

# Distribution of Strain Type and Antimicrobial Susceptibility of *Escherichia coli* Isolates Causing Meningitis in a Large Urban Setting in Brazil

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The clinical management of meningitis caused by *Escherichia coli* is greatly complicated when the organism becomes resistant to broad-spectrum antibiotics. We sought to characterize the antimicrobial susceptibilities, sequence types (ST), and presence of known drug resistance genes of *E. coli* isolates that caused meningitis between 1996 and 2011 in Salvador, Brazil. We then compared these findings to those for *E. coli* isolates from community-acquired urinary tract infections (UTI) that occurred during the same time period and in the same city. We found that 19% of *E. coli* isolates from cases of meningitis and less than 1% of isolates from UTI were resistant to third-generation cephalosporins. The sequence types of *E. coli* isolates from cases of meningitis included ST131, ST69, ST405, and ST62, which were also found among isolates from UTI. Additionally, among the *E. coli* isolates that were resistant to third-generation cephalosporins, we found genes that encode the extended-spectrum beta-lactamases CTX-M-2, CTX-M-14, and CTX-M-15. These observations demonstrate that compared to *E. coli* strains isolated from cases of community-acquired UTI, those isolated from cases of meningitis are more resistant to third-generation cephalosporins, even though the same sequence types are shared between the two forms of extraintestinal infections.

The prevalence of antimicrobial drug resistance in Gram-negative bacteria continues to increase worldwide, which complicates the clinical management of infections caused by these organisms (1). These infections include enteric as well as extraintestinal infections such as urinary tract infections (UTI), sepsis, and meningitis (2, 3). *Escherichia coli* is a Gram-negative bacillus that is capable of causing urinary tract infections (UTI) and meningitis, but it is also a normal constituent of the gut microbiota of mammals (2, 4).

*E. coli* isolates can be classified into pathomorphs based on the type of disease caused (2, 5). Johnson et al. termed *E. coli* strains isolated from extraintestinal sites as extraintestinal pathogenic *E. coli* (ExPEC) based on a distinct set of virulence genes (6). ExPEC can be further subdivided into pathomorphs that include those that cause urinary tract infection (uropathogenic *E. coli* [UPEC]) and those that cause neonatal meningitis, also known as neonatal meningitis-causing *E. coli* [NMEC] (2, 5, 7–9). This is an area of active research, but currently there are no virulence factors that unequivocally distinguish these groups of ExPEC.

It is thought that the *E. coli* strains that cause extraintestinal infections first colonize the gut (2). From the gut the *E. coli* organisms are then able to spread to the urinary tract and bloodstream (2). Animal models of *E. coli* meningitis have shown that once the *E. coli* organisms reach a high enough concentration in the bloodstream, they are able to cross the blood-brain barrier and cause meningitis (10, 11). The severity of meningitis has been correlated with the concentration of bacteria in the bloodstream (2). *E. coli* is the leading cause of UTI and second leading cause of neonatal meningitis in developed countries (2, 12). Although it is not as common, *E. coli* also causes meningitis in adults (12, 13).

Isolates of *E. coli* that have caused neonatal meningitis in developed countries have been extensively studied (10, 14–19). These studies have found that some serotypes and sequence types predominate among the *E. coli* strains that cause meningitis. How-

ever, these same serotypes and sequence types are found in *E. coli* strains isolated from other sources such as urinary tract infections, poultry, and healthy human digestive tracts (15, 16, 20). Serotypes of *E. coli* that have been isolated from cases of meningitis include O18:K1, O45:K1, O1:K1, and O83:K1 (14, 19, 21, 22). The sequence types isolated from cases of meningitis include ST95, ST62, ST131, and ST69 (14, 19, 20, 22–24). When combinations of typing methods and more discriminant typing methods are used, isolates that have caused meningitis can be separated from isolates from other sources (15, 16). However, most studies examined a limited number of collections of *E. coli* strains available for study obtained from different geographic sites and time periods, and hence, it is unclear how significant these differences are.

There are recent reports of meningitis caused by *E. coli* strains that are resistant to third-generation cephalosporins and produce extended-spectrum beta-lactamases (ESBL) (23, 25). The spread of *E. coli* strains that are resistant to broader-spectrum cephalosporin antibiotics is problematic because these antibiotics are recommended for the treatment of meningitis caused by *E. coli* (11, 12). We sought to characterize *E. coli* strains isolated from UTI and meningitis patients by both multilocus sequencing typing (MLST) and the presence of ESBL genes in order to better understand the spread and distribution of drug resistance in *E. coli* strains that cause extraintestinal infections.

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## MATERIALS AND METHODS

**Isolate collection.** The isolates characterized in this study were originally collected as part of three separate studies of meningitis and community-acquired UTI that occurred in Salvador, Brazil (26, 27). Due to sample loss, only a subset of the UTI isolates were characterized. All *E. coli* isolates from the meningitis collection were included in the current study.

As part of active hospital-based surveillance, *E. coli* isolates from cases of meningitis that occurred between 1996 and 2011 in Salvador were consecutively collected. The study was based at Courto Maia Hospital, which serves as the infectious disease referral hospital and reference laboratory. This laboratory is part of the public health system, and all cases of meningitis that occur in the region are required to be reported to this laboratory. As part of an ongoing study of the etiology of meningitis, limited epidemiologic information is recorded, and bacterial isolates are collected from this reference laboratory and then biobanked at the Gonçalo Moniz Research Center. The methodology and results of this study were previously published (27).

*E. coli* isolates from community-acquired UTI were collected as part of two separate studies, one that occurred between 2001 and 2002 and another that occurred between 2008 and 2009. The results and methodology of the study of *E. coli* isolates from UTI that occurred between 2001 and 2002 have been published elsewhere (26). The study of *E. coli* isolates collected from UTI from 2008 to 2009 is unpublished (M. G. Barberino, J. N. Reis, and E. D. Moreira, Jr., personal communication). The antimicrobial susceptibilities of the UTI *E. coli* isolates reported here were determined as part of this previous study. The 2008-2009 UTI study by Barberino et al. occurred at a private outpatient emergency room in Salvador, Brazil, and was conducted in accordance with all relevant protections for human subjects (institutional review board [IRB] 180/2008).

As previously published, the isolates from the 2001-2002 and the 2008-2009 UTI collections were classified as susceptible or nonsusceptible to each antimicrobial agent tested. Nonsusceptible isolates included isolates that showed either intermediate or resistant phenotypes.

**Antimicrobial susceptibility testing.** The susceptibilities of the *E. coli* isolates from cases of meningitis were determined with MicroScan BP38 panels (Siemens). ATCC 27853 and ATCC 25922 strains were used as reference strains for all tests.

According to the manufacturers' published breakpoints, isolates were classified as susceptible, intermediate, or resistant to each drug. Where indicated, isolates were classified as either susceptible or nonsusceptible. The nonsusceptible isolates contained all those with either the intermediate or resistant phenotype.

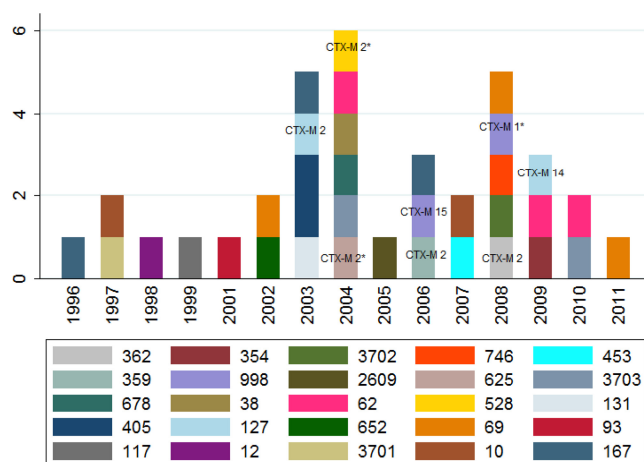
**DNA extraction, beta-lactamase detection, and sequencing.** DNA was extracted with a Promega Wizard genomic DNA kit according to the manufacturer's recommendations.

Meningitis isolates were screened for the presence of *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>OXA</sub>, and *bla*<sub>CTX-M</sub> gene sequences with multiplex PCR protocols previously published by Dallenne et al. (28). UTI isolates were screened for only *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>OXA</sub>. The primer sets used were Multiplex I TEM, SHV, and OXA-1-like and Multiplex II CTX-M group 1, group 2, and group 9 (28). All reactions and gel electrophoresis were performed according to previously published protocols (28). For each reaction, DNA known to contain the relevant gene sequence was used as a positive control, and water was used as a negative control.

The PCR products that tested positive for a beta-lactamase were submitted for sequencing to the University of California, Berkeley, DNA Sequencing Facility. The resulting sequences were then submitted to BLASTN for identification (29).

**Multilocus sequence typing and sequence analysis.** Multilocus sequence typing (MLST) was performed according to previously published protocols (20). The sequences were submitted to the online MLST database at <http://mlst.ucc.ie/mlst/dbs/Ecoli> for sequence type (ST) identification.

All sequencing was done at the University of California, Berkeley,



**FIG 1** Sequence types versus year of isolate collection. Isolates that were resistant to third-generation cephalosporins are labeled with the *bla*<sub>CTX-M</sub>-type gene they carry. Isolates marked with an asterisk did not yield an interpretable DNA sequence and are labeled with the name of the *bla*<sub>CTX-M</sub> group they tested positive for.

DNA Sequencing Facility. The resulting sequences were cleaned, assembled, and analyzed with Geneious 5.6.

**Data analysis.** All data analyses and graphs were generated with STATA 10. *P* values were calculated with Fisher's exact test.

## RESULTS

**Characterization of *E. coli* causing meningitis.** Thirty-six *E. coli* isolates from cases of meningitis were collected from patients in Salvador, Brazil, from 1996 to 2010. Figure 1 displays the year of isolation and sequence type of each of these isolates. The ages of the patients who had meningitis were known for 21 isolates. Thirteen (62%) of these isolates were from children <2 years of age. Three novel sequence types (ST3701, ST3702, and ST3703) were identified among these isolates. All of the isolates with novel sequence types were isolated from cases of meningitis in children who were <2 years old.

Among the meningitis isolates, 19% were resistant to ceftazidime and cefotaxime, 17% were resistant to aztreonam, and 14% were resistant to ciprofloxacin (Table 1). All isolates that were resistant to third-generation cephalosporins were found to have caused meningitis after 2002 (Fig. 1). We did not find resistance to carbapenems, cefotetan, or tigecycline.

Eighty-six percent of isolates carried *bla*<sub>TEM</sub>, 13% carried *bla*<sub>SHV</sub>, and none carried *bla*<sub>OXA</sub>. Thirty-six isolates tested positive for *bla*<sub>CTX-M</sub>. All isolates that were resistant to third-generation cephalosporins carried a *bla*<sub>CTX-M</sub>-type gene. Five percent of isolates tested positive for *bla*<sub>CTX-M</sub> group 1, 3% for *bla*<sub>CTX-M</sub> group 9, and 28% for *bla*<sub>CTX-M</sub> group 2. Of the 13 isolates that tested positive for CTX-M, 10 yielded interpretable sequences. *bla*<sub>CTX-M-2</sub> was identified in isolates ST652, ST62, ST405, ST127, ST167, ST359, and ST362. *bla*<sub>CTX-M-15</sub> was found in one ST998 isolate. *bla*<sub>CTX-M-14</sub> was found in one ST127 isolate (Fig. 1).

**Comparison of *E. coli* isolates that caused UTI and meningitis.** The *E. coli* strains isolated from cases of meningitis had a significantly (*P* < 0.001) higher frequency of resistance to third-generation cephalosporins than the *E. coli* strains that were isolated from UTI. The study of the 544 cases of UTI in Salvador that took place in 2001-2002 found that <1% of isolates were nonsus-

**TABLE 1** Results of antimicrobial susceptibility testing of 36 *E. coli* isolates from cases of meningitis<sup>a</sup>

Antimicrobial	<i>E. coli</i> isolates that were:					
	Susceptible		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Amikacin	32	89	1	4	3	8
Gentamicin	31	86	NA	NA	5	14
Tobramycin	31	86	NA	NA	5	14
Chloramphenicol	27	75	1	4	8	22
Tetracycline	15	42	NA	NA	21	58
Nitrofurantoin	35	97	NA	NA	1	3
Ciprofloxacin	31	86	NA	NA	5	14
Levofloxacin	32	89	NA	NA	4	11
Piperacillin	13	36	1	4	22	61
Ampicillin-sulbactam	17	47	5	19	14	39
Piperacillin-tazobactam	33	92	2	8	1	3
Aztreonam	30	83	NA	NA	6	17
Cefazolin	25	69	1	4	10	28
Cefepime	31	86	NA	NA	5	14
Cefotaxime	28	78	1	4	7	19
Cefoxitin	35	97	1	4	0	0
Ceftazidime	29	81	NA	NA	7	19
Ceftriaxone	28	78	1	4	7	19
Cefuroxime	28	78	NA	NA	8	22
Cephalothin	12	33	11	42	13	36
Trimethoprim-sulfamethoxazole	12	33	NA	NA	24	67

<sup>a</sup> The categories marked with NA were not tested. Beta-lactam and beta-lactamase inhibitor combinations are ampicillin-sulbactam and piperacillin-tazobactam. Aztreonam is a monobactam. First-generation cephalosporins are cephalothin and cefazolin, second-generation cephalosporins include cefoxitin and cefuroxime, third-generation cephalosporins are cefotaxime and ceftazidime, and the fourth-generation cephalosporin is cefepime.

ceptible (intermediate or resistant phenotypes) to third-generation cephalosporins (26). The study of the 411 cases of community-acquired UTI that took place between 2008 and 2009 found that all isolates were susceptible to third-generation cephalosporins. The current study of the 36 *E. coli* isolates from cases of meningitis found that 22% were nonsusceptible to third-generation cephalosporins and that 19% were resistant to third-generation cephalosporins.

We compared the *E. coli* strains that caused both UTI and meningitis by comparing the sequence types of isolates with identical drug-resistant phenotypes. The isolates were classified as susceptible or nonsusceptible to eight classes of antimicrobial drugs because different antimicrobial susceptibility testing methods, drugs, and breakpoints were used to characterize each collection of *E. coli* isolates. Only the classes of drugs that all isolates were tested with were included in this analysis. These classes were tetracyclines (TET), aminoglycosides (AMI), trimethoprim-sulfamethoxazole (TSX), penicillins (PEN), first-generation cephalosporins (CEPH1), third generation cephalosporins (CEPH3), fluoroquinolones (FLR), and nitrofurantoin (NIT). Each drug resistance phenotype was named after the classes of drugs for which the isolates were nonsusceptible.

The drug resistance phenotypes found in both the UTI and meningitis collections were TET<sup>+</sup> TSX<sup>+</sup> PEN<sup>+</sup> CEPH1, TSX<sup>+</sup> PEN<sup>+</sup> CEPH1, TET<sup>+</sup> TSX<sup>+</sup> PEN, PEN<sup>+</sup> CEPH1, TET<sup>+</sup> TSX<sup>+</sup> CEPH1, TET<sup>+</sup> TSX, TET<sup>+</sup> TSX<sup>+</sup> FLR, and TET<sup>+</sup> TSX<sup>+</sup> FLR<sup>+</sup> CEPH1 (Table 2). The available UTI isolates with these phenotypes were submitted for multilocus sequence typing and screened for the *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>OXA</sub> gene sequences. From these isolates, 10 sequence types were identified in both the meningitis and UTI collections. These sequence types were ST10, ST62, ST69, ST93, ST127, ST131, ST167, ST405, ST998, and ST2609.

Of these common sequence types, three types (ST10, ST62, and ST69) had the same drug resistance phenotypes in both the men-

**TABLE 2** Drug-resistant phenotypes, beta-lactamase (*bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, or *bla*<sub>OXA</sub>), and sequence types found in collections of *E. coli* from meningitis and UTI<sup>a</sup>

Phenotype	Results for meningitis			Results for UTI in:					
	No.	Sequence type(s)	<i>bla</i> gene(s)	2001-2002			2008-2009		
				No.	Sequence type(s)	<i>bla</i>	No.	Sequence type(s)	<i>bla</i> gene(s)
TET <sup>+</sup> TSX <sup>+</sup> PEN <sup>+</sup> CEPH1	5	167, 678, 69	<i>bla</i> <sub>TEM</sub>	6	394, 127, 998, 38, 73	<i>bla</i> <sub>TEM</sub>	18	38, 69, 93, 104, 31, 998, 14, 131, 73, 405, 62, 372, 3,928	<i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>SHV</sub>
TSX <sup>+</sup> PEN <sup>+</sup> CEPH1	4	3703, 2609, 62	<i>bla</i> <sub>TEM</sub>	4	62, 38, 73, 127	<i>bla</i> <sub>TEM</sub>	15	69, 62, 10, 12, 14, 23, 73, 38, 12, 2,609	<i>bla</i> <sub>TEM</sub>
TET <sup>+</sup> TSX <sup>+</sup> PEN	4	69, 746, 3702	<i>bla</i> <sub>TEM</sub>	24	550, 167, 976, 3,704, 73, 69, 131, 3,910, 1,312, 3,704, 205, 38, 10	<i>bla</i> <sub>TEM</sub>	7	69, 95, 14, 38, 74, 827	<i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>SHV</sub>
PEN <sup>+</sup> CEPH1	2	652, 62	<i>bla</i> <sub>TEM</sub>	3	127, 10, 73	<i>bla</i> <sub>TEM</sub>	6	3,705, 73, 3,706, 38, 127	<i>bla</i> <sub>TEM</sub>
TET <sup>+</sup> TSX <sup>+</sup> CEPH1	2	405	<i>bla</i> <sub>TEM</sub>	1	46	<i>bla</i> <sub>TEM</sub>	None	None	None
TET <sup>+</sup> TSX	1	10	<i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>SHV</sub>	10	10, 3903, 167, 73, 127, 46, 3,904, 12, 998	<i>bla</i> <sub>TEM</sub>	1	394	None
TET <sup>+</sup> TSX <sup>+</sup> FLR	1	117	<i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>SHV</sub>	1	533	None	0	None	None
TET <sup>+</sup> TSX <sup>+</sup> FLR <sup>+</sup> CEPH1	1	354	None	0	None	None	1	3,902	<i>bla</i> <sub>SHV</sub>

<sup>a</sup> UTI, urinary tract infection.

ingitis and UTI collections. Of these three sequence types, only ST69 and ST62 showed the same beta-lactamase genotype in the UTI and meningitis collections.

Isolates with the phenotype TET<sup>+</sup> SXT<sup>+</sup> PEN<sup>+</sup> CEPH1 that were ST69 and that carried the *bla*<sub>TEM</sub> gene were isolated from a case of meningitis in 2008 and from six cases of UTI from 2008 to 2009. Isolates with the phenotype TET<sup>+</sup> SXT<sup>+</sup> PEN that were ST69 and that carried the *bla*<sub>TEM</sub> gene were isolated from a case of meningitis in 2001, six cases of UTI that occurred between 2001 and 2002, and one case of UTI that occurred between 2008 and 2009. Isolates with the phenotype SXT<sup>+</sup> PEN<sup>+</sup> CEPH1 that were ST62 and that carried the *bla*<sub>TEM</sub> gene were isolated from two cases of meningitis in 2009 and 2010. ST62 with *bla*<sub>TEM</sub> and the same drug resistance phenotype was also isolated from one UTI that occurred between 2001 and 2002 and from one UTI that occurred between 2008 and 2009.

## DISCUSSION

To better understand the spread of drug-resistant *E. coli* strains, we compared MLST and presence of *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> beta-lactamase genes from *E. coli* strains isolated from UTI and meningitis cases. We strain typed the *E. coli* by an MLST protocol which is commonly used to characterize drug-resistant *E. coli* isolates (20, 30). We identified a number of internationally distributed sequence types that are frequently drug resistant. These sequence types have also previously been isolated from food products and include the ST10 complex, ST69, ST117, ST131, and ST405 (30). ST69, previously labeled CgA, was first reported from UTI patients in Berkeley, California, USA, and has also been reported from UTI patients in Rio de Janeiro, Brazil (31, 32). ST62 has previously been associated with K1 *E. coli*, a serotype that frequently causes neonatal meningitis (20). Our findings further highlight the worldwide distribution of these lineages and the fact that they are capable of causing meningitis.

The results of antimicrobial susceptibility testing of the *E. coli* strains that caused meningitis showed that 19% were resistant to third-generation cephalosporins. These drugs are currently recommended for the treatment of meningitis suspected to be caused by Gram-negative organisms (12). In this study, we also identified extended-spectrum beta-lactamase genes in all meningitis isolates that were resistant to third-generation cephalosporins. In addition to the presence of *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes, this study identified *bla*<sub>CTX-M-2</sub>, *bla*<sub>CTX-M-15</sub>, and *bla*<sub>CTX-M-14</sub> genes among the *E. coli* strains that caused meningitis. Cases of meningitis caused by ESBL-producing *E. coli* strains have been also reported from Turkey, France, Algeria, Thailand, Germany, and Brazil (23, 33).

The *E. coli* strains that caused meningitis were more resistant to third-generation cephalosporins than those that caused community-acquired UTI. While the numbers of *E. coli* strains that caused meningitis are small ( $n = 36$ ), this difference was still statistically significant. Unfortunately, the UTI isolates that were nonsusceptible to third-generation cephalosporins were lost, and we were unable to investigate these isolates further.

The results of this study support the findings of previous studies comparing *E. coli* strains from cases of meningitis and *E. coli* strains isolated from other sources (14, 15, 21). These studies showed that the same sequence types are frequently found in *E. coli* strains that are isolated from meningitis and other sources, and once further typing schemes are applied, differences can be found. In our study, these overlapping sequence types included

the most common *E. coli* sequence types in the UTI collection. When the drug resistance profiles and beta-lactamase genotypes were compared, many of the *E. coli* strains from the meningitis cases could be further differentiated from those that caused UTI. The meningitis-causing *E. coli* strains may express additional factors that render them more invasive.

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