *ompC* half-lives that ranged from <2 to 23 min. While OmpC transcript half-life generally correlated with the amount of OmpC protein produced (i.e. shorter half-lives correlated with less protein produced) in non-ST131 isolates, this trend was not observed for ST131 isolates, where longer half-lives correlated with decreased OmpC production.

Post-transcriptional regulation of OmpC and OmpF

The decrease in OmpC production but an extended *ompC* half-life for ST131 isolates suggested the involvement of sRNAs in the regulation of OmpC translation. Hfq is an RNA chaperone required by some sRNAs. The level and availability of Hfq could impact the regulation of OmpC translatability. To evaluate sRNA involvement, we first evaluated the level of Hfq transcripts and protein production. Hfq mRNA expression was higher and statistically significant in ST131 isolates compared with non-ST131 isolates (Table 1 and Figure 4). Expression of *hfq* in 5/7 ST131 isolates was 2- to 5-fold higher compared with XQ13 (Table 1). A 2-fold higher level of *hfq* expression was observed in 2/13 non-ST131 isolates. Three of 13 non-ST131 isolates had 2- to 3-fold lower levels of *hfq* expression and the remaining 8 non-ST131 isolates showed similar levels of *hfq* expression compared with XQ13. Although *hfq* expression was higher in ST131 isolates, no difference was observed in Hfq protein production between ST131 and non-ST131 isolates (Figure 4). Sequence analysis of Hfq in all evaluated isolates revealed no modifications in amino acid sequence, indicating that its functionality was not compromised in these isolates. However, an

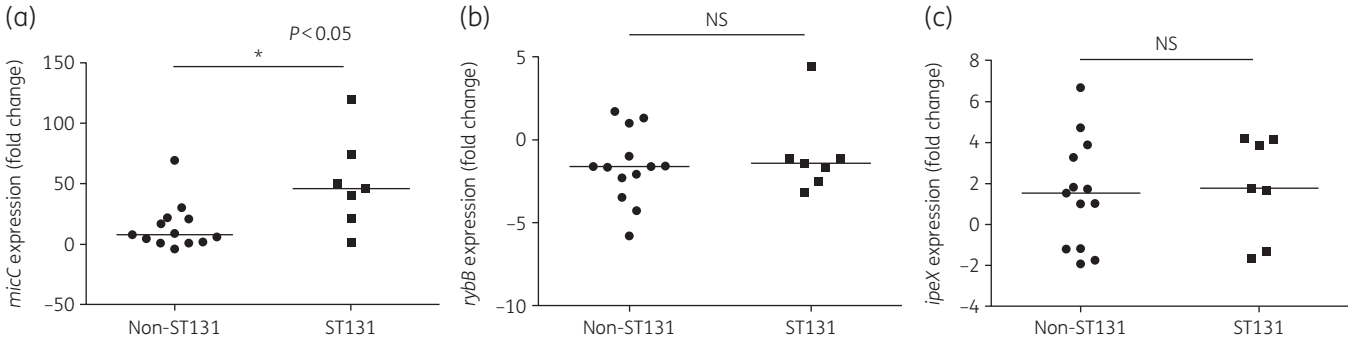

Figure 5. Expression of sRNA post-transcriptional regulators of OmpC in clinical isolates. (a) Comparison of *micC* expression between ST131 and non-ST131 clinical isolates. (b) Comparison of *rybB* expression between ST131 and non-ST131 clinical isolates. (c) Comparison of *ipeX* expression between ST131 and non-ST131 clinical isolates. Bars indicate median fold change. Statistical significance was evaluated using a *t*-test (two-tailed, unpaired). NS, not significant.

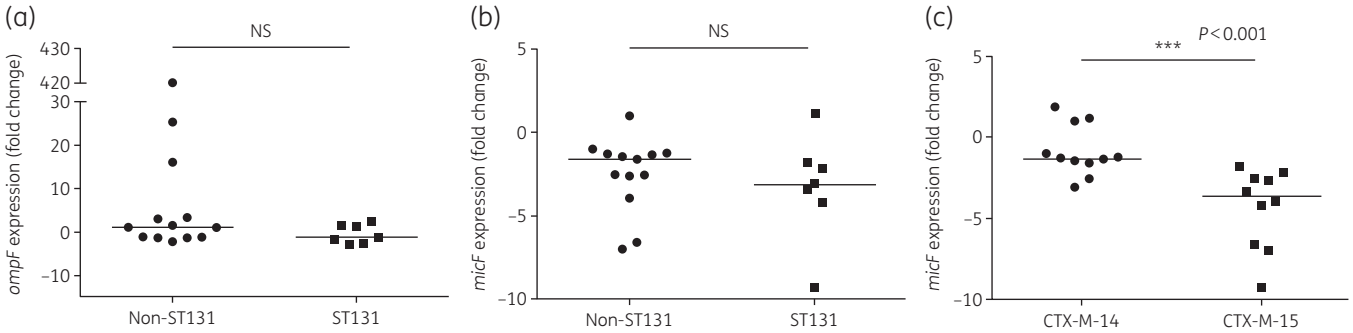

Figure 6. *ompF* and *micF* expression in clinical isolates. (a) Comparison of *ompF* expression between ST131 and non-ST131 clinical isolates. (b) Comparison of *micF* expression between ST131 and non-ST131 clinical isolates. (c) Comparison of *micF* expression between CTX-M-14- and CTX-M-15-producing clinical isolates. Bars indicate median fold change. Statistical significance was evaluated using a *t*-test (two-tailed, unpaired). NS, not significant.

~2.5-fold increase in OmpC and OmpF production was observed in the Hfq knockout (Δ*hfq*) (Figure S4). These data indicated a role for Hfq in the regulation of OmpC and OmpF protein production and implicated the involvement of sRNAs in the differential production of these porins among different STs.

Evaluation of sRNA expression

The lower OmpC production and extended mRNA half-life in ST131 isolates suggested that sRNAs may be involved in the translatability of the OmpC transcript. When expression of the sRNA MicC was evaluated, a clear difference was observed between ST131 and non-ST131 isolates (Figure 5). Seventeen of 20 isolates, regardless of ST, had higher levels of *micC* expression compared with XQ13, ranging from 2- to 120-fold (Table 1). Levels of *micC* expression in 6/7 ST131 isolates ranged from 21- to 120-fold higher. Ten of 13 non-ST131 isolates showed *micC* levels ranging from 2- to 70-fold higher compared with XQ13. These differences in *micC* expression correlated with OmpC protein production. For example, *micC* levels observed for ST131 isolates were associated with lower OmpC protein levels compared with more modest OmpC protein levels in non-ST131 isolates, which had lower levels of *micC* expression. Sequence analysis of the MicC promoter region in six isolates with varying levels of *micC* expression (D14, FS-ESBL014, FO44, CUMC247, RS059 and XQ12) revealed no differences in promoter sequence compared with XQ13. Therefore, mutations in the MicC promoter region were not responsible for the differential expression of MicC.

sRNAs IpeX and RybB were also evaluated for expression in these isolates. Expression analysis of IpeX showed no difference compared with XQ13 in 13/20 isolates (Table 1). Five of 20 isolates had 4-fold higher *ipeX* expression and 1 isolate had 7-fold higher *ipeX* expression. A similar trend was observed for *rybB* expression. Fourteen of 20 isolates showed no difference in expression compared with XQ13. Four isolates had lower *rybB* expression ranging from 3- to 6-fold and one isolate showed 4-fold higher *rybB* expression. Overall, no significant difference between ST131 and non-ST131 isolates was observed in the expression of *rybB* and *ipeX* (Figure 5).

Although *micC* expression levels varied widely in both ST131 and non-ST131 isolates, differences in *micF* expression levels were minimal and differences were more modest in comparison (Table 1). Ten of 20 of the isolates showed no difference in expression compared with XQ13 while the remaining isolates had lower *micF* expression levels ranging from 2- to 9-fold. These differences in *micF* expression correlated with the observed OmpF protein production and the differences were correlated not with ST but with whether the isolate produced a CTX-M-14 or CTX-M-15 enzyme (Figure 6). The *micF* expression levels ranged from 2- to 9-fold

lower in 9/10 CTX-M-15-producing isolates while 2/10 CTX-M-14-producing isolates had only 2- and 3-fold lower *micF* expression levels.

Discussion

The 20 clinical isolates evaluated in this study represent a wide geographical distribution, which increases the impact of these findings. Few studies have evaluated physiological differences between ST131 and non-ST131 *E. coli*. A previous study by Geyer *et al.*[20] evaluated CTX-M mRNA expression, mRNA half-life and protein production among various *E. coli* STs. It is interesting that the CTX-M-15 data from that study and the OmpC data in this study are strikingly similar. In both studies, the mRNA expression was much higher than the corresponding protein production. The difference, however, was that ST did not influence the disparity observed between CTX-M-15 mRNA or protein production. In that study, the plasmid encoding CTX-M-15 influenced the CTX-M-15 mRNA half-life. In the present study, the variability observed for OmpC mRNA and protein production was correlated with ST, but no pattern was observed for the clades of ST131 regarding the expression and production of OmpC, OmpF and Hfq, the expression of the sRNA post-transcriptional regulators of OmpC and OmpF, or the half-life of OmpC transcripts. However, the clade designation for the ST131 isolates of this study was consistent with the findings of Matsumura *et al.*;[5] the CTX-M-14-producing ST131 isolates evaluated in this study belonged to either clade C1 or subclade C1-M27, whereas the CTX-M-15-producing ST131 isolates belonged to clade C2 (Figure S1). Therefore, we focused on post-transcriptional regulatory mechanisms of mRNA half-life and sRNA expression, which could also be influenced by ST.

It was not surprising that many of the isolates had an extended OmpC transcript half-life as OmpA transcripts have a half-life of ∼14 min.[23] However, it was surprising that ST131 isolates, which produced the lowest amount of OmpC protein, had the longest mRNA half-lives compared with the majority of non-ST131 isolates. Non-ST131 isolates had shorter half-lives, which were associated with less protein produced. In addition, steady-state levels of OmpC transcript reflected the longevity of these transcripts and suggested that they were not being targeted for RNase degradation.

Our data indicated that levels of the sRNA MicC correlated in most isolates with the differences in OmpC mRNA and protein levels. The higher levels of *micC* expression in most ST131 isolates correlated with lower OmpC protein production, as measured by whole-cell lysates. To our knowledge, only two studies have evaluated *micC* expression and those studies evaluated laboratory strains, not clinical isolates.[13,24] Post-transcriptional regulation of outer membrane proteins by sRNAs are mediated by Hfq.[11] However, the differences observed among the *E. coli* isolates in this study were not the result of varying levels of Hfq. This is not surprising given the ubiquitous nature of Hfq in the cell.[25]

The present study advances the field by evaluating the level of MicC in clinical isolates and how those levels correlate with OmpC mRNA half-life and protein production. The higher *micC* expression found in many of the isolates could be a response to the amount and extended half-life of OmpC transcripts in ST131 isolates. Dam *et al.*[24] showed that β-lactams could influence *micC* expression so the increase observed in these clinical isolates may reflect a selective advantage during β-lactam exposure. While *rybB* and *ipeX* expression did not appear to play a major role in post-transcriptional regulation in these isolates, some interplay among the sRNAs and OmpC regulation was observed. For example, isolate FO44 (ST131) had 6-fold less OmpC protein and no difference in *micC* expression compared with XQ13, but had 4-fold higher *ipeX* and *rybB* expression.

Contrary to OmpC, OmpF mRNA expression and protein production did not statistically differ between ST131 and non-ST131. Instead, the observed differences correlated with the production of CTX-M-14 or CTX-M-15. Isolates producing CTX-M-15 had higher levels of OmpF protein production compared with CTX-M-14-producing isolates. In the current study, levels of *micF* expression also correlated with the production of CTX-M-14 or CTX-M-15. Isolates producing CTX-M-15 had lower levels of *micF* expression compared with CTX-M-14-producing isolates. No differences were found in the sequence of the MicF promoter region, suggesting that MicF promoter mutations were not responsible for the observed differential expression of *micF* between CTX-M-14- and CTX-M-15-producing isolates. As with MicC, investigation into *micF* expression has been limited to laboratory strains.[16,26] The study by Geyer *et al.*[20] identified that a factor(s) encoded on CTX-M-15 plasmids extended the half-life of CTX-M-15 mRNA compared with CTX-M-14 transcripts. Therefore, it is reasonable to suggest that a factor(s) encoded on CTX-M-15 plasmids may be influencing *micF* expression.

The data from this study demonstrated physiological differences in the regulation of OmpC between ST131 and non-ST131 *E. coli* clinical isolates. Porins play an important role in the emergence of drug-resistant Gram-negative bacteria. Therefore, the success of the pandemic clone *E. coli* ST131 may not only be attributed to the possession of virulence factors and acquired resistance mechanisms but to physiological differences in the regulation of porins. Upon further study, these physiological differences could be exploited to find targets for the development of novel antibiotics to be used in the treatment of MDR organisms.

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Transparency declarations

None to declare.

Supplementary data

Figures S1 to S4 are available as Supplementary data at *JAC* Online.

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