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Turnover of dominant *Escherichia coli* lineages and antimicrobial resistance variations in children gastrointestinal tract. A prospective study.

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DEDICATORIA

A Cecilia, María Soledad, Gioconda y Janella, las cuatro mujeres que son luz en mi camino. A Ramiro, por su amor, alegría y motivación constante. De manera especial, dedico este trabajo a todas las mujeres de ciencia.

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RESUMEN

El tracto gastrointestinal (TGI) constituye un ecosistema complejo y diverso, de donde *Escherichia coli* es uno de los microorganismos mejor estudiados y caracterizados. Desde hace varias décadas se ha pretendido determinar su diversidad y estructura poblacional a lo largo del tiempo dentro de su hospedador; caracterizando así las cepas residentes, transitorias, dominantes y minoritarias. Considerando que las bacterias intestinales suelen albergar genes de resistencia a los antibióticos (AMR) en diversos elementos genéticos móviles (EGM), resulta interesante analizar el dinamismo de las cepas dominantes en el TGI, al igual que los cambios en su perfil de resistencia antimicrobiana a largo plazo. En este estudio prospectivo, se obtuvieron muestras de heces frescas pertenecientes a 31 niños menores de cinco años de edad y se realizó el seguimiento de los participantes en diferentes periodos de muestreo. De cada muestra se seleccionaron 5 colonias de *Escherichia coli* de la morfología predominante y de cada uno se determinó el perfil de resistencia a 12 antibióticos utilizando la técnica de difusión de disco y el número de alelo perteneciente al gen *fumC,* mediante secuenciación Sanger. Aquellos aislados que provenían del mismo niño y compartían el mismo número de alelo *fumC* en diferentes periodos de muestreo se genotipificaron mediante MLST. En cada periodo de muestreo se observó que por lo menos 3 de los 5 aislados de cada individuo compartían el mismo perfil de resistencia y número de secuencia tipo (ST). No obstante, es común que dichas características no se mantengan a lo largo del tiempo ya que únicamente se identificaron 4 individuos con posibles cepas residentes, lo que indica que el recambio poblacional de *Escherichia coli* dentro del TGI es sumamente dinámico. La permanencia de cepas predominantes resistentes a los antibióticos podría contribuir a la diseminación de dicha resistencia a otros miembros de la microbiota, al ambiente e incluso a otros hospedadores humanos y no humanos.

Palabras clave: *Escherichia coli,* resistencia antimicrobiana, cepas residentes, cepas transitorias, cepas dominantes, cepas minoritarias, recambio.

ABSTRACT

The gastrointestinal tract (GIT) constitute a complex and diverse ecosystem whence *Escherichia coli* is one of the best studied and characterized microorganisms. For several decades, numerous attempts have been made to determine their diversity and population structure inside the host trough time, whence resident, transitory, predominant, and minority strains were distinguished. Due to intestinal bacteria often carry antimicrobial resistance (AMR) genes in multiple mobile genetic elements (MGE), it is interesting to analyze the predominant strains dynamism in the GIT, as well as their antimicrobial resistance profile variations overtime. In this prospective study, fresh fecal samples were obtained from 31 children less than five years of age, and the participants were followed up at different sampling periods. From each sample, 5 *Escherichia coli* colonies of the predominant morphology were selected. The resistance profile to 12 antibiotics was determined using the disc diffusion technique, and the allele number of the *fumC* gene, by Sanger sequencing. Those isolates which came from the same participant and shared the same *fumC* allele number at different sampling periods were genotyped using MLST. In 3/5 isolates obtained from each sample, the resistance profile and the sequence type (ST) were the same in each sampling period. Nevertheless, those characteristics do not remain trough time since only four individuals with possible resident strains were identified, which determines that *Escherichia coli* population's turnover within the GIT is highly dynamic. The permanence of predominant antibiotic-resistant strains may contribute to the resistance dissemination to some other microbiota members, to the environment, and even to other human or not-human hosts.

Key words: *Escherichia coli*, antimicrobial resistance, resident strains, transient strains, predominant strains, minority strains, turnover.

CONTENT INDEX

FIGURE INDEX

TABLE INDEX

PART I: GENERAL INTRODUCTION

Children gastrointestinal tract colonization

The gastrointestinal tract (GIT) is "a series of hollow organs joined in a long, twisting tube from the mouth to the anus" (Sing, 2014). Of those organs, the intestine constitutes a place of particular interest due to the billions of microorganisms it contains; it is well known that "there are more bacteria in our guts than there are stars in the Milky Way" (Yong, 2016). The community of microorganisms (Eukarya, Bacteria or Archaea) which coexists in the intestine and fulfills specific functions, is called microbiota (Pereira & Berry, 2017). Several of those functions are related to the breakdown of food substances, the releasement of nutrients that would otherwise be inaccessible to the host, the gut cell differentiation, the protection of the gut from colonization of pathogens, and the modulation of the immune system (Milani et al., 2017).

The composition and establishment of a stable gut microbiota take place during childhood (2.5-3 years old). This particular interaction, between microorganisms and the host, occurs for the first time during labor; when the maternal (vaginal or skin) microbiota, depending on the mode of delivery, is transferred to the newborn (Milani et al., 2017). Thenceforth, this interaction becomes highly personalized due to several aspects related to socioeconomic factors, such as dietary habits, level of hygiene, etc. of each family; and also geographical, climatic and host genetic conditions (Tenaillon et al., 2010).

"The intestine is considered as a dynamic ecosystem in which spatial and temporal heterogeneity in the nutrient landscape shape the composition of the microbiota" (Pereira &

Berry, 2017). This long structure also showed different physical-chemical features, which are related to multiple niches where several microbial communities can settle (Pereira & Berry, 2017). Thereby, the gut microbiota is constantly changing overtime as a response to the selective pressure set by the host and their interacting environment (Richter et al., 2018). Bearing this in mind, the human gut microbiota comprises of resident and transient microorganisms (Milani et al., 2017): the former, are related to strains with a better adaptation to a specific niche, which can be associated with a long permanence in the gut (from months to years) and also correlated with a numerical predominance (Tenaillon et al., 2010; Caugant, Levin & Selander, 1981; Selander et al., 1986). Meanwhile, the latter strains are related to a lower ability of adaptation and consequently, with a short permanence in the gut (from days to weeks) and also associated with a numerical minority (Tenaillon et al., 2010; Caugant, Levin & Selander, 1981; Selander et al., 1986).

In newborns, the colonization of the infant's gut represents a de novo assembly (Milani et al., 2017). During the first weeks, the gut is firstly dominated by Enterobacteriaceae, where "*Escherichia coli* is among the first bacterial species to colonize the intestine during infancy, reaching very high density (higher than 10^9 CFU per gram of feces) before the expansion of anaerobes" (Tenaillon et al., 2010), such as *Bifidobacterium* and *Bacteroides* (Yong, 2016). Colonizing first may bring and advantage, due to the possibility of proliferation without outcompeting for space nor nutrients before the introduction of other functionally-similar species (Fukami, 2015). Through time, the diversity of higher taxonomic levels in the microbiota decreases and then stabilizes, remaining in the gut for more than 50 years (Faith et al., 2013); while at population levels, the unit of natural selection (Martinson et al, 2019), the dynamism is high as well as the rate of strain-level turnover, with time scales of months to years (Priya & Blekhman, 2019).

Importance of Enterobacteriaceae family

The involvement of the family Enterobacteriaceae in the early gut colonization plays a crucial role in educating epithelial cells to become hypo-responsive to microbial ligands during microbiome development (Tingting, 2019). Among this diverse family, *Escherichia coli* in the healthy human gut is, by far, the predominant facultative anaerobe microorganism, establishing commensal and symbiotic relationships mainly (Milani et al., 2017; Tenaillon et al., 2010; Tingting, 2019; Wold et al., 1992; Escobar-Paramo et al., 2004). For many years, several studies were interested in describing the population structure of commensal *Escherichia coli* and also in evaluating their lineage persistence in the lower GIT of humans (Caugant, Levin & Selander, 1981; Sears, Brownlee & Uchiyama, 1950; Adlerberth et al., 1998). Nonetheless, this Gram-negative microorganism is often recognized as an important source of disease (Richter et al.,2018), whereby most investigations have focused on pathogenic strains and their virulence factors (Tenaillon et al., 2010; Wold et al., 1992; Kaper et al., 2004). Lately, some studies have reconsidered the importance of commensal *Escherichia coli* residency and temporal variability (Tenaillon et al., 2010; Martinson et al., 2019; Priya & Blekhman, 2019; Richter et al., 2018), even though this microorganism represents only around 1% of the intestinal microbiota (Delmas, Dalmasso & Bonnet, 2015).

As time goes by, the molecular techniques used for studying the population genetics of non-pathogenic *Escherichia coli* have become considerably more sophisticated. During the pre-sequencing era, serotyping (O, K and H antigens) in the 1940s (Wallick & Stuart, 1943) and multilocus enzyme electrophoresis in the 1980s (Caugant, Levin & Selander, 1981) principally, identifies polymorphisms (Tenaillon et al., 2010; Ochman & Selander, 1984). Thereupon, at the beginnings of the sequencing era, multilocus sequence typing (housekeeping genes) in the late 1990s (Urwin & Maiden, 2003), and phylogrouping triplex PCR in the 2000s (Doumith et

al., 2012), identifies the sequence data for individual genes from PCR amplicons (Tenaillon et al., 2010; Gordon et al., 2008). Nowadays, next-generation sequencing techniques screen the entire genome (WGS) of hundreds of strains rapidly, opening the era of population genomics (Tenaillon et al., 2010; Richter et al., 2018; Shapiro, 2016).

Gastrointestinal tract strain status

Since the very beginning, fecal samples were used to perform these analyses, as they constitute a close representation of the lower digestive tract (Milani et al., 2017). From these, numerous studies report that, in a specific time point, each host carries a predominant strain and many different strains in a minority level (Yong, 2016; Tenaillon et al., 2010; Tingting, 2019; Priya & Blekhman, 2019; Ramberg, 2016; Lautenbach et al., 2008; Gordon, O'Brien & Pavli, 2015); moreover, resident strains can persist through time in the gut, while transient strains just come and go (Yong, 2016; Tenaillon et al., 2010; Priya & Blekhman, 2019; Ramberg, 2016; Gordo, Demengeot & Xavier, 2014; Gumpert, 2014). Since the intestine comprises a complex, diverse and challenging ecosystem, due to, principally, the constant competition for space and resources, the heterogeneity of nutrient niches, the high rate of incoming strains and natural selection; the genetic composition of *Escherichia coli* populations changes dramatically over short timespans, as a result of clonal lineages turnover (Pereira & Berry, 2017; Caugant, Levin & Selander, 1981; Shapiro, 2016; Gordo, Demengeot & Xavier, 2014).

Those strains better adapted to the host may persist and may also remain as the numerically dominant (Richter et al., 2018). There are some molecular factors involved in the prevalence of the strains in this dynamic environment. Considering *Escherichia coli* as a versatile microorganism with a special capacity of adaptation to new niches, even as a pathogen

(Wold et al., 1992; Kaper et al., 2004) or a saprophytic bacterium outside the host (Barrera et al., 2018; Vasco et al., 2015); many strains have some clusters of genes associated to virulence in the chromosome that may have emerged to adapt to an extraintestinal ecosystem (Tenaillon et al., 2010), which abilities are frequently associated with uropathogenic characteristics such as adhesins, siderophores and hemolysins (Martinson et al., 2019; Wold et al., 1992); while some others are associated with toxins and protectins production (Tenaillon et al., 2010).

Antimicrobial-resistance impact

Escherichia coli can acquire or lose foreign genetic material by the promiscuous flow of mobile genetic elements (MGE), principally through conjugation (Richter et al., 2018; Gumpert, 2014). In general, those MGE carry antibiotic-resistance genes that can be easily spread in the gut microbiota and become popular if those MGE confer any adaptive advantage in the current gut environment, making it possible to find the same clone with a different antibiotic-resistance profiles (Levin et al., 1997), which certainly contribute to the current antibiotic resistance crisis (Courvalin, 2016).

Little is known about the dynamics of this interaction between sensitive and resistant strains within the host (Davies et al., 2019) since there are limited longitudinal studies that elucidate that dynamism (Priya & Blekhman, 2019; Richter et al., 2018). Commensal antibiotic-resistant strains that persist in the human gastrointestinal tract "may become a reservoir for virulence and antibiotic resistance genes" (Richter et al.,2018).

PART II: SCIENTIFIC ARTICLE

Dominance and antimicrobial resistance variations in *Escherichia coli* lineages in the children gastrointestinal tract. A prospective study.

Introduction

Escherichia coli represents a very versatile microorganism: this Gram-negative bacterium harbor an impressive genetic and phenotypic diversity (Gordo, Demengeot & Xavier, 2014), which may explain why it could be found as a saprophytic in the environment (out of their host) (Barrera et al., 2018), as a commensal in the gastrointestinal tract (TGI) of warm-blooded animals (Tenaillon et al., 2010) or as a pathogen causing intestinal or extraintestinal disease (Kaper et al., 2004). Moreover, this microorganism can acquire or lose foreign genetic material by the promiscuous flow of mobile genetic elements (MGE), principally through conjugation (Ritcher et al., 2018; Gumpert., 2014). In general, those MGE carry antimicrobial-resistance (AMR) genes that can be easily spread which certainly contribute to the current antibiotic resistance crisis (Courvalin, 2016; Reygaert, 2018).

Inside the human TGI, Escherichia coli is the predominant facultative anaerobe microorganism, establishing commensal and symbiotic relationships mainly (Tenaillon et al., 2010; Milani et al., 2017; Tingting, 2019; Wold et al., 1992; Escobar-Paramo et al., 2004). Since 1940, several investigations report that, in a specific time point, each host carries a predominant strain "which constitutes more than half of the colonies isolated" (Tenaillon et al., 2010) and many different strains in a minority level (Tenaillon et al., 2010; Tingting, 2019; Yong, 2016; Priya & Blekhman, 2019; Ramberg, 2016; Lautenbach et al., 2008; Gordon,

O'Brien & Pavli, 2015; Caugant, Levin & Selander, 1981); moreover, resident strains can persist through time in the gut, while transient strains just come and go (Gordo, Demengeot & Xavier, 2014; Tenaillon et al., 2010; Gumpert, 2014; Yong, 2016; Priya & Blekhman, 2019; Ramberg, 2016; Caugant, Levin & Selander, 1981). The presence of dominant *Escherichia coli* strains holding AMR genes may represent a public health problem due to a greater possibility of transferring genes to other bacteria, including opportunistic pathogens (Reygaert, 2018; WHO, 2018; Levin et al., 1997). Furthermore, considering inadequate hygienic and sanitation conditions of the low-and middle-income countries (LMICs) (Salinas et al., 2019; Vasco, Graham & Trueba, 2016), those resistant microorganisms may persist in the environment and return to humans through water sources and animal products (Barrera et al., 2018; Collignon et al., 2018). Therefore, recent investigations have focused on The One Health strategy to "understand better the dissemination of resistant bacteria and genes among humans, animals, and the environment at a global scale" (Rousham, Unicomb & Islam, 2018). The population genetic dynamics of *Escherichia coli* in the intestine has received little attention even though i) *Escherichia coli* is probably the intestinal bacteria that transfers the most among hosts (Barrera et al., 2018; Salinas et al., 2019; Montealegre et al., 2010); ii) it is very active in horizontal gene transfer of antimicrobial resistance genes (Richter et al., 2018; Brito et al., 2016; Cloeckaert et al., 2018); iii) some lineages of this bacterium are multi-resistant pathogens of high importance (Cloeckaert et al., 2018; Nicolas, Bertrand & Madec, 2014; Reid, DeMaere & Djordjevic, 2018). Here we screened the *Escherichia coli* dominant strains obtained from children less than five years of age and analyze the turnover of these strains during one year.

Experimental procedures

Study locations.

This one-year longitudinal study was carried out in six semi-rural communities belonging to the parishes of Yaruquí, Pifo, Tumbaco, Checa, Puembo, and Tababela; all of them located near Quito, Ecuador.

For household enrollment, some inclusion criteria must be met: (i) households with a child ages six months to four years, (ii) households with a child care provider who was over eighteen years, and (iii) informed consent provided by the primary child care provider.

61 households were enrolled at the beginning of the study but, only 27 finished it.

Ethical considerations.

The study protocol was approved by the Committee for Protection of Human Subjects (CPHS) and the Office for Protection of Human Subjects (OPHS) at the University of California, Berkeley (Federalwide Assurance # 6252), the Human Research Ethics Committee at the Universidad San Francisco de Quito (no. 2017-178M) and the Ministry of Public Health, Ecuador (MSPCURI000243-3).

Sample collection.

We collected a single fecal sample from each child during three sampling periods (SP): from October to December of 2018 (SP I), from January to May of 2019 (SP II) and from July to December of 2019 (SP III), obtaining a total of 120 stool samples. Every time a sample was collected, the child care provider completed a survey related to the current family lifestyle and their recent exposition to antimicrobials.

We followed during SP I and SP II to nine children (18 samples) and, during SP I, SP II and SP III to 27 children (81 samples). The remaining 21 samples were discarded.

Each stool sample was collected into sterile tubes, stored at 4ºC, transported to the laboratory, and immediately processed.

Escherichia coli isolation.

Each fecal sample was plated on MacConkey agar and incubated at 37 ºC for 18 hours. To ensure the selection of the dominant *Escherichia coli* strain (≥ 50% of all colonies) (Lautenbach et al., 2008), we collected 5 colonies that correlate with the predominant morphology.

Additionally, each colony was transferred to Chromocult® coliform agar for the identification of *Escherichia coli* through its β-D-glucuronidase activity.

Those strains were incubated in Brain Heart Infusion (BHI) medium + glycerol (15%) at 37 ºC for 18 hours to perform the antimicrobial susceptibility test. After that, the tubes were stored at -80 ºC.

Antimicrobial susceptibility test.

We used the Kirby Bauer technique (disc diffusion in Muller Hinton agar) to determinate the strains antimicrobial susceptibility using the following discs: Cefazolin (CZ; 30 µg), Ciprofloxacin (CIP; 5 µg), Ampicillin (AM; 10 µg), Chloramphenicol (C; 30 µg), Imipenem (IPM; 10 µg), Trimethoprim-sulfamethoxazole (SXT; 1.25/23.75 µg), Gentamicin (GM; 10 µg), Ceftazidime (CAZ; 30 µg), Cefepime (FEP; 30 µg), Cefotaxime (CTX; 30 µg), Tetracycline (TE; 30 µg) and Amoxicillin + Clavulanic Acid (AMC; 20/10 µg).

The resistance or susceptibility was determined according to Clinical and Laboratory Standards Institute (CLSI) guidelines (2018).

DNA extraction.

Each isolate was grown on MacConkey agar at 37 ºC for 18 hours and 5-6 colonies were placed into Eppendorf® tubes with 500 µl of sterile distilled water. We followed the heat-shock protocol to release the DNA (Dashti et al., 2009).

The quality of the DNA was monitored by gel electrophoresis.

Genotyping.

The clonal relationship of the isolates was screened by amplifying and sequencing the *fumC* gene (Johnson et al., 2004) using the Master Mix Go Taq. Those isolates coming from the same individual and sharing the same allele were subjected to full multi-locus sequence-typing (MLST).

Briefly, the PCR conditions were: 180 secs at 95ºC, 30 cycles of 30 secs at 94ºC, 30 secs at the annealing temperature of each primer (*adk*: 52 ºC; *fumC*: 55 ºC; *gyrB* and *mdh*: 58 ºC; *icd* and *recA*: 54 ºC; *purA*: 50 ºC) and 60 secs at 72 ºC (*fumC* and *icd*) or 45 secs at 72 ºC (*adk*, *gyrB*, *mdh*, *purA*, and *recA*), and a final extension of 7 minutes at 72 ºC.

DNA sequencing.

All PCR products were sequenced at Macrogen Inc. using the Sanger sequencing method.

The sequences were analyzed using the program Geneious Prime 2020 and were screened in the Enterobase database (Zhou, Alikham & Mohamed, 2020).

Statistical analysis.

Significant differences between phenotypic antimicrobial resistance prevalence of the individuals through time were tested using a chi-square test.

Results

As we mentioned before, 61 households were enrolled in the first sampling period (SP I) of this prospective study. Of those households, 32 enrolled again for SP II and only 27, for SP III. We followed a total of 31 children: nine during SP I and II, and 22 during the three sampling periods (SP I, SP II and SP III).

A total of 413 *Escherichia coli* isolates were recovered: 153 in SP I, 150 in SP II, and 110 in SP III. 125 (30.3%) were susceptible to all the antimicrobials tested. The remaining fell into one of the 76 unique antibiotic resistance profiles: 54 isolates (13.1%) were resistant to only one antibiotic, 38 isolates (9.2%) were resistant to two antibiotics, and 196 (47.5%) were resistant to three or more antimicrobials.

The resistance to Ampicillin (AM) + Trimethoprim-sulfamethoxazole (SXT) + Tetracycline (TE) were the most common profile (n=36, 8.7%), followed by Tetracycline (TE) resistance (n=21, 5.1%), Ampicillin (AM) resistance and also Ampicillin (AM) + Trimethoprim-sulfamethoxazole (SXT) + Amoxicillin-clavulanic acid (AMC) resistance (n=15, 3.6%, respectively).

A total of 41 *fumC* alleles were analyzed, whence the most prevalent was allele 11 (n=109, 26.4%), followed by allele 35 (n=40, 9.7%) and allele 4 (n=38, 9.2%).

Sampling periods I and II.

The predominant *Escherichia coli* strains recovered from nine children in SP I and SP II were analyzed: 43 from SP I and 40 from SP II. The samples belonged to three females and six males (**Figure 1**).

The highest percentages of resistance in SP I isolates were to Tetracycline (TE) (46.5%), Trimethoprim-sulfamethoxazole (SXT) (34.9%) and Ampicillin (AM) (32.6%), while in SP II isolates were to Ampicillin (AM) (60%), Trimethoprim-sulfamethoxazole (SXT) (35%) and Tetracycline (TE) (27.5%). There are significant statistical differences in the resistance to Chloramphenicol (C) between SP I and SP II, whence is higher in SP I; in the same way as the resistance to Ampicillin (AM), whence in SP II is higher (**Table 1**).

We identified 17 different *fumC* alleles, whence allele 11 was the most prevalent (SP I=12 and SPII=11). *fumC* alleles 4, 11, and 27 were found in both sampling periods, but solely *fumC* allele 11 were found in the individual 18 through time. However, MLST analysis showed that these isolates belonged to a different sequence type (ST): ST43 in SP I and ST34 in SP II. Moreover, we identified seven unique alleles present in each sampling period (**Figure 2)**.

Sampling periods I, II and III.

We followed to 22 children (11 females and 11 males) during SP I, SP II, and SP III to obtain the predominant *Escherichia coli* strains: 110 *Escherichia coli* isolates were recovered in each sampling period (**Figure 3**).

The highest percentages of resistance in SP I and SP II isolates corresponded to Ampicillin (AM) (SP I=58.2% and SP II=54.5%), Trimethoprim-sulfamethoxazole (SXT) (SP I and SP II=52.7%) and Tetracycline (TE) (SP I=50% and SP II=47.3%), while in SP III isolates

were to Trimethoprim-sulfamethoxazole (SXT) (45.4%), Tetracycline (TE) (45.4%) and Ampicillin (41.8%). There are significant statistical differences in the resistance to Ampicillin (AM), Imipenem (IPM), and Amoxicillin-clavulanic acid (AMC) between sampling periods, whence in SP I is higher; while the resistance to Ceftazidime (CAZ) and Ciprofloxacin (CIP), whence in higher in SP II (**Table 2**).

A total of 33 different *fumC* alleles were identified, whence allele 11 was the most prevalent (SP I=23, SP II=34, and SP III=29); *fumC* alleles 4, 11, 23, 26, 35, and 40 were present in all the sampling periods; nine alleles, in two sampling periods and 18 only in one (**Figure 4).**

Strains showing the same *fumC* allele were subjected to MLST analysis showing that, overtime, ST34 was the predominant genotype recovered from individual 26 (7 isolates), ST10 was the predominant genotype in individuals 20 and 30, and ST131 was the predominant genotype in individual 23 (6 isolates). The remaining isolates belonged to a different sequence type (**Tables S1 and S2**).

Discussion

During this prospective study, we followed to 31 children during several sampling periods and screened the predominant *Escherichia coli* strains present in fresh fecal samples. At least three of the five selected colonies from each individual showed the same antimicrobial resistance profile, as well as the allele number from the *fumC* gene, which ensured us the selection of the predominant strains (Lautenbach et al., 2008). We observed that few strains persisted overtime in the individuals, suggesting a high turnover rate of *Escherichia coli* strains. We also observed significant differences in antimicrobial resistance in the strains collected

during different periods of time, denoting that the majority of dominant *Escherichia coli* strains in human intestines are transient colonizers, which is coincidental with a previous report (Richter et al., 2018).

Our results agree with several studies that reject the stability of the microbiome over time, at least at a population level, whence the strains turnover is highly dynamic (Tenaillon et al., 2010; Milani et al., 2017; Priya & Blekhman, 2019; Martinson et al., 2019; Richter et al., 2018). Several changes could happen in the lifestyle of each child amongst the sampling periods that could be related to the high strain turnover. Besides, there is limited information related to strain residency in the gastrointestinal tract. According to Martinson (2019), any strain would be considered a resident if it overcomes at least three times the maximum transit time (~14 days). Due to the sampling periods were pretty far from each other (~ 4 - 5 months), we probably missed the opportunity to recover the strains detected previously.

In this research we identified only four children with possible dominant resident strains: ST10 (individuals 20 and 30), ST34 (individual 26) and ST131(individual 23), isolates which persisted for 7, 5, 11, and 4 months respectively. The most remarkable of them is ST131 which is a known human extra-intestinal pathogen, characterized by their resistance to extended-spectrum cephalosporins (ESC) and fluoroquinolones (FQ) (Yamaji et al., 2018; Nicolas, Bertrand & Madec, 2014). This genotype is strongly related to extra-intestinal infections, from the urinary tract (UTI) principally (Martinson et al., 2019; Wold et al., 1992), which can be related to a higher persistence inside the intestine; are globally reported as residents of human, companion, and food-production animals intestine; and have been identified in the environment: in soil and water sources mainly (Cloeckaert et al., 2018; Reid, DeMaere & Djordjevic, 2018; Yamaji et al., 2018; Nicolas, Bertrand & Madec, 2014). Moreover, ST10 and ST34 belonged to clonal complex 10 (CC10), whence ST10 is known as one of the most

dominant commensal STs in *Escherichia coli* (Reid, DeMaere & Djordjevic, 2018) however, strains belonging to this ST are usually not clonal (Pietsch et al., 2018).

Regarding the antimicrobial resistance, among the 413 isolates analyzed, 30.3% (125/413) were susceptible to whole the antimicrobials tested. The resistance to Ampicillin (AM), Trimethoprim-sulfamethoxazole (SXT), and Tetracycline (TE) were the most prevalent, while Gentamycin (GM) was the least because only six strains showed resistance. Moreover, it is interesting to find several commensal *Escherichia coli* isolates resistant to Imipenem (IPM) (24/413) and Cefepime (FEP) (40/413) which are not part of first-line treatment (CLSI, 2018).

Even though numerous studies have focused on the turnover of human microbiome at the level of taxa such as families, genera or species, little is known about the turnover of bacterial lineages. There are two regimes within the human microbiota: one is driven by external fluctuations (host behavior) and the other by internal processes (microbial-microbial interaction) (Gibbons et al., 2017). In the former, many factors, such as diet, antimicrobials, gut physiology, and host immune system, substantially influence the microbiota biogeography within the host (Donaldon, Lee & Mazmanian, 2015; Gibbons et al., 2017); while in the latter, specific interplays between the microbiota members, such as competition and cooperation, determine the emergence of a define community structure (Gordo, Demengeot & Xavier, 2014; Caugant, Levin & Selander, 1981; Perreira & Berry, 2017; Shapiro, 2016). Recent evidence has indicated that besides major taxon modifications (bacterial families, genera or species), diet can also favor the proliferation of some strains (Brito et al., 2016).

According to the surveys, 31 households report access to potable water 24/7, and 29 had sewerage. Moreover, during the sampling periods, 20 children changed their dietary habits (stop breastfeeding), 14 children were exposed to antimicrobials at least in one sampling period, 29 has animal contact (pets, livestock, poultry, etc.) and 15 children had diarrhea at least in one

sampling period. Although we identified several changes, it is difficult to understand the specific metabolic and ecological interactions between the microorganisms and the host (Vracken, 2019). The omics era has allowed us to identify the microbiota members and their conduct at a particular time point (Selzer, Marhöfer & Koch, 2018; Sonda et al., 2018) but in a long-term "the use of culture isolates with mathematical modeling will enable the ultimate goal: translating mechanistic and ecological knowledge into new and effective therapies" (Vracken, 2019); in this way, we can understand, overtime and in a host-specific manner, why a particular strain could overcome a specific selective pressure and become dominant or persistent in the lower gastrointestinal tract complex ecosystem (Tenaillon, 2010; Milani et al., 2017; Vracken, 2019; Davies et al., 2019; Perreira & Berry, 2017; Kerr et al., 2002).

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SUPPLEMENTARY MATERIAL INDEX

FIGURES

FIGURE 1. Summary of the nine individuals followed during SP I and SP II: host ID, sampling time point, host age, host sex, host diet habits, sample clinical presentation, exposition to antibiotics and isolate identification.

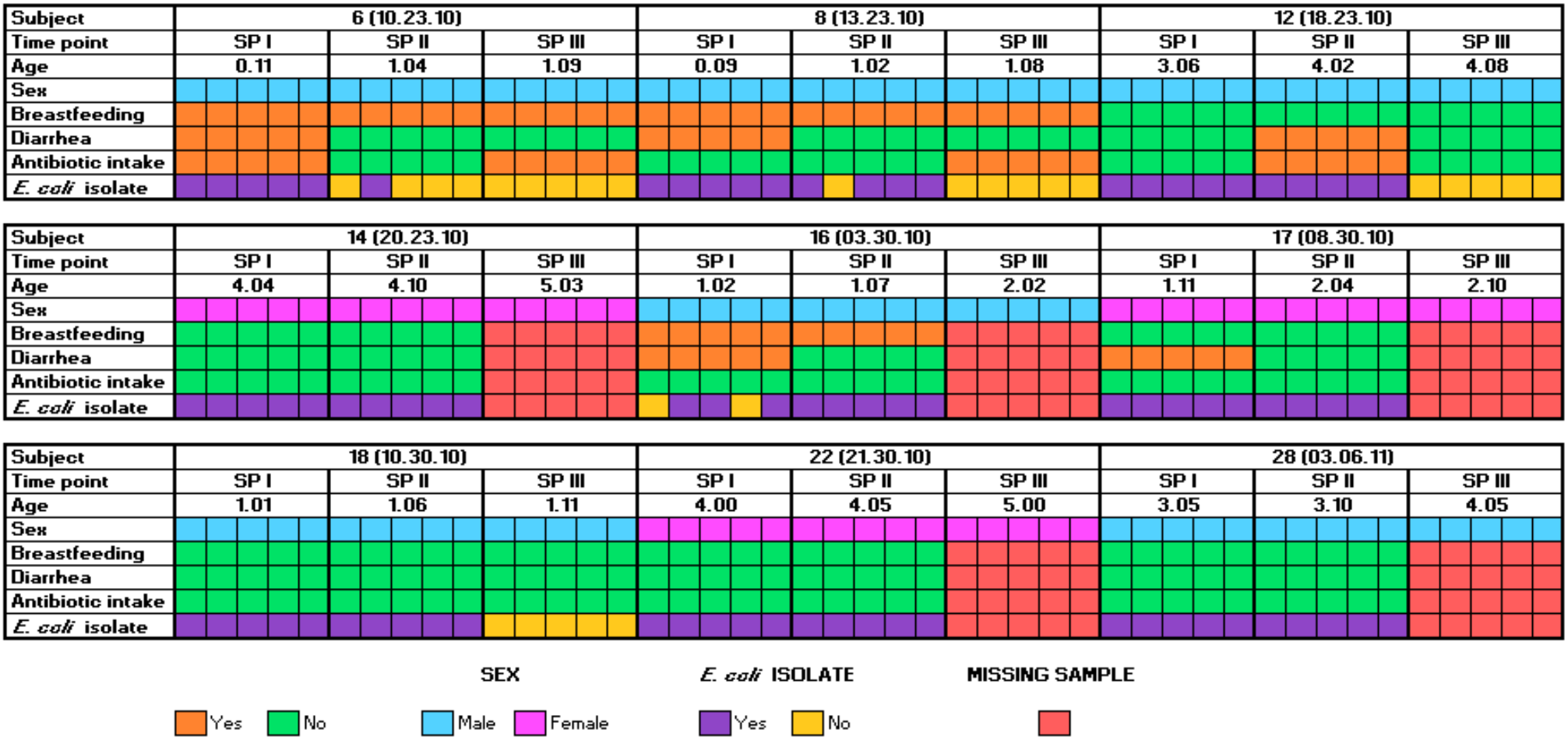


FIGURE 2. Venn diagram showing the shared *fumC* alleles identified in nine children during SP I and SP II.

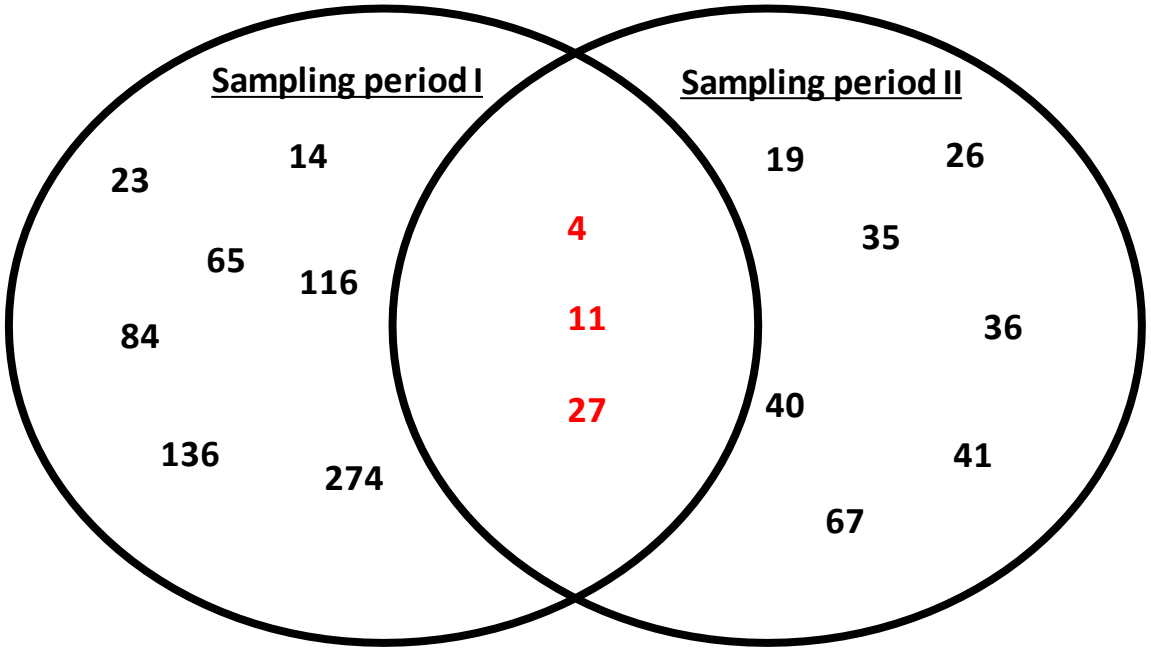


FIGURE 3. Summary of the 22 individuals followed during SP I, SP II and SP III: host ID, sampling time point, host age, host sex, host diet habits, sample clinical presentation, exposition to antibiotics and isolate identification (1/2).

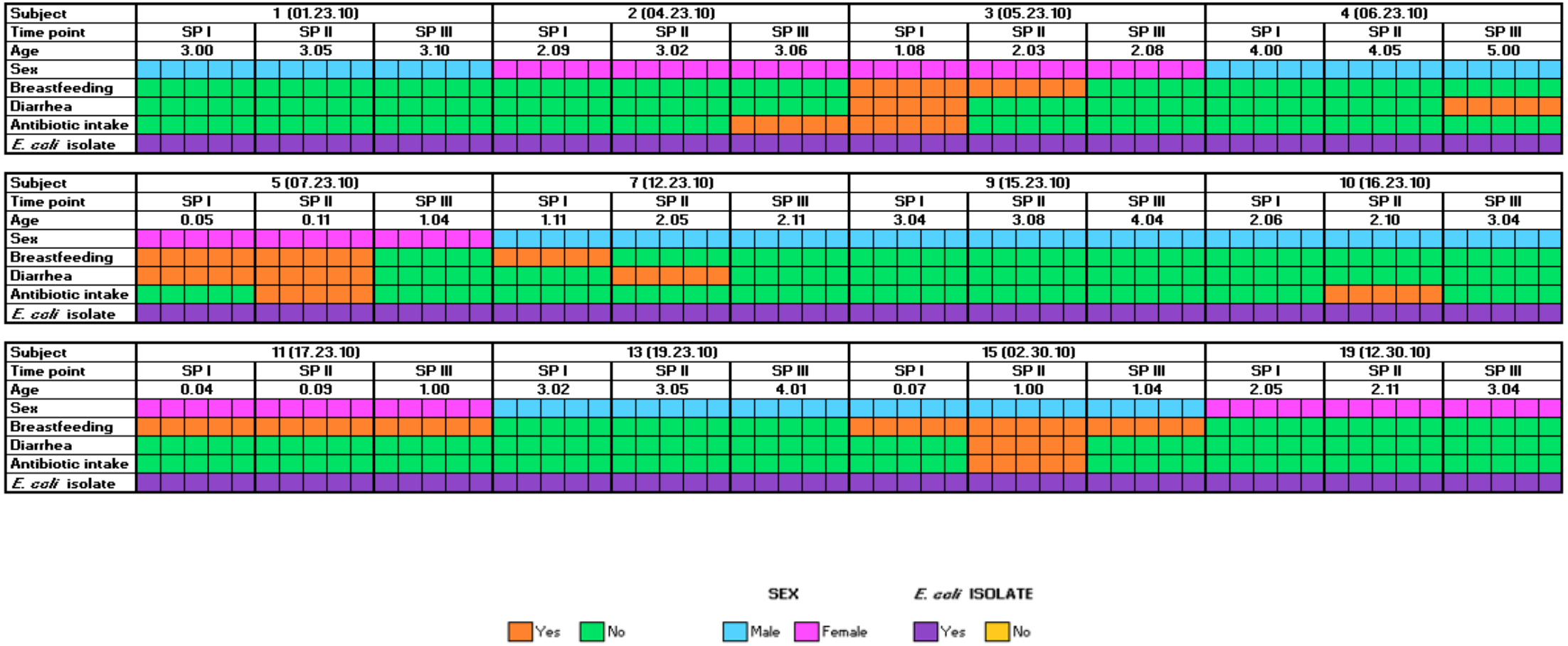


FIGURE 3. Summary of the 22 individuals followed during SP I, SP II and SP III: host ID, sampling time point, host age, host sex, host diet habits, sample clinical presentation, exposition to antibiotics and isolate identification (2/2).

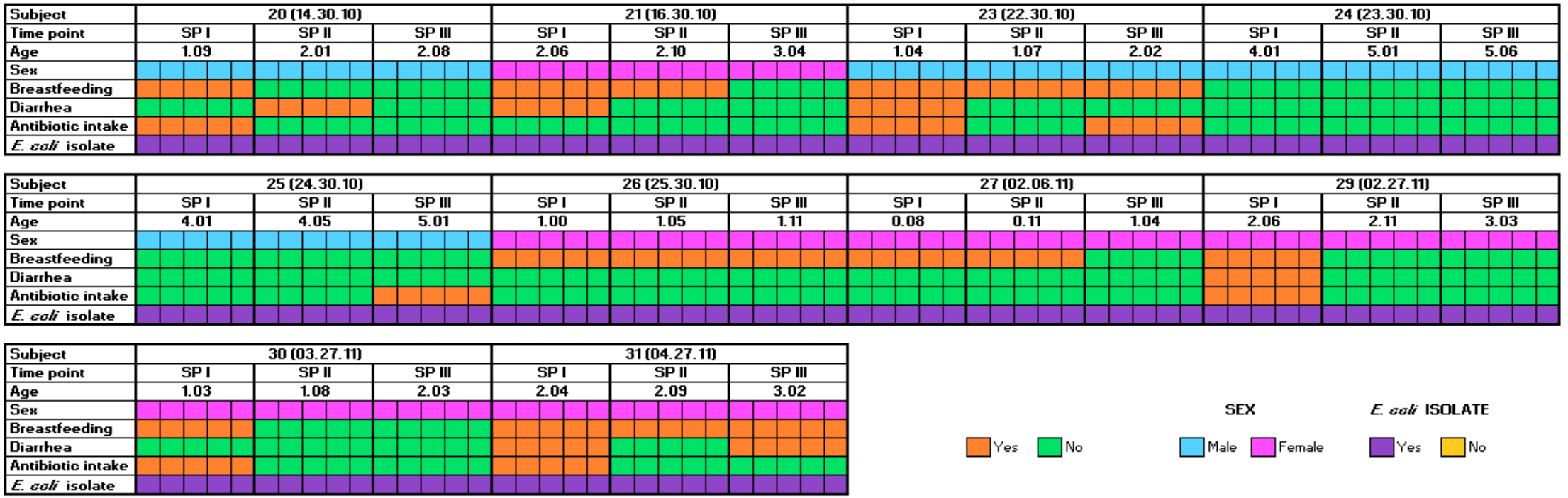
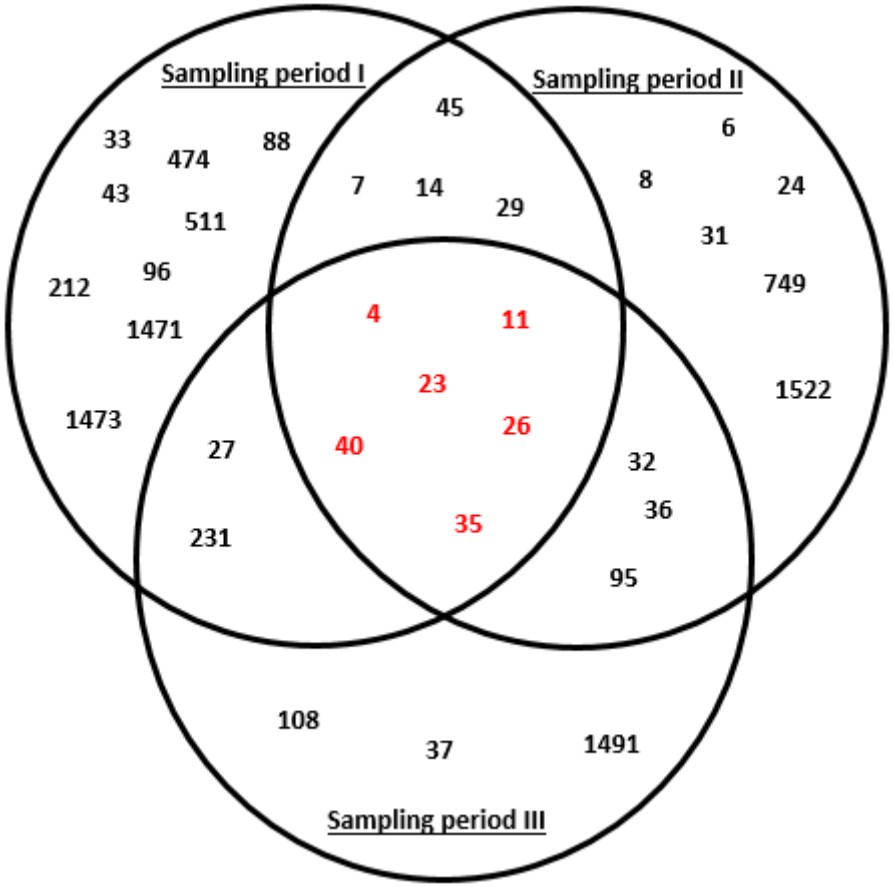


FIGURE 4. Venn diagram showing the shared *fumC* alleles identified in 22 children during SP I, SP II and SP III.



TABLES

TABLE 1. Prevalence of phenotypically resistance of *Escherichia coli* isolates obtained from nine children during SP I and SP II.

Antimicrobials	n= 43 isolates	n= 40 isolates		
	SP I	**SP II**	**Chi square test**	**P value**
Ampicillin (AM)	14 (32.6 %)	24 (60 %)	**6.287**	**0.012**
Chloramphenicol (C)	11 (25.6 %)	1 (2.5 %)	**8.927**	**0.003**
Imipenem (IPM)	4 (9.3 %)	0 (0 %)		
Trimethoprim-sulfamethoxazole (SXT)	15 (34.9 %)	14 (35 %)	0.000	0.991
Gentamicin (GM)	0 (0 %)	0 (0 %)		
Ceftazidime (CAZ)	1 (2.3 %)	1 (2.5 %)	0.003	0.959
Cefepime (FEP)	2 (4.6 %)	1 (2.5 %)	0.275	0.510
Cefazolin (CZ)	1 (2.3 %)	3 (7.5 %)	1.210	0.271
Ciprofloxacin (CIP)	12 (27.9 %)	5 (12.5 %)	3.020	0.082
Cefotaxime (CTX)	1 (2.3 %)	2 (5 %)	0.426	0.514
Amoxicillin + Clavulanic Acid (AMC)	13 (30.2 %)	10 (25 %)	0.283	0.594
Tetracycline (TE)	20 (46.5 %)	11 (27.5 %)	3.201	0.074

(*P* value < 0.05)

TABLE 2. Prevalence of phenotypically resistance of *Escherichia coli* isolates obtained from 22 children during SP I, SP II and SP III.

Antimicrobials	n= 110 isolates (each one)			Chi square test	*P* value
	SP I	SP II	SP III		
Ampicillin (AM)	64 (58.2 %)	60 (54.5 %)	46 (41.8 %)	**6.502**	**0.039**
Chloramphenicol (C)	5 (4.5 %)	12 (10.9 %)	9 (8.2 %)	3.090	0.213
Imipenem (IPM)	12 (10.9 %)	1 (0.9 %)	7 (6.4 %)	**9.687**	**0.008**
Trimethoprim-sulfamethoxazole (SXT)	58 (52.7 %)	58 (52.7 %)	50 (45.4 %)	1.552	0.460
Gentamicin (GM)	0 (0 %)	6 (5.4 %)	0 (0 %)		
Ceftazidime (CAZ)	2 (1.8 %)	14 (12.7 %)	10 (9.1 %)	**9.352**	**0.009**
Cefepime (FEP)	13 (11.8 %)	15 (13.6 %)	9 (8.2 %)	1.705	0.426
Cefazolin (CZ)	18 (16.4 %)	14 (12.7 %)	15 (13.6 %)	0.645	0.724
Ciprofloxacin (CIP)	22 (20 %)	21 (19.1 %)	9 (8.2 %)	**7.168**	**0.028**
Cefotaxime (CTX)	14 (12.7 %)	18 (16.4 %)	14 (12.7 %)	0.808	0.668
Amoxicillin + Clavulanic Acid (AMC)	35 (31.8 %)	28 (25.4 %)	15 (13.6 %)	**10.376**	**0.006**
Tetracycline (TE)	55 (50 %)	52 (47.3 %)	50 (45.4 %)	0.462	0.794

(*P* value < 0.05)

SUPPLEMENTARY MATERIAL

TABLE SM1. Multilocus sequence typing (MLST) and antibiotic resistance profile of selected *Escherichia coli* isolates obtained from 9 children during SP I and SP II (1/2).

			Sampling period I			Sampling period II		
Individual	Sample ID	Isolate	Resistance profile	*fumC* allele	Sequence type	Resistance profile	*fumC* allele	Sequence type
6	10.23.10	1	AM-AMC	27				
6	10.23.10	2	AM-IPM-AMC	27		SUSCEPTIBLE	11	
6	10.23.10	3	AM-IPM-AMC	27				
6	10.23.10	4	AM-IPM-AMC	27				
6	10.23.10	5	AM-C-IPM-CAZ-FEP-CZ-CIP-CTX-AMC-TE	27				
8	13.23.10	1	SXT-CIP-TE	14		SUSCEPTIBLE	67	
8	13.23.10	2	SXT-CIP-TE	14				
8	13.23.10	3	SXT-CIP-TE	14		SUSCEPTIBLE	36	
8	13.23.10	4	SXT-CIP-TE	14		SUSCEPTIBLE	67	
8	13.23.10	5	SXT-CIP-TE	14		SUSCEPTIBLE	67	
12	18.23.10	1	SUSCEPTIBLE	274		SUSCEPTIBLE	11	
12	18.23.10	2	SUSCEPTIBLE	274		SUSCEPTIBLE	11	
12	18.23.10	3	TE	274		SUSCEPTIBLE	11	
12	18.23.10	4	SUSCEPTIBLE	274		SUSCEPTIBLE	11	
12	18.23.10	5	SUSCEPTIBLE	274		SUSCEPTIBLE	11	
14	20.23.10	1	SUSCEPTIBLE	11		AM-SXT-AMC	4	
14	20.23.10	2	SUSCEPTIBLE	11		AM-SXT-AMC	4	
14	20.23.10	3	SXT	136		AM-SXT-AMC	4	
14	20.23.10	4	SXT	136		AM-SXT-AMC	4	
14	20.23.10	5	SXT	136		AM-SXT-AMC	4	
16	03.30.10	1				AM	11	
16	03.30.10	2	SUSCEPTIBLE	65		AM	26	
16	03.30.10	3	SUSCEPTIBLE	65		AM	26	
16	03.30.10	4				AM	26	
16	03.30.10	5	SUSCEPTIBLE	65		AM-CTX	26	

No *E. coli* isolate

Does not apply

Same *fumC* allele

TABLE SM1. Multilocus sequence typing (MLST) and antibiotic resistance profile of selected *Escherichia coli* isolates obtained from 9 children during SP I and SP II (2/2).

			Sampling period I			Sampling period II		
Individual	Sample ID	Isolate	Resistance profile	*fumC* allele	Sequence type	Resistance profile	*fumC* allele	Sequence type
17	08.30.10	1	SUSCEPTIBLE	11		AM-SXT-AMC-TE	35	
17	08.30.10	2	SUSCEPTIBLE	14		AM-SXT-AMC-TE	35	
17	08.30.10	3	SUSCEPTIBLE	11		AM-SXT-CZ-AMC-TE	35	
17	08.30.10	4	CIP-TE	84		AM-SXT-AMC-TE	35	
17	08.30.10	5	SUSCEPTIBLE	14		AM-SXT-CZ-AMC-TE	35	
18	10.30.10	1	SUSCEPTIBLE	4		AM-CIP	11	
18	10.30.10	2	AM-SXT-AMC-TE	11		AM-SXT-CIP-TE	11	34
18	10.30.10	3	SUSCEPTIBLE	4		AM-SXT-CIP-TE	11	
18	10.30.10	4	SUSCEPTIBLE	4		AM-C-CIP	41	
18	10.30.10	5	AM-SXT-AMC-TE	11	43	SUSCEPTIBLE	11	
22	21.30.10	1	C-SXT-CIP-TE	23		SXT	27	
22	21.30.10	2	C-SXT-CIP-TE	11		SUSCEPTIBLE	35	
22	21.30.10	3	AM-C-SXT-CIP-TE	23		SXT	27	
22	21.30.10	4	AM-C-SXT-CIP-AMC-TE	116		SUSCEPTIBLE	35	
22	21.30.10	5	C-SXT-CIP-TE	23		SUSCEPTIBLE	35	
28	03.06.11	1	AM-C-AMC-TE	11		AM-TE	35	
28	03.06.11	2	AM-C-FEP-AMC-TE	11		AM-TE	35	
28	03.06.11	3	AM-C-AMC-TE	11		AM-CAZ-FEP-CZ-CTX-TE	40	
28	03.06.11	4	AM-C-AMC-TE	11		AM	19	
28	03.06.11	5	AM-C-AMC-TE	11		AM-CIP-TE	35	

No *E. coli* isolate

Does not apply

Same *fumC* allele

TABLE SM2. Multilocus sequence typing (MLST) and antibiotic resistance profile of selected *Escherichia coli* isolates obtained from 22 children during SP I, SP II and SP III (1/4).

			Sampling period I			Sampling period II			Sampling period III		
Individual	Sample ID	Isolate	Resistance profile	*fumC* allele	ST	Resistance profile	*fumC* allele	ST	Resistance profile	*fumC* allele	ST
1	01.23.10	1	SUSCEPTIBLE	7		AM-SXT-CIP-TE	11		SUSCEPTIBLE	36	
1	01.23.10	2	SUSCEPTIBLE	40		AM-SXT-CIP-TE	11		SUSCEPTIBLE	36	
1	01.23.10	3	SUSCEPTIBLE	40		CTX	95		SUSCEPTIBLE	36	
1	01.23.10	4	AM-C-SXT-CAZ-FEP-CZ-CIP-CTX-TE	88		C-TE	11		SUSCEPTIBLE	36	
1	01.23.10	5	SUSCEPTIBLE	7		AM-SXT-CIP-TE	11		SUSCEPTIBLE	36	
2	04.23.10	1	AM-SXT-TE	11		SUSCEPTIBLE	11		C-SXT-CIP-TE	23	
2	04.23.10	2	AM-SXT-TE	11	10	SUSCEPTIBLE	11		C-SXT-CIP-TE	23	
2	04.23.10	3	AM-SXT-TE	11		AM	11	8125	C-SXT-CIP-TE	23	
2	04.23.10	4	AM-SXT-TE	11		SUSCEPTIBLE	11		C-SXT-CIP-TE	23	
2	04.23.10	5	AM-SXT-FEP-CZ-CTX-TE	26		SUSCEPTIBLE	11		C-SXT-CIP-TE	23	
3	05.23.10	1	AM-SXT-AMC-TE	1471		AM-SXT-CAZ-FEP-CZ-CTX-AMC-TE	26		SUSCEPTIBLE	11	
3	05.23.10	2	CIP	7		AM-SXT-CAZ-FEP-CZ-CTX-AMC-TE	26		SUSCEPTIBLE	11	
3	05.23.10	3	AM-SXT-AMC-TE	1471		AM-CAZ-FEP-CZ-CTX-TE	26		SUSCEPTIBLE	11	
3	05.23.10	4	SXT-CIP	35		AM-SXT-CAZ-FEP-CZ-CTX-TE	26		SUSCEPTIBLE	11	
3	05.23.10	5	SXT-CIP	35		AM-SXT-CAZ-FEP-CZ-CTX-AMC-TE	26		SUSCEPTIBLE	11	
4	06.23.10	1	AM-SXT-FEP-CZ-CIP-CTX-AMC	40		SUSCEPTIBLE	11		AM-IPM-SXT-CAZ-FEP-CZ-CTX-AMC-TE	35	
4	06.23.10	2	AM-SXT-AMC-TE	26		FEP	11		AM-TE	35	
4	06.23.10	3	AM-SXT-FEP-CZ-CIP-CTX-AMC	40		SUSCEPTIBLE	7		AM-IPM-SXT-CAZ-CZ-CTX-AMC-TE	35	
4	06.23.10	4	AM-SXT-FEP-CZ-CIP-CTX-AMC	40		SUSCEPTIBLE	7		AM-IPM-SXT-CAZ-CZ-CTX-AMC-TE	35	
4	06.23.10	5	AM-SXT-FEP-CZ-CIP-CTX-AMC	40		CTX	7		AM-IPM-SXT-CAZ-CZ-CTX-AMC-TE	35	
5	07.23.10	1	TE	45		AM-SXT-FEP-CTX-AMC	24		TE	40	
5	07.23.10	2	TE	45		AM-C-SXT-CAZ-CZ-CIP-CTX-AMC-TE	31		TE	40	
5	07.23.10	3	TE	45		SUSCEPTIBLE	26		TE	40	
5	07.23.10	4	TE	45		AM-SXT-AMC	24		TE	40	
5	07.23.10	5	TE	45		AM-C-SXT-CAZ-CZ-CIP-CTX-AMC-TE	31		TE	40	
7	12.23.10	1	SUSCEPTIBLE	14		AM-SXT-AMC-TE	35	69	SUSCEPTIBLE	35	
7	12.23.10	2	SUSCEPTIBLE	23		AM-SXT-AMC-TE	35		CTX	35	
7	12.23.10	3	SXT-TE	11		TE	11		SUSCEPTIBLE	35	
7	12.23.10	4	SXT-TE	11	757	TE	11	665	FEP-CTX	35	
7	12.23.10	5	SXT-TE	11		TE	11		IPM-CTX	35	1380

Does not apply Same *fumC* allele Same ST

TABLE SM2. Multilocus sequence typing (MLST) and antibiotic resistance profile of selected *Escherichia coli* isolates obtained from 22 children during SP I, SP II and SP III (2/4).

			Sampling period I			Sampling period II			Sampling period III		
Individual	Sample ID	Isolate	Resistance profile	*fumC* allele	ST	Resistance profile	*fumC* allele	ST	Resistance profile	*fumC* allele	ST
9	15.23.10	1	AM-SXT-TE	35		SUSCEPTIBLE	4		AM-SXT-TE	11	
9	15.23.10	2	AM-SXT-TE	35		SUSCEPTIBLE	4		AM-SXT-TE	11	
9	15.23.10	3	AM-SXT-TE	35		SUSCEPTIBLE	4		AM-SXT-TE	11	
9	15.23.10	4	AM-SXT-AMC-TE	35		SUSCEPTIBLE	4		AM-SXT-TE	11	
9	15.23.10	5	AM-SXT-TE	35		SUSCEPTIBLE	4		AM-SXT-TE	11	
10	16.23.10	1	AM-SXT-TE	45		AM-SXT-AMC	6		SUSCEPTIBLE	11	
10	16.23.10	2	AM-SXT-TE	45		AM-SXT-AMC	6		SXT-TE	4	
10	16.23.10	3	AM-SXT-TE	45		AM-SXT-AMC	6		SXT-TE	4	
10	16.23.10	4	AM-SXT-TE	45		AM-SXT-CZ-AMC	6		SXT-TE	4	
10	16.23.10	5	AM-IPM-SXT-TE	45		AM-SXT-AMC	6		SXT-TE	4	
11	17.23.10	1	AM-IPM	33		AM-SXT-CIP-TE	14		SUSCEPTIBLE	36	
11	17.23.10	2	AM-IPM-AMC	33		AM-SXT-CIP-AMC-TE	14		SUSCEPTIBLE	36	
11	17.23.10	3	AM-AMC	33		AM-SXT-CIP-AMC-TE	14		AM	36	
11	17.23.10	4	AM-AMC	33		AM-SXT-CIP-TE	14		AM	36	
11	17.23.10	5	AM-AMC	33		AM-SXT-CIP-AMC-TE	14		AM-IPM-CTX-AMC	36	
13	19.23.10	1	AM-AMC	26		SUSCEPTIBLE	11		AM-CAZ-FEP-CZ-CIP-CTX-AMC-TE	95	
13	19.23.10	2	AM-AMC	26		C-SXT-TE	7		AM-CZ-CIP-AMC-TE	95	
13	19.23.10	3	AM	26		SUSCEPTIBLE	4		AM-FEP-CZ-CIP-AMC-TE	95	
13	19.23.10	4	AM	26		AM-C-SXT-AMC-TE	23		AM-CZ-CIP-AMC-TE	95	
13	19.23.10	5	AM	26		AM-SXT-FEP-CIP-CTX-AMC-TE	1522		IPM	37	
15	02.30.10	1	AM-SXT-AMC-TE	40		SUSCEPTIBLE	11		C-SXT-TE	27	
15	02.30.10	2	AM-SXT-CZ-AMC-TE	40		SUSCEPTIBLE	11		C-SXT-TE	27	
15	02.30.10	3	AM-SXT-AMC-TE	40		SUSCEPTIBLE	35		AM-SXT	4	
15	02.30.10	4	AM-SXT-CZ-AMC-TE	40		SUSCEPTIBLE	11		C-SXT-TE	27	
15	02.30.10	5	AM-SXT-CZ-AMC-TE	40		SUSCEPTIBLE	11		C-SXT-TE	27	
19	12.30.10	1	AM-C-SXT-TE	45		AM-SXT-TE	11	3877	AM-SXT-TE	11	
19	12.30.10	2	SXT-AMC	1473		SUSCEPTIBLE	29		SXT	11	
19	12.30.10	3	AM-IPM-SXT-AMC	1473		TE	95		SXT	11	
19	12.30.10	4	AM-SXT-AMC	1473		SUSCEPTIBLE	8		SXT	11	4238
19	12.30.10	5	AM-IPM-SXT-CTX-AMC	1473		SUSCEPTIBLE	29		SXT	11	

Does not apply Same *fumC* allele Same ST

TABLE SM2. Multilocus sequence typing (MLST) and antibiotic resistance profile of selected *Escherichia coli* isolates obtained from 22 children during SP I, SP II and SP III (3/4).

			Sampling period I			Sampling period II			Sampling period III		
Individual	Sample ID	Isolate	Resistance profile	*fumC* allele	ST	Resistance profile	*fumC* allele	ST	Resistance profile	*fumC* allele	ST
20	14.30.10	1	SUSCEPTIBLE	7		AM-SXT-GM-TE	11		SUSCEPTIBLE	1491	
20	14.30.10	2	SUSCEPTIBLE	7		AM-SXT-GM-CIP-AMC-TE	11		SUSCEPTIBLE	1491	
20	14.30.10	3	SUSCEPTIBLE	7		AM-SXT-GM-TE	11	10	SUSCEPTIBLE	1491	
20	14.30.10	4	SUSCEPTIBLE	7		AM-SXT-GM-TE	11		SUSCEPTIBLE	11	10
20	14.30.10	5	SUSCEPTIBLE	7		AM-SXT-GM-CIP-TE	11		SUSCEPTIBLE	1491	
21	16.30.10	1	SUSCEPTIBLE	11		SUSCEPTIBLE	749		AM-SXT-CAZ-FEP-CZ-CTX-TE	26	
21	16.30.10	2	SUSCEPTIBLE	11		SUSCEPTIBLE	749		AM-SXT-CAZ-FEP-CZ-CTX-TE	26	
21	16.30.10	3	SUSCEPTIBLE	11		SUSCEPTIBLE	749		AM-SXT-CAZ-FEP-CZ-CTX-TE	26	
21	16.30.10	4	SUSCEPTIBLE	11		SUSCEPTIBLE	749		AM-SXT-CAZ-FEP-CZ-CTX-TE	26	
21	16.30.10	5	SUSCEPTIBLE	11		SUSCEPTIBLE	749		AM-SXT-CAZ-FEP-CZ-CTX-TE	26	
23	22.30.10	1	AM-SXT-FEP-CZ-CIP-CTX-AMC	40		AM-AMC-TE	32		AM-SXT-CZ-AMC	108	
23	22.30.10	2	AM-SXT-FEP-CZ-CIP-CTX-AMC	40		AM-IPM-SXT-CAZ-FEP-CZ-CIP-CTX-AMC	40	131	AM-SXT-CZ-AMC	108	
23	22.30.10	3	AM-SXT-FEP-CZ-CIP-CTX-AMC	40		AM-SXT-TE	36		AM-SXT-AMC	108	
23	22.30.10	4	AM-SXT-FEP-CZ-CIP-CTX-AMC	40		SUSCEPTIBLE	11		AM-SXT-AMC	108	
23	22.30.10	5	AM-IPM-SXT-CAZ-FEP-CZ-CIP-CTX-AMC	40	131	AM-CTX-AMC-TE	32		AM-SXT-AMC	108	
24	23.30.10	1	AM-SXT-CIP-TE	231		AM-SXT-CIP-TE	11		SUSCEPTIBLE	32	
24	23.30.10	2	TE	11	34	AM-CIP-TE	11		SUSCEPTIBLE	32	
24	23.30.10	3	AM-SXT-CIP-TE	231		TE	11		SUSCEPTIBLE	32	
24	23.30.10	4	SUSCEPTIBLE	4		TE	11	8125	SUSCEPTIBLE	32	
24	23.30.10	5	AM-SXT-CIP-TE	231		TE	11		SUSCEPTIBLE	32	
25	24.30.10	1	SUSCEPTIBLE	11		AM-SXT	6		SUSCEPTIBLE	11	
25	24.30.10	2	SUSCEPTIBLE	11	10	AM-SXT	6		SUSCEPTIBLE	11	
25	24.30.10	3	SXT-TE	43		AM-SXT	6		TE	11	48
25	24.30.10	4	TE	43		AM-SXT	6		SUSCEPTIBLE	11	
25	24.30.10	5	SUSCEPTIBLE	11		AM-SXT	6		SUSCEPTIBLE	11	
26	25.30.10	1	SUSCEPTIBLE	11	34	AM-SXT-CAZ-FEP-CZ-CIP-CTX	4		SUSCEPTIBLE	231	
26	25.30.10	2	SUSCEPTIBLE	11		AM-SXT-CAZ-FEP-CZ-CIP-CTX	4		SUSCEPTIBLE	231	
26	25.30.10	3	SUSCEPTIBLE	11		AM-SXT-CAZ-FEP-CZ-CIP-CTX	4		AM-SXT-TE	11	
26	25.30.10	4	SUSCEPTIBLE	11		AM-SXT-CAZ-FEP-CZ-CIP-CTX	4		SUSCEPTIBLE	231	
26	25.30.10	5	SUSCEPTIBLE	11		AM-C-SXT-GM-CAZ-FEP-CZ-CIP-CTX-AMC	4		AM-SXT-TE	11	34

Does not apply Same *fumC* allele Same ST

TABLE SM2. Multilocus sequence typing (MLST) and antibiotic resistance profile of selected *Escherichia coli* isolates obtained from 22 children during SP I, SP II and SP III (4/4).

			Sampling period I			Sampling period II			Sampling period III		
Individual	Sample ID	Isolate	Resistance profile	*fumC* allele	ST	Resistance profile	*fumC* allele	ST	Resistance profile	*fumC* allele	ST
27	02.06.11	1	AM-SXT-CIP-TE	231		SUSCEPTIBLE	7		AM-SXT-TE	4	
27	02.06.11	2	AM-SXT-CIP-TE	231		SUSCEPTIBLE	7		AM-SXT-TE	4	
27	02.06.11	3	AM-SXT-CIP-TE	231		AM-AMC	4	120	AM-SXT-TE	4	
27	02.06.11	4	AM-IPM-SXT-CIP-CTX-TE	231		AM-C-SXT-AMC-TE	36		AM-SXT	4	590
27	02.06.11	5	AM-IPM-SXT-CIP-TE	231		SUSCEPTIBLE	7		AM-SXT-TE	4	
29	02.27.11	1	AM-SXT-FEP	11	10	AM-CAZ-TE	45		SUSCEPTIBLE	11	
29	02.27.11	2	C-SXT-CTX-TE	7		AM-SXT-AMC	4		SUSCEPTIBLE	11	
29	02.27.11	3	C-SXT-TE	511		AM-SXT-FEP-AMC	4		SUSCEPTIBLE	11	34
29	02.27.11	4	SXT-FEP-TE	474		SUSCEPTIBLE	35		SUSCEPTIBLE	11	
29	02.27.11	5	IPM	29		AM-TE	35		SUSCEPTIBLE	11	
30	03.27.11	1	AM-SXT-AMC-TE	11	10	AM-SXT-TE	35		AM	27	
30	03.27.11	2	AM-C-SXT-CIP-AMC-TE	27	1716	AM-SXT-TE	35		AM	27	
30	03.27.11	3	IPM	96		AM-SXT-TE	11		AM	27	301
30	03.27.11	4	AM-TE	212		AM-SXT-TE	11	10	AM	27	
30	03.27.11	5	IPM	96		AM-SXT-TE	11		AM-AMC	27	
31	04.27.11	1	AM-CZ-AMC-TE	35		C-SXT-TE	7		AM-SXT-TE	4	
31	04.27.11	2	AM-CZ-AMC-TE	35		C-SXT-TE	7		AM-SXT-TE	4	
31	04.27.11	3	AM-CZ-AMC-TE	35		C-SXT-TE	7		AM-SXT-TE	4	
31	04.27.11	4	AM-AMC-TE	35		C-SXT-TE	7		AM-SXT-TE	4	
31	04.27.11	5	AM-IPM-CZ-AMC-TE	35		C-SXT-TE	7		AM-SXT-TE	4	

Does not apply Same *fumC* allele Same ST