# Antimicrobial resistance, multilocus sequence types and virulence profiles of ESBL producing and non-ESBL producing uropathogenic Escherichia coli isolated from cats and dogs in Switzerland

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Abstract: Among 64 uropathogenic Escherichia coli (UPEC) isolated from 13 cats and 51 dogs, 35 were extended-spectrum beta-lactamase (ESBL) producers, and 29 were non-ESBL producers. Forty-six (71.9%) of the isolates were multidrug resistant (MDR). Among the ESBL producers, blaCTX-M-15 (n = 17/48.6% of the blaESBLs), blaCTX-M-1 (n = 10/28.6%), blaCTX-M-55 (n = 4/11.4%), blaCTX-M-14 (n = 3/8.6%), and blaCTX-M-27 (n = 1/2.9%) were identified. The plasmid-mediated fluoroquinolone resistance genes aac(6')-Ib-cr, qnrB and the azithromycin resistance gene mph(A) were detected in 17 (26.6% of all isolates), one (1.6%) and in 13 (20.3%) respectively. The most frequent phylogenetic groups were C (n = 19) and B2 (n = 15). Twenty-six different sequence types (STs) were identified, with two being novel. The most frequent STs were ST410 (n = 16/25%), ST131, and ST73 (both n = 5/7.8%), and ST361 (n = 4/6.3%). Ten (15.6%) of the STs have been associated with urinary tract infection (UTI) in humans, suggesting zoonotic potential. Among seven virulence-associated genes, fyuA was the most prevalent. The overall aggregate virulence factor (VF) score was highest for isolates belonging to phylogenetic group B2 (median aggregate VF score 6, mean score 5,5, range 3-7), and lowest for isolates belonging to phylogenetic group C (0/ 0.5/0-3). The most frequent ST in this study, ST410, harboured the lowest number of VF (0/0,3/0-2). VF scores were higher in NDR (4/3.8/3-4) than in MDR (1/1,9/0-7), and higher in non-ESBL producing isolates (3/3/0-7) than in ESBL producers (1/1,7/0-7). Our data advance our knowledge of the phenotypic and genotypic characteristics of UPEC in companion animals and their potential for infection, zoonotic transmission and dissemination of antimicrobial resistance determinants.

DOI: https://doi.org/10.1016/j.vetmic.2018.02.011

# Antimicrobial Resistance, Multilocus Sequence Types and Virulence Profiles of ESBL producing and non-ESBL producing uropathogenic *Escherichia coli* isolated from Cats and Dogs in Switzerland

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**Abstract**

Among 64 uropathogenic *Escherichia coli* (UPEC) isolated from 13 cats and 51 dogs, 35 were extended-spectrum beta-lactamase (ESBL) producers, and 29 were non-ESBL producers. Forty-six (71.9%) of the isolates were multidrug resistant (MDR). Among the ESBL producers, $bla_{\text{CTX-M-15}}$ (n=17/48.6% of the $bla_{\text{ESBLs}}$), $bla_{\text{CTX-M-1}}$ (n=10/28.6%), $bla_{\text{CTX-M-55}}$ (n=4/11.4%), $bla_{\text{CTX-M-14}}$ (n=3/8.6%), and $bla_{\text{CTX-M-27}}$ (n=1/2.9%) were identified. The plasmid-mediated fluoroquinolone resistance genes *aac(6')-Ib-cr*, *qnrB* and the azithromycin resistance gene *mph(A)* were detected in 17 (26.6% of all isolates), one (1.6%) and in 13 (20.3%) respectively. The most frequent phylogenetic groups were C (n=19) and B2 (n=15). Twenty-six different sequence types (STs) were identified, with two being novel. The most frequent STs were ST410 (n=16/25%), ST131, and ST73 (both n=5/7.8%), and ST361 (n=4/6.3%). Ten (15.6%) of the STs have been associated with urinary tract infection (UTI) in humans, suggesting zoonotic potential. Among seven virulence-associated genes, *fyuA* was the most prevalent. The overall aggregate virulence factor (VF) score was highest for isolates belonging to phylogenetic group B2 (median aggregate VF score 6, mean score 5,5, range 3-7), and lowest for isolates belonging to phylogenetic group C (0/ 0.5/0-3). The most frequent ST in this study, ST410, harboured the lowest number of VF (0/0,3/0-2).

VF scores were higher in NDR (4/3.8/3-4) than in MDR (1/1,9/0-7), and higher in non-ESBL producing isolates (3/3/0-7) than in ESBL producers (1/1,7/0-7). Our data advance our knowledge of the phenotypic and genotypic characteristics of UPEC in companion animals and their potential for infection, zoonotic transmission and dissemination of antimicrobial resistance determinants.

**Key words**: uropathogenic, *Escherichia coli*, cats, dogs, MLST, virulence

## 1. Introduction

*Escherichia coli* occurs naturally in the digestive tract of humans and animals as a commensal, however, some strains of *E. coli* can cause extraintestinal diseases, such as pneumonia, sepsis, meningitis and urinary tract infections (UTI) (Kaper et al., 2004).

Commensal strains have been shown by phylogenetic analyses to be associated with phylogenetic group A, B1 or C, while virulent extraintestinal *E. coli* strains belong mainly to group B2 and, to a lesser extent, to group D, E, or F (Clermont et al., 2013).

Uropathogenic *E. coli* (UPEC) is the most common etiologic agent of UTI in humans and animals (Foxman, 2010; Olin and Bartges, 2015). The ability of UPEC to cause disease is associated with the expression of specific virulence factors (VF) encoding adhesins, invasins, toxins and other proteins (Kaper et al., 2004).

Among companion animals, *E. coli* clones that are found in the intestine may cause urinary tract infection (UTI) (Johnson et al., 2008b). Furthermore, UPEC may be acquired via external reservoirs: previous studies have demonstrated that humans and their companion animals can share pathogenic types of *E. coli,* including UPEC, indicating zoonotic as well as anthroponotic transmission (Johnson et al., 2008b; Johnson et al., 2008c; Johnson et al., 2009).

Other significant sources of pathogenic *E. coli* include raw meat canine diets and raw or inadequately cooked chicken meat (Glaser 2012; Schmidt et al., 2015).

For uncomplicated UTI in cats and dog, the first-line therapeutic option is amoxicillin, with sulfamethoxazole/trimethoprim and fluoroquinolones remaining appropriate for complicated infections and pyelonephritis, respectively (Barsanti, 2012). The emergence of antimicrobial resistance among UPEC is of great concern and increases the risk of antimicrobial treatment failure in companion animals, as in humans.

Understanding the prevalence of antimicrobial resistance and pathogenicity of UPEC isolated from cats and dogs is important both from veterinary medicine and public health perspectives,

79 but information regarding the population structure and virulence association of UPEC from
80 cats and dogs is still limited.
81 The aim of this study was therefore to (i) assess the antimicrobial susceptibility of
82 uropathogenic *E. coli* isolates obtained throughout 2012–2016 from Swiss dogs and cats, (ii)
83 to detect and characterize plasmid-mediated resistance to third-generation cephalosporins,
84 fluoroquinolones and azithromycin, (iii) to characterize the strains by phylogenetic grouping,
85 multilocus sequence typing (MLST) and virulence profiling.
86
87 **2. Materials and Methods**
88
89 **2.1. Bacterial isolates**
90
91 From a total of 205 *Escherichia coli* strains isolated between 2012 and 2016 from urine
92 samples of companion animals admitted to the University of Zürich veterinary clinic, a total
93 of 64 (35 ESBL-producing and 29 randomly selected non-ESBL producing) strains from 13
94 cats and 51 dogs were analysed. Strain identification and preliminary antimicrobial
95 susceptibility profiling was performed using VITEK 2 Compact system with AST GN38
96 cards (Biomérieux, Marcy l'Etoile, France) according to the manufacturer's instructions.
97
98 **2.2. Phenotypic and genotypic antimicrobial resistance testing**
99
100 Antimicrobial susceptibility testing was performed using the disk-diffusion method and the
101 antibiotics ampicillin (AM), cefazolin (CZ), cefotaxime (CTX), amoxicillin with clavulanic
102 acid (AMC), cefepime (FEP), nalidixic acid (NA), ciprofloxacin (CIP),
103 sulfamethoxazole/trimethoprim (SXT), fosfomycin (FOS), nitrofurantoin (F/M), streptomycin
104 (S), kanamycin (K), gentamicin (G), chloramphenicol (C) and tetracycline (TE) (Becton

105 Dickinson, Allschwil, Switzerland). Results were interpreted according to Clinical and
106 Laboratory Standards Institute (CLSI) performance standards (CLSI, 2016). Isolates
107 displaying resistance to three or more classes of antimicrobials (counting beta-lactams as one
108 class) were defined as multidrug-resistant (MDR). Synergistic effects between AMC and
109 CTX and FEP were regarded as an indication of the presence of an ESBL producer (Kaur et
110 al., 2013).
111 For genotypic analyses, bacterial DNA was extracted using a standard heat lysis protocol. The
112 presence of $bla_{\text{ESBL}}$ genes belonging to the $bla_{\text{TEM}}$, $bla_{\text{SHV}}$, and $bla_{\text{CTX-M}}$ families was
113 confirmed by PCR and amplicons were sequenced as described previously (Woodford et al.,
114 2006; Zurfluh et al., 2015). Ciprofloxacin resistant strains were examined for mutations in
115 quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC*, using previously
116 described PCR and sequencing primers (Zurfluh et al., 2014). Screening all isolates for the
117 plasmid-mediated fluoroquinolone resistance genes *aac(6')-Ib-cr*, *qnrA*, *qnrB*, *qnrC*, *qnrD*,
118 *qnrS*, and *qepA* was carried out as described previously (Zurfluh et al., 2014). Further, all
119 strains were screened for the plasmid-mediated azithromycin resistance gene *mph(A)* using
120 previously described primers (Ojo et al., 2004). DNA samples from isolates described
121 previously were used as positive controls (Nüesch-Inderbinen et al., 2017; Zurfluh et al.,
122 2014).
123 Synthesis of primers and DNA custom sequencing was carried out by Microsynth (Balgach,
124 Switzerland) and nucleotide sequences were analysed with CLC Main Workbench 6.6.1. For
125 database searches the BLASTN program of NCBI (http://www.ncbi.nlm.nih.gov/blast/) was
126 used.
127

128 **2.3. Phylogenetic characterization and multilocus sequence typing**

129

130 The distribution of phylogenetic groups amongst the isolates was determined by the

131 improved phylotyping PCR approach described by Clermont *et al.*, targeting the genes *chuA,*
132 *yjaA, arpA* and TspE4.C2 (Clermont et al., 2013). Isolates were thereby classified as
133 belonging to one of the eight phylogenetic groups A, B1, B2, C, D, E, F, (*E. coli sensu*
134 *stricto)*, or *Escherichia* clade I.
135 For multilocus sequence typing of *E. coli* isolates, internal fragments of the seven
136 housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA*, and *recA*) were amplified by PCR
137 from DNA, as described by Wirth *et al*. (Wirth et al., 2006). Sequencing of the amplification
138 products was performed by Microsynth (Balgach, Switzerland). Sequences were imported
139 into the *E. coli* MLST database website (http://mlst.ucc.ie/mlst/dbs/Ecoli) to determine
140 sequence types (STs). Alleles and STs that had not been previously described were designated
141 new ST, but not assigned numerical designations, since whole-genome sequencing was not
142 performed.
143
144 **2.4. Virulence factor (VF) determination**
145
146 All 64 strains were tested by conventional PCR for the presence of genes of VF that mediate
147 adhesion (p-fimbrial adhesion genes *papAH* and *papEF*, and the chaperone-usher fimbria
148 *yfcv*), toxins (α-haemolysin *hlyA*), siderophores (the ferric yersiniabactin uptake protein *fyuA*),
149 serum resistance (*traT*), and the right-hand terminus of pathogenicity island (PAI) from *E.*
150 *coli* strain CFT073, using primers and conditions described previously (Johnson and Stell,
151 2000; Spurbeck et al., 2012). The aggregate VF score was defined as the number of unique
152 VF detected for each isolate, counting the PAI marker as one.
153
154

155 **2.5** eBURST

156

157 The Based Upon Related Sequence Types (eBURST) clustering algorithm in phyloviz

158 (www.phyloviz.net) was used to visualise relationships between the isolates.

159

160 **3. Results**

161

162 **3.1. Antimicrobial resistance**

163

164 Between 2012-2016, 205 *E. coli* strains were collected (41 in 2012, 121 in 2013, 17 in 2014,

165 17 in 2015 and 9 in 2016). During these years, ESBL producers were detected in 10 (24.4%),

166 12 (9.9%), 4(23.5%), 6 (35.3%), and 3 (33.3%) of the isolates, respectively. Resistance

167 profiles of the 35 ESBL producing and 29 non-ESBL producing isolates to all 15

168 antimicrobials tested are shown in detail in supplementary Table S1. Resistance rates to

169 classes of antimicrobials commonly used for treating feline and canine *E. coli* UTIs are

170 summarized in Table 1.

171 Overall, 46 (71.9%) of the isolates were MDR (88.6% of the ESBL-producers, and 51.7% of

172 the non-ESBL producers, respectively). The presence of $bla_{\text{ESBL}}$ genes was confirmed and the

173 genes were identified in 35 phenotypic ESBL producers as $bla_{\text{CTX-M-15}}$ (n=17/48.6% of the

174 $bla_{\text{ESBLs}}$), $bla_{\text{CTX-M-1}}$ (n=10/28.6%), $bla_{\text{CTX-M-55}}$ (n=4/11.4%), $bla_{\text{CTX-M-14}}$ (n=3/8.6%), and

175 $bla_{\text{CTX-M-27}}$ (n=1/2.9%). Except for one $bla_{\text{CTX-M-1}}$, all $bla_{\text{ESBLs}}$ were detected in canine isolates.

176 Overall, 51 (79.7%) of the isolates were resistant to nalidixic acid, and 47 thereof were

177 ciprofloxacin resistant (Table 1 and supplementary Table S1). Among the isolates displaying

178 ciprofloxacin resistance, all revealed chromosomal mutations that result in amino acid

179 substitutions in GyrA and ParC (Table 2 and supplementary Table S1). The plasmid-mediated

180 fluoroquinolone resistance genes *aac(6')-Ib-cr* and *qnrB* were detected in 17 (26.6% of all

isolates), and one (1.6%) isolate, respectively, and were restricted to canine isolates (Table 3 and supplementary Table S1). The plasmid-mediated azithromycin resistance gene *mph(A)* was found in 13 (20.3%) of the isolates. The majority (n= 15/88.2%) of the 17 isolates with plasmid-mediated fluoroquinolone resistance and the majority (n= 34/69.4%) of the 49 isolates with chromosomally mediated quinolone resistance were ESBL producers (Table 3). Only 4 (6.3%) isolates were non-drug resistant (NDR) (supplementary Table S1).

**3.2. Phylogenetic groups and ST**

As shown in Table 3, the most prevalent phylogenetic group was C (n=19 /29.7% of all isolates), followed by group B2 (n=15 /23.4%), B1 (n=10/15.6%), group A (n=9/14%), group F (n=8/12.5%), and group D (n=3/4.7%). None of the isolates belonged to phylogenetic group E or clade I. Among feline isolates, phylogenetic group B2 predominated (n=7 /53.8% of all feline isolates), whilst among canine isolates, the majority (n=18/35.3%) belonged to group C. MLST assigned the majority (n=39/60.9%) of the isolates to 9 different clonal complexes (CC): CC10 (n=3), CC12 (n=2), CC23 (n=17), CC73 (n=5) and CC131 (n=5), CC156 (n=1), CC354 (n=2), CC469 (n=1), and CC648 (n=3).

Twenty-six different STs were identified, the three most common represented by ST410 (n=16/25%), ST131, and ST73 (both n=5/7.8%). Less frequently occurring STs included ST533 (n=4/6.3%), and ST361, ST744 and ST648 (each n=3/4.7%, respectively). All other STs were represented by one or two isolates only (Table 3 and supplementary Table S1). Two new STs were detected, which however were not assigned numerical designations by the *E. coli* MLST database (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). Figure 1 illustrates eBURST showing relationships between the isolates. The prevalence of the most common ST, ST410, was variable, declining from 50% in 2012 to 1% in 2015, with an increase to 33.3%

observed for 2016. Of the 64 isolates, a total of 10 (15.6%) belonged STs frequently associated with human UTI (ST73 and ST131).

The MDR, ESBL- and non-ESBL producers were distributed throughout phylogroups A, B1, B2, C, D and F.

### 3.3. Distribution of VF genes

Overall, 47 (73.4%) of the isolates tested positive for one or more of the VF genes (Table 3). Among the 64 isolates, the prevalence of individual VF genes was 53.1% for *fyuA,* 48.4% for *traT,* 34.4% for *yfcv* and PAI, 23.4% for *hlyA*, 20.3% for *papAH*, and 18.8% for *papEF*.

The overall median aggregate VF score was highest for isolates belonging to phylogenetic group B2 (median aggregate VF score 6, mean score 5,5, range 3-7), followed by isolates from phylogenetic group F (3/ 2,9/1-4). Isolates assigned to phylogenetic group D scored 2/2/2-2, and isolates of phylogenetic group A scored 1/1,8/ 0-6. The overall median aggregate VF scores were lowest for isolates belonging to phylogenetic group C (0/ 0.5/0-3).

Among the most common STs in this study, ST131 harboured the highest number of VF (7/6,4/4-7), followed by ST73 (4/4,6/4-7), and ST648 (4/3,7/3-4). Of the most prevalent ST in this study, ST410 harboured the lowest number of VF (0/0,3/0-2).

VF scores were higher in NDR (4/3.8/3-4) than in MDR (1/1,9/0-7), higher in non-ESBL producing isolates (3/3/0-7) than in ESBL producers (1/1,7/0-7), and higher in isolates that did not harbour *mph(A)* (2/2,6/0-7), than those that harboured *mph(A)* (1/1,1/0-4).

## 4. Discussion

In this study, we assessed antimicrobial resistance, phylogenetic background, and virulence profiles of ESBL producing, and non-ESBL producing UPEC isolated from cats and dogs

232 admitted to the veterinary clinic of the University of Zürich, Switzerland. In veterinary as in
233 human medicine, antimicrobials are the cornerstone of treatment of bacterial UTI.
234 Sulfamethoxazole/trimethoprim, amoxicillin/clavulanate, quinolones and chloramphenicol are
235 antimicrobial compounds that are recommended for the treatment of feline and canine UTI
236 due to infection with *E. coli* (Barsanti, 2012). Our data show that infection management may
237 be compromised by the high rate of resistance to these drugs, similar to the situation /as
238 described for UPEC in humans (Gibreel et al., 2012; Nicolle 2013; Nolan et al., 2015;
239 Lavigne et al. 2016). Furthermore, of the 205 isolates from our strain collection, 35 (17%)
240 were ESBL producers and the annual prevalence ranged from 9.9% to 35.3%. This is a
241 remarkable increase compared to the prevalence of 7.5% ESBL producers among UPEC
242 isolated from companion animals at our institution between 2010–2011 (Huber et al., 2013),
243 and poses a threat to the efficacy of third-generation cephalosporins approved for use in
244 veterinary medicine, such as cefovecin (Stegemann et al., 2006). Further, the high prevalences
245 of 26.6% *aac(6')-Ib-cr* and 13.6% *mph(A)* among the UPEC analysed in this study contrast
246 noticeably with the 2.3% and 13.6%, respectively, observed for these genes in UPEC from
247 human primary care patients in Switzerland (Nüesch-Inderbinen et al., 2017), and indicate
248 that companion animals, particularly dogs, may serve as reservoirs for *E. coli* harbouring
249 these resistance genes.
250 In this study, we identified the phylogenetic groups based on the new Clermont method which
251 enabled, besides the phylogenetic groups A, B1, B2, and D, the identification of groups C and
252 F among the feline and canine UPEC. Our results show that in accordance to previous reports,
253 UPEC isolates from cats frequently belonged to group B2 (Wagner et al., 2014; Liu et al.,
254 2015). However, overall, phylogroup C had the highest prevalence, a finding which is in
255 contrast to previous studies of UPEC in animals as well as in humans that indicate the
256 predominance of extraintestinal, pathogenic group B2 (Barsanti, 2012; Gibreel et al., 2012).
257 Data on the prevalences of phylogroups C, E, F and clade I among UPEC are rare, but a

recent study shows that among human isolates, approximately 25% belong to these groups (Iranpour et al., 2015). Since 42.2% of the isolates belonged to the newly described phylogroups C and F, further studies are needed to provide a more refined understanding of the prevalences of phylogenetic groups among *E. coli* isolates of animal and human origin. Antimicrobial resistant as well as susceptible UPEC in humans are frequently associated with specific clonal complexes such as CC69, CC73, CC95, ST127, and CC131 (Lau et al., 2008; Gibreel et al., 2012). Some of these CCs or STs, such as CC73, ST127 and CC131 have also been described as etiological agents of UTI in companion animals (Johnson et al., 2008a; Johnson et al., 2009). Corresponding well with reports on human UPEC (Bengtsson et al., 2012), we found 80% of the *E. coli* ST131 isolates to ESBL producers, and all of the ST73 isolates to be non-ESBL producers. Overall, our data specified that 15.6% of UPEC from cats and dogs belong to STs commonly associated with UTI in humans and harbour urovirulence genes that are identical to those found in human isolates (Gibreel et al., 2012). Other STs found in this study, such as ST648 and ST410 are also associated with extraintestinal disease in humans and animals, but not specifically with UTI (Ewers et al., 2014; Falgenhauer et al. 2016; Schaufler et al. 2016; Timofte et al., 2016). Therefore, although major UPEC clones described in human infection were not predominant, such strains exhibit reciprocal zoonotic potential, and should be considered a threat to pet owners and companion animals alike.

The high prevalence of ST410 in ESBL-producing UPEC from dogs has been observed previously in Switzerland (Huber et al., 2013). This ST, assigned to phylogroup C, displayed inverse association of VF scores with MDR and with plasmid-mediated resistance to third-generation beta-lactams, aminoglycosides and macrolides. This phenomenon was observed among other isolates belonging to phylogenetic groups A and C, suggesting that selective pressure, rather than virulence may be the driving force behind the high prevalence of ST410 among canine UPEC. However, the panel of VFs selected for this study was limited in number and represents only a subset of known VFs. Other determinants of virulence may

have been missed that may have shown a different tendency. Nevertheless, the high prevalence of isolates belonging to phylogenetic groups A, B1 and C indicate that the commensal flora of companion animals may play an important role in the aetiology of UTI. The results of this study illustrate the need for further observation of *E. coli* causing disease in companion animals, as well as the prudent use of antimicrobials in veterinary medicine, including the use of third generation cephalosporins, fluoroquinolones and macrolides.

## 5. Conclusion

In conclusion, our findings showed that feline and canine UPEC population was characterized by a high prevalence of MDR. ESBLs producing UPEC were detected almost exclusively among canine isolates. Phylogenetic groups B2 and F were associated more frequently with VF than other groups. In this study, group C was the most predominant phylogenetic group and displayed the lowest number of VF. MLST revealed a diverse clonal structure, and detected STs that may pose an infectious threat to humans. The most frequent MLST was ST410, which was characterized by a high prevalence of plasmid-mediated resistance genes *bla*$_{\text{CTX-M-15}}$, *aac(6')-Ib-cr* and *mph(A),* and a low aggregate VF score.
These results increase our current knowledge of the phenotypic and genotypic characteristics of feline and canine UPEC, and may be advantageous for future infection management and prevention.

## Conflict of Interest Statement

Conflicts of interest: none.

## Funding

309 This work was partly supported by the Swiss Federal Office of Public Health, Division
310 Communicable Diseases.
311

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**Figure legend**

MLST-based eBURST diagram depicting non-ESBL and ESBL producing UPEC isolated from cats and dogs. Each ST is represented by a circle, the size of which correlates to the frequency of the ST. Pie parts represent the number of strains within one ST. Exact numbers of isolates per ST are given in Table 3 and supplementary Table S1. Black outer rings show percentage of dog-derived strains from dogs, white outer rings show percentage of strains from cats. Isolates that tested negative by PCR for $bla_{\text{ESBL}}$ genes are coloured grey. Isolates harbouring $bla_{\text{CTX-M-1}}$ are depicted in blue,$bla_{\text{CTX-M-14}}$ in red, $bla_{\text{CTX-M-15}}$ in orange, $bla_{\text{CTX-M-27}}$ in turquoise, $bla_{\text{CTX-M-55}}$ in green.