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. The sRNAs involved in post-transcriptional regulation of OmpC and OmpF require the RNA chaperone protein Hfq to facilitate the sRNA/transcript interaction. The result of this interaction is the inhibition of OmpC and OmpF translation through blockade 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. 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. 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 (ΔompC), JW0912-1 (ΔompF) and JW6130-1 (Δhfq) were obtained from the Keio collection. 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/ecoll/allele_st_search). To determine which clade the evaluated ST131 isolates belonged to, multiplex PCR was done according to Matsumura et al. (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. Relative mRNA expression was calculated using the 2−ΔΔCT method using the clinical isolate QX13 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. To determine which clade the isolates belonged to, multiplex PCR was done according to Matsumura et al. (Figure S1, available as Supplementary data at JAC Online). PCR amplicons were sequenced by Functional Biosciences™ (Madison, WI, USA).

Evaluation of OmpC mRNA half-life

Cultures were grown to mid-logarithmic growth (OD600 = ~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 T0 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 $\lambda$ 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 (OD600 = ~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 SKGNLGRGYDDED, the anti-OmpF antibody was directed toward the peptide sequence GKNERTARRSNGD and the anti-Hfq antibody was directed toward the peptide sequence SAONTSAQDSEEET. 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 QX13 as the comparator. The data represent the mean of three independent isolates 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. 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 QX13 (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 QX13 to 638-fold. However, three isolates (JJ2235S (ST167), FS-ESBL014 (ST10) and JJ1231 (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 QX13, 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 QX13 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 QX13 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 | Location of isolation | blαCTX-M allele | ST | Relative fold change | ompC | OmpC | micC | rybB | ipeX | Relative fold change | ompF | OmpF | micF | hfq | Hfq |
|--------|-----------------------|-----------------|----|----------------------|------|------|------|------|------|----------------------|------|------|------|-----|-----|
| XQ13   | Seattle, WA, USA      | CTX-M-14        | 68 |                      | 1    | 1    | 1    | 1    | 1    | 17                   | 1    | 1    | 1    | 1   | 1   |
| (comparator) |                      |                 |    |                      |      |      |      |      |      |                      |      |      |      |     |     |
| 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 ± 3.14 ± 0.37   | -2.53 | 1.34 | 1.20 |
| Lo14   | 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 |
| F5-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 |
| F5-ESBL014 | Denmark            | CTX-M-14        | 10 | 171.96 ± 0.33       | 6.00  | 1.31  | 6.68  | 8    | 2.18 ± 3.25 ± 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 | 4.61  | -1.61 | 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 | -1.14 | 3.89  | 28   | 1.44 ± 4.77 ± 0.21  | -9.26 | 1.20 | 1.66 |
| RS099  | 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| 1136.762.11          | -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 |

aData obtained from Geyer et al.20
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.
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
2.5-fold increase in OmpC and OmpF production was observed in the Hfq knockout (Δhfq) (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 translatibility 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. 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 designations for the ST131 isolates of this study was consistent with the findings of Matsumura et al.; 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. 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. Post-transcriptional regulation of outer membrane proteins by sRNAs are mediated by Hfq. 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. 

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. 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. The study by Geyer et al. 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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