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High Metabolic Potential May Contribute to the Success of ST131 Uropathogenic *Escherichia coli*

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Uropathogenic *Escherichia coli* (UPEC) is the predominant cause of urinary tract infection in both hospital and community settings. The recent emergence of multidrug-resistant clones like the O25b:H4-ST131 lineage represents a significant threat to health, and numerous studies have explored the virulence potential of these organisms. Members of the ST131 clone have been described as having variable carriage of key virulence factors, and it has been suggested that additional unidentified factors contribute to virulence. Here we demonstrated that ST131 isolates have high metabolic potential and biochemical profiles that distinguish them from isolates of many other sequence types (STs). A collection of 300 UPEC isolates recovered in 2007 and 2009 in the Northwest region of England were subjected to metabolic profiling using the Vitek2 Advanced Expert System (AES). Of the 47 tests carried out, 30 gave a positive result with at least one of the 300 isolates examined. ST131 isolates demonstrated significant association with eight tests, including those for peptidase, decarboxylase, and alkalinization activity. Metabolic activity also correlated with antibiotic susceptibility profiles, with resistant organisms displaying the highest metabolic potential. This is the first comprehensive study of metabolic potential in the ST131 lineage, and we suggest that high metabolic potential may have contributed to the fitness of members of the ST131 clone, which are able to exploit the available nutrients in both the intestinal and urinary tract environments.

Escherichia coli is a very diverse member of the normal intestinal flora of humans and other mammals. Some *E. coli* bacteria are considered uropathogenic (UPEC), and this subset differs substantially from the majority of enteric *E. coli* in terms of virulence profiles, phylogenetic grouping, and serotype (31).

Urinary tract infection (UTI) usually occurs following the movement of UPEC from the intestinal tract to the periurethral area (13). In a study of urovirulence characteristics of fecal *E. coli*, it was demonstrated that the phylogenetic groups B2 and D, which are strongly associated with UPEC, tend to dominate, leading to low clonal diversity, suggesting the presence of unknown bacterial factors that contribute to fitness within the intestine prior to transfer to the urinary tract (20).

Generally, the ability of UPEC to colonize and cause infection in the urinary tract results from the cumulative action of specific virulence factors (VFs). These include determinants that aid resistance to host defenses, mediate adhesion, increase iron acquisition, or paralyze ureteric peristalsis (10); however, these VFs are not sufficient to explain the success of UPEC clones like ST131 (14, 21), which shows moderate VF profiles compared to other successful sequence types (STs), such as ST69 and ST127 (8). It has recently been suggested that bacterial metabolic capability enhances fitness and contributes to pathogenesis, which could provide an alternative explanation for the success of prevalent UPEC clones (16), and previous reports have shown that certain metabolic enzymes may also enhance virulence (22). For example, the ability of UPEC to catabolize the amino acid D-serine during UTI supports bacterial growth and acts as a signaling mechanism to trigger virulence gene expression (2, 24).

Biotyping, like most phenotypic methods, is considered to be an unreliable epidemiological tool because of its modest reproducibility and poor discriminatory power, though this depends greatly on the pathogen in question. Indeed, *E. coli* strains can have considerable biochemical variability (9), and biotyping can be a dependable method for their characterization (3, 12, 17).

Recent advances in automated biotyping methods provide more reliable tools that rely on a variety of novel substrates and precise interpretation techniques that increase reproducibility and discriminatory power (27). In this study, we examined the metabolic potential of UPEC and demonstrated an association between members of the ST131 clone and certain metabolic profiles.

MATERIALS AND METHODS

Bacterial strains. 300 UPEC isolates were included in this study, 150 isolates being collected in June 2007 and 150 in June 2009. Isolates were recovered from nonduplicate, consecutive urine samples received by the microbiology laboratories at hospitals in Manchester (200 isolates) and Preston (100 isolates), United Kingdom. The multilocus sequence typing (MLST) of isolates and identification of virulence capacity and antibiotic susceptibility profiles have been previously described (8).

Metabolic profiling. The Vitek 2 compact Automated Expert System (AES) (bioMérieux) was used for metabolic profiling. Using the Vitek 2 ID-GNB card (bioMérieux), identification of Gram-negative bacilli occurs through testing the organism's metabolic activity in 47

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TABLE 1 Biochemical tests used to differentiate between UPEC isolates

Test	Abbrev.[a]
Adonitol	ADO
Beta-galactosidase	BGAL
D-Glucose	dGLU
Gamma-glutamyl-transferase	GGT
Fermentation/glucose	OFF
D-Maltose	dMAL
D-Mannitol	dMAN
D-Mannose	dMNE
L-Proline arylamidase	ProA
Tyrosine arylamidase	TyrA
Urease	URE
D-Sorbitol	dSOR
Saccharose/sucrose	SAC
D-Tagatose	dTAG
D-Trehalose	dTRE
Malonate	MNT
5-Keto-D-gluconate	5KG
L-Lactate alkalinization	ILATk
Succinate alkalinization	SUCT
Alpha-galactosidase	AGAL
Phosphatase	PHOS
Glycine arylamidase	GlyA
Ornithine decarboxylase	ODC
Lysine decarboxylase	LDC
Coumarate	CMT
Beta-glucoronidase	BGUR
O/129 resistance	O129R
L-Malate assimilation	IMLTa
Ellman	ELLM
L-Lactate assimilation	ILATa

[a] Abbreviation.

biochemical tests designed to measure carbon source utilization and enzymatic activity.

Correlation analyses. The metabolic profiles of the isolates were compared in a pairwise fashion, and the similarity matrix obtained was clustered according to the unweighted pair group method using average linkages (UPGMA). Associations between different parameters were analyzed by using Fisher's exact or Mann-Whitney U tests, and the threshold for statistical significance was a P value of ≤ 0.05. Where appropriate, more stringent criteria for statistical significance were used.

Aggregate scores for metabolic activities (Bio) and antimicrobial resistance (AST) were calculated for each isolate as the sum of all parameters for which the isolates tested positive divided by the total number of parameters tested. Correlation analysis between Bio and AST scores were analyzed using Pearson's correlation coefficients. Correlation analysis was also used to describe the association of different STs with the aggregate scores calculated for metabolic activities and the resistance to different antimicrobial drugs.

RESULTS

Although the MLST data relating to the isolate collection have been previously described, a summary is given here. Among the 100 STs identified, 9 (ST73, ST131, ST69, ST95, ST10, ST127, ST14, ST88, and ST405) accounted for 59% of the collection (50, 37, 27, 19, 13, 11, 8, 6, and 5 isolates, respectively). Of the 37 ST131 isolates identified, 11 belonged to the O25b-CTX-M-15 subclone, all of which been characterized as multidrug resistant (resistant to at least three antibiotic groups), and the remaining 26 were non-O25b-CTX-M-15 ST131, of which only seven were considered multidrug resistant (8).

TABLE 2 Aggregate Bio scores in relation to STs

ST	Median (range) aggregate Bio score	P value[a]
ST10	0.55 (0.48–0.65)	0.670
ST14	0.59 (0.55–0.72)	0.145
ST69	0.55 (0.48–0.72)	0.967
ST73	0.59 (0.45–0.65)	0.770
ST88	0.52 (0.51–0.58)	0.216
ST95	0.55 (0.45–0.62)	0.973
ST127	0.52 (0.45–0.55)	**0.003**
ST131	0.62 (0.48–0.76)	**0.0001**
ST405	0.55 (0.41–0.69)	0.771

[a] P values (Mann-Whitney U test) show the significance of the different Bio scores of each ST compared to the other STs. Bold indicates a significant result.

ST131 UPEC demonstrate higher metabolic capabilities than those of other STs. Of the 47 tests employed by the Vitek AES, 30 gave a positive result with at least 1 of the 300 isolates examined. These tests were included in the following analyses for differentiation between UPEC isolates (Table 1). Tests that were found to be negative for all strains tested are shown in Table S1 in the supplemental material.

To assess whether isolates of certain STs share specific metabolic characteristics, two analytical approaches were used. First, a nonparametric comparison analysis using the Mann-Whitney U test was used to compare the aggregate Bio scores generated for each ST against all others. Isolates of ST131were significantly more likely to have a higher Bio score than isolates of all other STs ($P \leq 0.0001$), and ST127 isolates showed a significant association with low Bio scores ($P \leq 0.003$) (Table 2).

Second, the statistical association of activity in individual metabolic tests with STs was assessed using Fisher's exact test. Of the 30 metabolic activities tested, isolates of common STs were significantly associated with 11 tests. Remarkably, ST131 exhibited significant associations (six positive and two negative) with eight tests, indicating distinctive biochemical characteristics (Table 3), and ST73 had a significant association with four tests. In contrast, isolates of ST14 and ST405 showed no difference in their metabolic activity compared to the rest of the tested isolates.

In ST131, metabolic potential is correlated with antibiotic susceptibility. UPGMA cluster analysis based on the biochemical profiles of isolates from the major STs revealed a large cluster comprised almost entirely of ST131 (19/21 cluster members) and included 51% of the total ST131 isolates (Fig. 1). The cluster was defined at the 70% similarity level and contained isolates previously identified as being resistant to multiple antibiotics (8). The remaining ST131 isolates were found scattered through the dendrogram. Although there was a tendency for isolates with the same ST to cluster together, only ST73 and ST69 gathered in clusters consisting primarily of their respective STs. Other than ST131, no correlation was found between the defined clusters and antibiotic resistance profiles (Fig. 1).

No significant correlation was observed when Pearson's correlation coefficient was determined between Bio and AST scores ($r^2 = 0.1476$). However, the comparison revealed an appreciable correlation coefficient that justified further investigation.

Subsequent examination of the correlations at the level of each ST revealed a weak but significant positive association between Bio and AST scores ($P \leq 0.01$) among ST131 isolates, which could explain the correlation observed between Bio and AST scores in the general population (Table 4).

TABLE 3 Statistical analysis of association between STs and individual metabolic tests[a]

ST	*P* value for biochemical test[b]										
	ADO	AGAL	BGUR	ILATa	ILATk	5KG	ODC	O129R	PHOS	ProA	SAC
ST10	0.0012						**0.0001**				
ST14											
ST69						0.0017	**0.0001**				**0.0001**
ST73						0.0001	0.0001		**0.0014**		**0.0001**
ST88											
ST95								**0.0001**			**0.0001**
ST127											
ST131		0.0041	0.0002	**0.0017**	0.0041	**0.0001**	0.0034			0.0001	0.0001
ST405											

[a] *P* values (by Fisher's exact test) were calculated for each ST compared to the rest of the population, shown only where $P \leq 0.005$. Bold *P* values indicate negative associations. For abbreviations, see Table 1.

[b] No. (%) of positive isolates is as follows: for ADO, 6 (2); for AGAL, 208 (69); for BGUR, 275 (92); for ILATa, 3 (1); for ILATk, 120 (40); for 5KG, 123 (41); for ODC, 227 (76); for O129R, 175 (58); for PHOS, 36 (12); for ProA, 28 (9); for SAC, 167 (56).

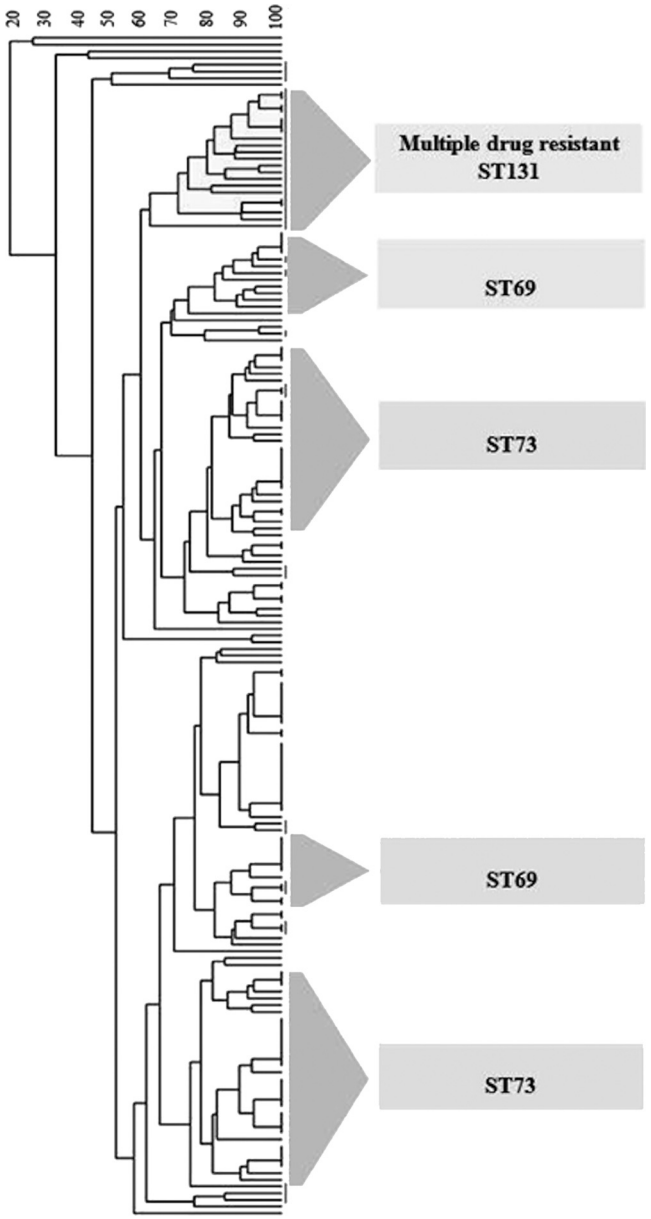

FIG 1 UPGMA cluster analysis based on the biochemical profiles of major sequence types.

Cluster analysis of a simple matching coefficient based on the metabolic profiles was used to generate a dendrogram describing the association of ST131 and antibiogram profiles. This revealed a large ST131 cluster defined at the 88% similarity level and comprised of 22 (60%) isolates that were characterized as having a multiple resistance antibiogram (Fig. 2).

A nine-digit numerical code (three tests per digit) was generated using the results of 27 biochemical tests (3 tests being positive for all isolates) to yield the Bio score, with a high score indicating increased metabolic potential. A total of 149 different profiles were detected, of which 19 were solely associated with ST131 isolates. Figure 3 shows the distribution of isolates on the basis of biochemical profiles. Interestingly, isolates of ST131 were divided into 2 groups, susceptible and multidrug resistant, where multidrug-resistant isolates and those of CTX-M-15 O25b were significantly associated with high Bio scores.

To investigate whether the observed distinctive characteristics of ST131 are general characteristics of the clone or are only the result of O25b-CTX-M-15 ST131 clonal spread, a comparative analysis of incidence of these characteristics in O25b-CTX-M-15 ST131 and non-O25b-CTX-M-15 ST131 isolates compared with non-ST131 isolates was carried out. Although numbers of representative strains are small in some cases and most of the characteristics are general features of the ST131 clone, some biochemical traits were significantly associated with O25b-CTX-M-15 ST131,

TABLE 4 Correlation between different phenotypic characteristics and different STs[a]

ST	Correlation coefficient (r^2), Bio vs. AST	*P* value, Bio vs. AST	
		Statistical difference	Correlation significance
ST10	0.0000	—	—
ST14	0.1123	—	—
ST69	0.0041	—	—
ST73	0.0018	—	—
ST88	0.0132	—	—
ST95	0.0325	—	—
ST127	**0.1032**	—	—
ST131	**0.3689**	*<0.0001*	*< 0.01*
ST405	**0.0608**	—	—

[a] *P* values (by Fisher's exact test) are shown only where $P \leq 0.05$. Bold indicates positive correlation.

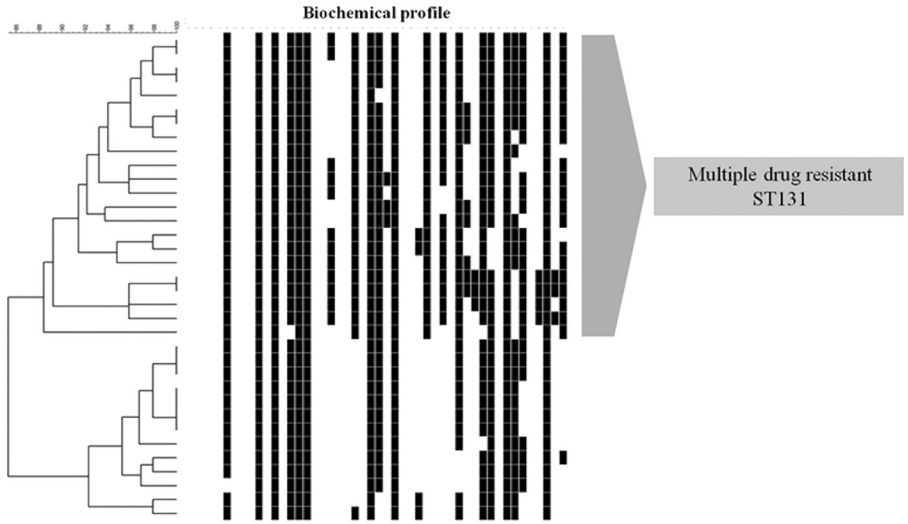

FIG 2 Biotype profiling of ST131 isolates: cluster analysis based on metabolic profiles.

such as tyrosine arylamidase, L-lactate alkalinization, phosphatase, and succinate alkalinization ($P < 0.005$) (Table 5).

DISCUSSION

UPEC bacteria have long been recognized as distinct clones of *E. coli*, which exhibit specific characteristics, such as virulence-associated traits, distinctive O antigens, selected genotypes, and multidrug resistance phenotypes (31). This is the first report of metabolic profiling being used to describe the globally disseminated ST131 clone. The isolates examined were collected at time points 2 years apart and represent UPEC from community- and hospital-associated infections in patients living in rural and urban areas.

In our collection, UPEC isolates showed biochemical profiles similar to those of the general *E. coli* population with one exception: α-galactosidase was observed in 69% of UPEC isolates compared to 99% of the general *E. coli* population, as reported by other groups (5, 6, 17). The reason for the discrepancy in the relatively low incidences of α-galactosidase among UPEC reported in the current study is not clear. However, the low pH and low glucose concentration of urine could present a different environmental selective pressure regulating metabolic pathway expression (19).

Some inferences could also be drawn regarding the correlation between biochemical tests and STs. Although production of ornithine decarboxylase and assimilation of sucrose appeared to be key characteristic in many successful STs, ST131 showed significant correlations with three types of metabolic reactions: peptidase (proline arylamidase), decarboxylase (ornithine decarboxylase), and alkalinization (L-lactate). Among ST131 isolates, the O25b-CTX-M-15 isolates showed a significant correlation with

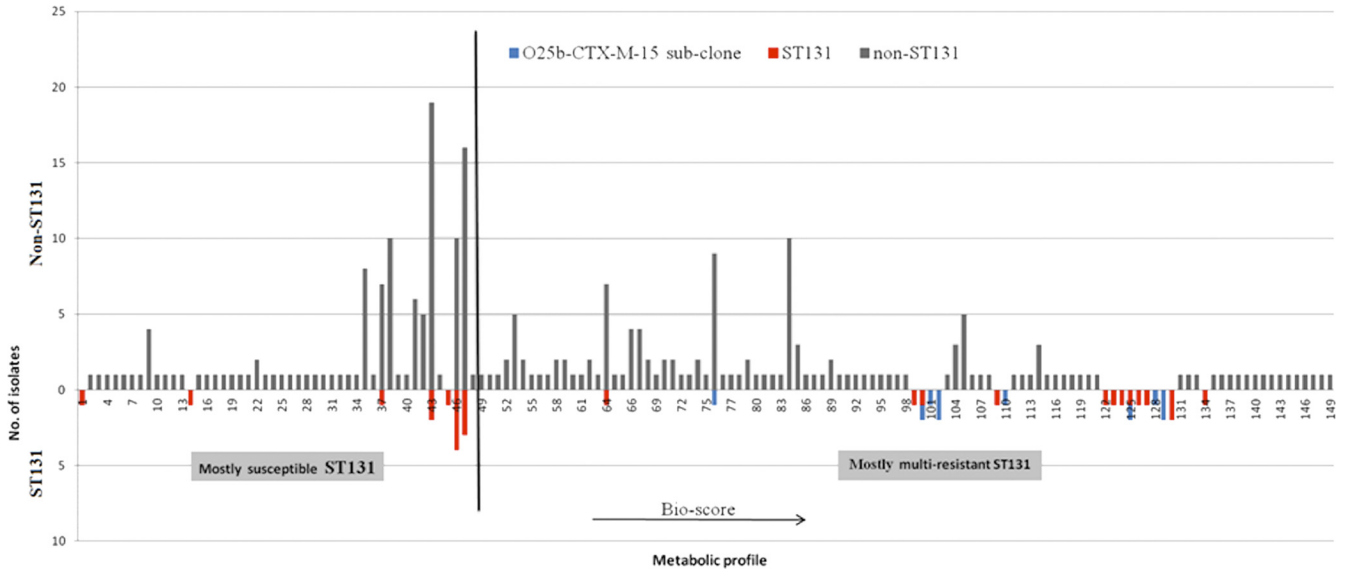

FIG 3 Distribution of metabolic profiles of UPEC isolates. ST131 isolates displayed at the left side of the *x* axis (left of the black line crossing the *x* axis) were predominantly susceptible, and those on the right side were resistant (as projected earlier by cluster analysis).

TABLE 5 Characteristics of *E. coli* ST131 isolates compared to non-ST131 isolates[a]

Biochemical test	No. of ST131 isolates with trait[b]			No. of non-ST131 isolates with trait[c]	
	O25b-CTX-M-15	Non-O25b-CTX-M-15	*P* value		*P* value
AGAL	11	22		175	0.0042
BGUR	7	20		248	0.0001
ILATa	2	1		0	0.0018
ILATk	11	12	0.0022	97	0.0042
5KG	0	4		119	0.0001
ODC	10	25		192	0.0034
PHOS	7	1	0.0002	28	
ProA	4	9		15	0.0001
SAC	10	24		133	0.0001
SUCT	10	10	0.0043	108	
TyrA	11	13	0.0032	138	

[a] *P* values (Fisher's exact test) are shown where $P < 0.005$ for metabolic reactions. For abbreviations, see Table 1.
[b] For O25b-CTX-M-15, $n = 11$; for non-O25b-CTX-M-15, $n = 26$.
[c] $n = 263$.

two other metabolic reactions, tyrosine arylamidase and succinate alkalinization. Proline and tyrosine arylamidase are specific peptidase enzymes that hydrolyze proline- and tyrosine-rich proteins (7). Similarly, ornithine decarboxylase is also involved in protein metabolism.

During growth in human urine, *E. coli* scavenges amino acids and peptides, and disruption of peptide import in UPEC significantly compromises fitness, suggesting that short peptides taken up by UPEC are degraded to amino acids and catabolized as intermediates for the tricarboxylic acid (TCA) cycle and a substrate for the gluconeogenesis pathway (1). The other metabolic reactions, such as alkalinization of lactate and succinate, observed with an elevated incidence could be related to bacterial efforts to relieve acid stress generated by amino acid metabolism.

We suggest that the high metabolic capacity of ST131 compared to that of other STs allows isolates to compensate for their relatively low virulence capacity and explains the apparent virulence reported for members of ST131 (8). In contrast, ST127 showed the lowest metabolic capacity, but we have previously shown that members of this clone have a high virulence potential (8). The association of clonal groups with specific biochemical profiles was previously reported for the O157:H7 clone (17) and the O15:K52:H1 and O25:H4-ST131 clones (4) using API 20E tests, though we cannot compare our data with the findings of these studies.

Although no significant association was observed between the Bio and AST scores in the entire UPEC collection, reexamining the correlation at the ST level revealed an appreciable positive correlation between metabolic scores and the antibiotic resistance scores of members of the ST131 clone. Cluster analysis based on biochemical profiles demonstrated that more than half of the ST131 isolates shared 70% similarity, forming a single large cluster that was also characterized by multiple antibiotic resistances, compared to the rest of the ST131 isolates. This suggests ongoing subclonal variation and spread of ST131 in the Northwest region of England.

Several factors may play a role in the success of the ST131 clone; in 2010, Peirano and Pitout suggested a combination of a phylogenetic group B2 background with certain virulence factors and fluoroquinolone resistance as important factors in the success of the ST131 clone in causing UTI, whereas the acquisition of CTX-M-15-carrying plasmids has facilitated the rapid global spread of the clone (23). Lavigne and colleagues recently suggested that members of the ST131 clone may represent pathogens with an optimal balance of limited virulence and antibiotic resistance (15). Johnson suggested that undefined phylogenetic group B2-associated factors provide fitness advantages to ST131, independent of its virulence traits (11). Recent draft genome sequence determination for ST131 strain EC958 revealed that the chromosomal backbone is most similar to that of the commensal *E. coli* O150:H5 strain SE15 (28, 29). SE15 is a B2 strain lacking several important genomic islands and prophage elements seen in EC958. The similarity between EC958 and a commensal strain supports our suggestion that metabolic potential is important for the fitness of ST131 strains with respect to gut colonization. Previous reports have also highlighted high levels of intestinal carriage of ST131, indicating fitness for this environment (18, 25, 30). In a similar manner, Roos et al. attributed the ability of ABU isolates to become established in the human bladder to their metabolic fitness (26).

We suggest that further investigation of the metabolic potential of ST131 isolates, using additional phenotypic assays and genome sequence determination, will provide new perspectives in understanding the success of this and other clones (16). It may also be relevant to examine the metabolic potential of ST131 isolates from other infection sites and non-UPEC *E. coli* to investigate the specificity of the profiles identified in the current study.

The high metabolic potential may facilitate adaptation of strains to different host environments, taking advantage of available nutrients present in the gut or in urine, such as amino acids and small peptides, promoting colonization and increasing pathogenic potential (16). In a diagnostic sense, it may also be possible to exploit specific metabolic traits in the development of differential media for recovery and presumptive identification of ST131 isolates.

In conclusion, this study determined the metabolic capacity of members of successful STs among a well-defined UPEC population. Several correlations were observed between the successful STs and key traits, and these patterns could help to explain why isolates from certain STs, including ST131, are successful extraintestinal pathogenic *E. coli* clones.

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