Journal of Pediatric Infectious Diseases 9 (2014) 157–162
DOI 10.3233/JPI-140430
IOS Press

Antibiotic susceptibility of *Escherichia coli* strains isolated in a pediatric population from South Eastern Romania

Simona Claudia Cambrea*
Clinic of Infectious Diseases, Faculty of Medicine, Ovidius University, Constanta, Romania

Received 24 August 2014
Revised 6 October 2014
Accepted 12 October 2014

Abstract. In Constanta during the summer hospital admission of children especially with diarrheal diseases is increased because of the influx of tourists. We determine retrospectively the antibiotic susceptibility of *E. coli* strains isolated from a pediatric population hospitalized during 2012 at Constanta Clinical Infectious Diseases Hospital. Microbiology samples were inoculated on selective media (Blood Agar and Drigalscki Lactose Agar) and incubated at 37^{o}C, for 16 h, and subsequently on average multitest media (MIU, TSI, and Simmons). For the final identification we used API 20E Biomerieux tests. During 2012 we isolated 112 strains of *E. coli* – 85 strains from stool, 16 from urine, 10 from gastric fluid, 8 from throat, and 3 from other secretions. All *E. coli* strains isolated from our patients were *E. coli* enteropathogens. Male: Female ratio was 1:1.33. Median age was 5.5 years (range from 3 months–17.4 years). The antibiotic sensitivity of E. coli revealed a low sensitivity to ampicillin (19.6%), tetracycline (29.5%), and amoxicillin (37.5%). The highest sensitivity was to Carbapenems (93%). Among diseases caused by *E. coli* in children hospitalized at Constanta Clinical Infectious Diseases Hospital during 2012 intestinal tract infections were the most frequent, followed by urinary tract infections. Antibiotic susceptibility of *E. coli* was low to ampicillin, and tetracycline, while the majority of *E. coli* strains were sensitive to carbapenems.

Keywords: *Escherichia coli*, antibiotic, susceptibility

1. Introduction

E. coli is a diverse group of facultative anaerobic gram-negative bacilli of the genus Escherichia in the family Enterobacteriaceae, and contains a variety of strains ranging from commensal organisms to highly pathogenic variants. *E. coli* tends to affect the intestine and the urinary tract but almost any extra-intestinal site may be involved. *E. coli* is often used as a marker of faecal contamination, e.g., in food and water testing, as it does not survive outside the animal body. *E. coli* which causes diarrhea can be transmitted through contaminated water or food, or through contact with infected animals or persons. Six types of pathogenic *E. coli* strains are of associated with diarrhea: shiga toxin-producing (STEC) or enterohemorrhagic (EHEC); enterotoxigenic (ETEC); enteropathogenic (EPEC); enteroaggregative (EAEC), enteroinvasive (EIEC) and diffusely adherent *E. coli* (DAEC) [1–3].

After Bucharest the capital of Romania, Constanta is the second largest city of Romania in the south east of the country. In summer, due to tourism on the Black Sea coast, the city's population doubles and paediatric hospital admissions increase at Constanta Clinical Infectious Diseases Hospital from late spring to late summer. During these months acute diarrheal diseases represent an important public health problem; trans-

*Correspondence: Simona Claudia Cambrea, 100 Ferdinand Avenue, 900709, Constanta, Romania. Tel.: +40 723 47 28 44; Fax: +40 241 61 54 91; E-mail: cambrea.claudia@gmail.com.

Table 1
Distribution by age, sex and pathological products

Month	No. cases	Sex		Environment area		Age				
		Male	Female	Urban	Rural	< 1 yr.	1–4 yrs.	5–10 yrs.	11–14 yrs.	15–18 yrs.
January	4	1	3	3	1	0	3	1	0	0
February	5	1	4	4	1	0	1	2	2	0
March	8	4	4	6	2	1	6	1	0	0
April	10	5	5	6	4	4	5	0	0	1
May	15	5	10	11	4	0	9	2	3	1
June	17	8	9	14	3	1	12	2	1	1
July	38	21	17	25	13	0	21	6	6	5
August	10	3	7	7	3	0	7	1	0	2
September	2	0	2	1	1	0	0	1	1	0
October	2	0	2	1	1	0	1	0	0	1
November	1	0	1	0	1	0	1	0	0	0
December	0	0	0	0	0	0	0	0	0	0
Total	112	48	64	78	34	6	66	16	13	11
%	100	42.9	57.1	69.6	30.4	5.3	58.9	14.4	11.6	9.8

mission is through contaminated food or water or related to sharing contaminated toys.

2. Material and method

We included 112 *E. coli* isolates from stool, urine, and throat or from other purulent secretion (ear, eye, and skin) from children hospitalized at Constanta Clinical Infectious Diseases Hospital, over the year 2012.

Urine, gastric fluid, throat and purulent secretions were inoculated on Blood Agar and Drigalscki Lactose Agar and incubated for 16–24 h at 37^{o}C [4]; for identification we used biochemical tests: TSI (Triple Sugar Iron), MIU (Motility, Indol, and Urea), Citrate Simmons. For purulent secretions after initial inoculation we used Nutrient Broth for 24 h, and then transferred to Blood Agar and Drigalscki Lactose Agar, made in house [5].

Stools were inoculated on Drigalscki Lactose Agar and incubated for 16–24 h at 37^{o}C; and then we use specific sera to agglutination for E. coli: EPEC-Polyvalent and Monovalent; EHEC-O: 157 H7. For the final identification we used API 20E Biomerieux tests [4,5].

Antibiotic susceptibility was performed by disc diffusion method (Kirby-Bauer). Antibiotic tested were: ampicillin, amoxicillin-clavulanic acid, nalidixic acid, ciprofloxacin, norfloxacine, gentamicin, amikacine, cefuroxime, ceftriaxone, ceftazidime, meropenem, piperacillin, chloramphenicol, trimethoprim-sulfamethoxazol (TMP-SMX), tetracycline, colistine and nitrofurantoin disc were used.

Antibiotic sensitivity was evaluated according to Clinical and Laboratory Standards Institute for year 2012 [6].

3. Results

Over a period of one year, between 1st January and 31st December 2012, we isolated 112 *E. coli* from different samples: stool –75, urine –16, gastric fluid –10, throat –8, ear –1, eye –1, and skin –1. All *E. coli* strains isolated from our patients were EPEC. The sex ratio M:F was 1:1.33. Disaggregation by environmental area showed that patients from urban area (69.6%) were more frequently hospitalized than patients from rural area (30.4%). The median age was 5.5 years, (3 months to17.5 years) (Table 1).

The seasonal distribution of *E. coli* isolated from the hospitalized pediatric population showed that the highest numbers of cases were in summer (65 occasions) and the lowest number of cases was isolated during the autumn: 5 (Table 1).

E. coli was isolated most often from feces and gastric fluid (85 cases) related to gastrointestinal infections and, in a smaller number from urine (16 cases) in urinary tract infections (UTI). *E. coli* UTI were observed in only 15 cases: two in children 1–4 years old, 6 in children 5–10 years old and 5 for each age group of 11–14 years and 15–18 years old, respectively. Of the total UTI only 3 cases were found in

Table 2
Antibiotic susceptibility of E coli strain

Antibiotic tested	Total strain – number			Total strain (%)		
	S	I	R	S	I	R
Ampicillin	22	7	83	19.6	6.3	74.1
Amoxicillin	42	29	41	37.5	25.9	36.6
Amoxicillin- Clavulanic acid	49	12	51	43.8	10.7	45.5
Ceftazidim	89	9	14	79.5	8	12.5
Ceftriaxon	84	12	16	75	10.7	14.3
Cefotaxim	72	13	27	64.3	11.6	24.1
Chloramphenicol	80	5	27	71.4	4.5	24.1
Trimethoprim-sulfamethoxazol	43	9	60	38.4	8	53.6
Meropenem	105	4	3	93.8	3.6	2.7
Ertapenem	104	4	4	92.9	3.6	3.6
Nalidixic acid	74	13	25	66.1	11.6	22.3
Ciprofloxacin	92	10	10	82.2	8.9	8.9
Norfloxacin	94	7	11	83.9	6.3	9.8
Amikacine	96	10	6	85.7	8.9	5.4
Gentamycin	61	12	39	54.5	10.7	34.8
Piperacilline + Tazobactam	67	24	21	59.8	21.5	18.7
Nitrofurantoin	88	7	17	78.6	6.3	15.2
Colistine	80	8	24	71.4	7.2	21.4
Tetracycline	33	32	47	29.5	28.6	41.9

S = sensitive, I = intermediate, R = resistant.

boys. A total of 57 gastrointestinal infections (enterocolitis, food poisoning) were identified, the highest number was children between 1–4 years of age.

July was the peak month of isolation with most hospitalized cases – 34 (M : F = 1 : 1.2). In 8 cases with symptoms and clinical findings suggestive of acute tonsillitis (enlarged red tonsils, vomiting), *E. coli* was isolated from throat swabs, but after hospitalization other gastrointestinal symptoms (abdominal pain, diarrhea) were identified.

There was an association between *E. coli* and respiratory infections in 29 cases and with childhood diseases (varicella, rubella, rubeola and scarlet fever) in 6 cases. *E. coli* gastrointestinal infections were concomitant with other intestinal infections in 10 cases, and with other urinary infections (Klebsiella, Pseudomonas aeruginosa), in 3 cases.

E. coli infections were associated with iron deficiency anemia in 53 cases Antibiotic sensitivity of *E. coli* isolated from various pathological samples revealed a low sensitivity to ampicillin (19.6%), and tetracycline (29.5%), and also significant decreases in sensitivity to TMP-SMX (38.4%), amoxicillin (37.5%) and amoxicillin-clavulanic acid (43.8%) (Table 2).

Sensitivity to first-generation aminoglycosides, such as gentamicin, was 54.5%, while the sensitivity to amikacin, a third generation aminoglycoside, was 85.7%. Third generation cephalosporins preserved sensitivity between 70–80%, the highest sensitivity being to ceftriaxone and ceftazidime at 75% and 79.5%, respectively. The highest antibiotic susceptibility of *E. coli* strain was observed with carbapenems at 93% (Table 2).

When we analyzed the antibiotic susceptibility of *E. coli* isolated from stool (Table 3) we noticed a lower sensitivity to ampicillin (18.66%) and TMP-SMX (33.33%). Nalidixic acid, amoxicillin-clavulanic acid and TMP-SMX were some of the most used antimicrobials, in primary care for children, although we noticed an important decrease in susceptibility of *E. coli*. Tetracycline has been extensively used in adults with diarrheic diseases in the past, and as a consequence we observed an important decrease in sensitivity (28%).

4. Discussions

In a publication by Diaconu et al. in 2008 regarding the sensitivity of *E. coli* to antibiotics at our hospital the sensitivity of *E. coli* to ampicillin was 89.4% to nalidixic acid, 93.3%, and to TMP-SMX, 77.5% in

Table 3
Antibiotic susceptibility of E coli strain isolated from stool, urine and throat culture

Antibiotic tested	Stool culture (75 strains)			Urine culture (16 strains)			Throat/Vomiting culture (18 strains)		
	S	I	R	S	I	R	S	I	R
Ampicillin	14	4	57	1	1	14	6	1	11
Amoxicillin	24	20	31	8	4	4	9	5	4
Amoxicillin- Clavulanic acid	28	7	40	10	2	4	11	2	5
Ceftazidim	67	2	6	7	3	6	15	1	2
Ceftriaxon	60	6	9	6	4	6	16	1	1
Cefotaxim	56	6	13	4	0	12	10	6	2
Chloramphenicol	53	1	21	13	1	2	13	1	4
Trimethoprim-sulfamethoxazol	25	4	46	8	0	8	10	2	6
Meropenem	73	1	1	13	2	1	18	0	0
Ertapenem	74	1	0	13	2	1	17	1	0
Nalidixic acid	51	6	18	10	4	2	11	3	4
Ciprofloxacin	62	8	5	12	2	2	18	0	0
Norfloxacin	65	2	8	13	2	1	17	0	1
Amikacine	69	5	1	12	3	1	15	2	1
Gentamycin	45	5	25	6	0	10	10	4	4
Piperacilline +Tazobactam	41	17	17	8	8	0	18	0	0
Nitrofurantoin	62	0	13	10	6	0	16	1	1
Colistine	56	4	15	10	2	4	14	2	2
Tetracycline	21	23	31	6	5	5	6	4	8

S = sensitive, I = intermediate, R = resistant.

2003 [7]. If we compare these data with our data we observe major changes in the sensitivities of these 3 drugs in 2012.

In recent years third generation cephalosporins were often used in hospitals, as consequence we observed slight decreased sensitivity to these drugs. Sensitivities to Ceftriaxone and Cefotaxim were 80% and 74.66% respectively (Table 3). Ceftazidime is less used and *E. coli* remained sensitive (89.33%) compared with other cephalosporins of the same generation.

Comparing our data with those obtained from Mos et al. antibiotic susceptibility of *E. coli* strains isolated from infected skin wounds in the northwest area of Romania, we note that the sensitivity of these strains to ampicillin is only 6.8%, while susceptibility to ciprofloxacin and carbapenems is about 50%, much lower than our data [8].

In another study of isolates from acute diarrheal disease in under 5 years old, at the Laboratory of Enteric Bacterial Infections of the National Institute of Research Development for Microbiology and Immunology “Cantacuzino” Bucharest, the authors found that of 250 strains of E. coli isolated, only 61 were pathogenic after PCR examination. Among these *E. coli*, 29 were classified as enteroaggregative, 22 were atypical enteropathogenic, 8 as enterotoxigenic isolates with only one *E. coli* verotoxin-producing isolate and another one isolate categorized as unconventional DAEC [8]. This study demonstrated the limited efficacy of serotyping, used extensively in Romanian laboratories. More than half of the pathogenic strains analyzed in this study showed phenotypic resistance to multiple drugs. Seven of the multidrug-resistant strains were confirmed to be extended spectrum beta-lactamase (ESBL) producers belonging to ETEC (3 strains), atypical EPEC (2 strains) and EAEC (2 strains) respectively. Given the increasing resistance of E coli in Romania, efforts are needed to improve laboratory methodologies in order to detect resistance on a regional basis to help guiding a national strategy for the prevention and control human infection with E. coli in Romania [9].

In the study done by Hristea et al. the presence of class 1 integrons in resistant strains of *E. coli* and Klebsiella pneumonia was assessed. Strains were isolated from bacteremia in hospitalized patients at three hospitals in Bucharest-Romania between January 2009 and May 2011. Class 1 integrons were detected by PCR targeting the integrase gene. Class 1 integrons were identified in 42 of 77 strains of E. coli resistant to antibiotics. The presence of these integrons in *E. coli* isolated from blood cultures was significantly associated with resistance to TMP-SMX ($p < 0.01$). Strains which were integrons positive were more likely to be multi-resistant. In this study class 1 integrase genes were detected in 17 ESBL-producing *E. coli* strains and 10 ESBL-negative *E. coli* strains. The conclusion of this study was that most strains of resistant *E. coli* isolates from community acquired, health care associated or hospital acquired bacteremias carry class 1 integrons, which are easily transferable [10].

Another study by Baciu et al. performed in the East area of Romania between May 2011–February 2012, identified ESBLs enzymes in Enterobacteriaceae isolated from the samples of children hospitalized in The Clinical Emergency Hospital for Children “Sfântul Ioan”. Galati and evaluated susceptibility of the strains to selected antibiotics. A total of 2 *E. coli* (6.6%) and 18 *K. pneumonia* (41.86%) were producing ESBLs. Most of the ESBL-producing strains were also resistant to aminoglycoside antibiotics and to tetracycline. High sensitivity of ESBL-producing *E. coli* strains (100%) to fluoroqinolones was demonstrated [11].

Similar resistance patterns were identified in studies from other countries around the world. In a study by Kibret M and Abera B, on the susceptibility patterns of *E. coli* from clinical sources in northeast Ethiopia, high resistance rates were found to erythromycin (89.4%), amoxicillin (86.0%) and tetracycline (72.6%). In the same study they noticed a significantly high degree of sensitivity rates to nitrofurantoin (96.4%), norflaxocin (90.6%), gentamicin (79.6%) and ciprofloxacin. Multiple antimicrobial resistances at 74.6% and increased resistance rates to all antimicrobials except ciprofloxacin were also recorded [12].

In a study by Nguyen et al. concerning antibiotic resistance in *E. coli* and Shigella strains isolated from children in Hanoi, Vietnam, among 162 *E. coli* isolates, 86.4% were resistant to ampicillin, 77.2% were resistant to chloramphenicol, 29.6% were resistant to Cefuroxime, 24.1% were resistant to cefotaxime, 19.1% were resistant to nalidixic acid, 3.7% were resistant to ciprofloxacine, 88.3% were resistant to TMP-SMX, and all were sensitive to imipenem. Of the few *E. coli* strains resistant to ciprofloxacin, five were EPEC and one strain was an ETEC. The traditional antibiotics, including ampicillin, chloramphenicol, and TMP-SMX, showed low activity against the diarrheagenic *E. coli* strains [13].

In the study by Zemlickova et al. from the National reference laboratory for antibiotics, National Institute of Public Health, Prague, Czech Republic concerning the prevalence of antibiotic resistance among *Escherichia coli* isolated from community-acquired urinary tract infections, the resistance rates to ampicillin and co-trimoxazole were rather high (48.1%, and 24.1% respectively). Resistance to norfloxacin was markedly different in children and adults, 2.7% in children versus 13.4% in adults. On the contrary, resistance to ampicilin and TMP-SMX was higher in children than in adults (48.1% versus 42.4% in ampicilin and 28.3% versus 23.2% in co-trimoxazole). Five ESBL isolates were recovered from children, and all of the isolates belonged to the pathogenic B2 phylogenetic group [14].

5. Conclusions

Among diseases caused by *E. coli* in children hospitalized in Constanta clinical Infectious Diseases Hospital during 2012, intestinal tract infections were the most frequently observed, followed by urinary tract infections and infections from other sites.

Children between 1–4 years were most frequently affected.

Antibiotic susceptibility of *E. coli* was low to ampicillin (19.6%), Tetracycline (29.8%) and Trimethoprim/Sulfamethoxazole (38.4%).

In Romania extensive studies are required to identify the real pathogenicity of strains of *E. coli* and ESBL-producing strains. To date, studies have been only sporadic involving small numbers of infections.

References

[1] Torok ME, Cooke FJ, Moran E. Escherichia coli in Handbook of Infectious Diseases and Microbiology, Oxford University Press 2010: 324-6.

[2] Mos I, Micle O, Zdrâncă M, Mureşan M, Vicaş L. Antibiotic Sensitivity of the Escherichia coli Strains Isolated from Infected Skin Wounds. Farmacia 2010; 58 (5): 637-44.

[3] Torok ME, Cooke FJ, Moran E. Infectious diarrhoea in Handbook of Infectious Diseases and Microbiology. Oxford University Press 2010: 688-9.

[4] Buiuc D, Negut M. Tratat de Microbiologie Clinica. 3th ed. Editura Medicala, Bucuresti; 2009: 269-270.

[5] Buiuc D, Negut M. Tratat de Microbiologie Clinica. 3th ed. Editura Medicala, Bucuresti; 2009: 1049-100.

[6] CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. CLSI document M100-S22. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

[7] Diaconu S, Cambrea SC, Ilie MM, Tanase DE, Marcas C, Cretu-Stancu I et al. Evolution of susceptibility to antibiotics of strains of E. coli at children with acute diarrheal disease, 10 th National Congress of Infectious Diseases, Cluj Napoca 4-7 iunie 2008, Clujul Medical vol. LXXXI, supplement 2008, pp. 233-234.

[8] Mos I, Micle O, Zdranca M, Muresan M, Vicas L. Antibiotic Sensitivity of the E. coli strains isolated from infected skin wounds. Farmacia 2010; 58 (5): 637-45.

[9] Usein CR, Tatu-Chitoiu D, Ciontea S, Condei M, Damian M. Escherichia coli pathotypes associated with diarrhea in Romanian children younger than 5 years of age. Jpn J Infect Dis 2009; 62(4): 289-93.

[10] Hristea A, Ion M, Maxim D, Banica L, Nica M, Buzea M et al. Class 1 integrons in drug-resistant E. coli and K. pneumonia from blood stream infections. Revista Romana de Medicina de Laborator vol. 20, nr. ¾, Septembrie 2012, pp. 279-286.

[11] Baciu G, Gurau G, Florea C, Zisu M. Antimicrobial susceptibility of Enterobacteriaceae clinical isolates producing extended-spectrum beta-lactamase (ESBLs) in a pediatric hospital in Romania. 30th Annual Meeting of the European Society For Paediatric Infectious Diseases 2012 Thessaloniki, Greece, P 1310.

[12] Kibret M, Abera B. Antimicrobial susceptibility patterns of E. coli from clinical sources in northeast Ethiopia. Afr Health Sci 2011; 11 Supp l1: 40-5.

[13] Nguyen TV, Le PV, Le CH, Weintraub A. Antibiotic Resistance in Diarrheagenic Escherichia coli and Shigella Strains Isolated from Children in Hanoi, Vietnam Antimicrob. Agents Chemother 2005; 49(2): 816-19.

[14] Zemlickova H, Hrabak J, Fridrichova M, Jakubu V, Chudackova E. The prevalence of antibiotic resistance among Escherichia coli isolated from community-acquired urinary tract infections. 30th Annual Meeting of the European Society For Paediatric Infectious Diseases 2012 Thessaloniki, Greece, P 1220.