treated for 7 days with a combination of ranitidine, bismuth, metronidazole and doxycycline. In 44 of these patients (10.86%), Hp infection was not eradicated at follow-up 6 weeks after treatment. In these patients a 14-day combination therapy with omeprazole (40 mg/day), roxithromycin (200 mg b.i.d.) and bismuth (240 mg b.i.d.) was used. Therapeutic success using this triple combination was achieved in 43 of 44 patients (97.7) who were Hp negative at 6 weeks and 6 months after the end of this therapy.

Conclusions: The proposed therapeutic approach with high efficacy and good tolerance can be recommended for further clinical evaluation and the therapy of recurrent symptomatic Hp infections.

P674 Diagnosis of *Helicobacter pylori*-associated chronic duodenitis

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Objectives: To investigate chronic duodenitis with persistence of *Helicobacter pylori* (HP) on gastric metaplasia of duodenal bulbus.

Methods: Fifty-seven patients with chronic duodenitis have been examined. The diagnosis was made on the base of tissue biopsy data. *H. pylori* status was confirmed by the morphologic, immunologic method (*H. pylori* IgG ELISA, Diagnostic Automation, Inc., USA) and urease test (Jatrox-H.p. Test, Germany). The density of bacteria was determined according to the four-step scale. Regions of gastric metaplasia of duodenum were confirmed by alcian blue (Serva) staining.

Results: HP was present in the stomachs of 25 (43.9%) patients, on the gastric metaplasia of duodenal bulbus in 12 (21.1%) patients. Seven (12.3%) patients had BP only in duodenal bulbus, without persistence of microorganisms in gastric mucosa. There was a previous history of peptic ulcer disease in 42.9% of patients and erosive process in 28.6%. Endoscopically, in addition to acute chronic superficial duodenitis with gastric metaplasia of duodenal bulbus, peptic ulcer was found in 57.1% of patients and multiple erosive process in 28.6%. Histology of duodenal tissue revealed chronic inflammation, activity, superficial duodenitis and gastric metaplasia of bulbus in 1000% of patients, and erosive process in 28.6%. All of the patients with chronic duodenitis had a high pH of gastric juice on an empty stomach (1.25–1.95). Histology of gastric tissue revealed chronic inflammation (91.4%), activity (76.7%), superficial gastritis (60.0%), gland atrophy of the stomach (40.0%), intestinal metaplasia (26.7%) and erosive process (16.7%).

Conclusions: Indications for duodenal tissue biopsy for determination of *Helicobacter pylori* are: (1) ulcer dyspepsia and duodenal ulcer in anamnesis; (2) acute duodenitis with gastric metaplasia, and multiple erosive and ulcer processes by endoscopy.

P675 The determination of IgG anti-*Helicobacter pylori* antibodies in pregnant women and Venezuelan children

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Objectives: Evaluation of the seroprevalence of IgG anti-*H. pylori* antibodies in asymptomatic pregnant women and serum samples from umbilical cord blood at the moment of the baby's birth, compared to the seroprevalence in the juvenile population.

Methods: Serum samples from 160 healthy children (age: 1–10 years) from different states of Venezuela, 50 serum samples from pregnant women without gastroduodenal infection and 50 serum samples from umbilical cord blood were studied by ELISA test for the determination of IgG anti-*H. pylori* antibodies.

Results: The prevalence of IgG anti-*H. pylori* antibodies was high in the pediatric population (62.5%); only 37.5% of the children in the group were seronegative ($p<0.0001$). The seroprevalence observed in the serum samples from pregnant women and umbilical cord samples was 54% in both groups. 46% of the mothers and of the matched-up samples of umbilical cord blood were negative for IgG anti-*H. pylori* antibodies.

Conclusions: These results suggest that anti-*H. pylori* specific antibodies pass the placental barrier and have a protective role during the first months of life. The prevalence of IgG anti-*H. pylori* antibodies in samples from asymptomatic women probably indicates an infected group. The serologic test indicates a high IgG anti-*H. pylori* immunoresponse in the pediatric population. This study was supported by grant S1-96001408-CONICIT.

Enteric pathogens

P676 BBL CHROMagar O157, a new chromogenic medium for the isolation of *E. coli* O157

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Objectives: To create a chromogenic medium for the detection of *E. coli* O157, based on ß-glucuronidase negativity, which is suitable for human clinical, animal and food samples, performing at least as well as selective Sorbitol-MacConkey Agar (CT-SMAC).

Methods: CHROMagar O157 dehydrated medium was supplemented with selective agents to inhibit most of the undesired species such as *Proteus*, *Pseudomonas*, and *Aeromonas*. The manufacturing procedures were modified to allow the industrial preparation as a plated medium with a shelf-life of at least 8 weeks. The resulting medium (BCA) was tested with both O157 and undesired strains. A larger field investigation with 1104 samples from bovine and ovine carcasses and human fecal specimens was conducted at Sheffield PHL. For comparison, CT-SMAC from both BDMS (BCT) and Sheffield PHL (SCT) were used. Specimens were plated onto the media after immunomagnetic separation (DYNAL, Oslo, Norway).

Results: All of the O157 reference and clinical strains produced excellent growth of rose-violet ('mauve') colonies on BCA. The recovery rate of low CFUs was higher than on the non-selective reference medium. Among the 40 non-O157 strains tested, only one strain of *Enterobacter sakazakii* produced weak growth of mauve-colored colonies. Most of the other strains were fully inhibited, and only a few produced blue or colorless colonies. In the field investigation, the positivity rates of the three media were nearly identical. The number of false positives was higher on SCT than on BCT and BCA.

Conclusions: BBL CHROMagar O157 is an excellent alternative to Sorbitol-MacConkey-based media.

P677 Evaluation of O157:H7 ID, a new chromogenic medium for isolation and detection of *Escherichia coli* O157:H7

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O157:H7 ID is a chromogenic selective medium for isolation and detection of *Escherichia coli* O157:H7. On this medium, *E. coli* O157:H7 strains appear as blue–green colonies, whereas other strains produce colorless or pink to purple colonies, or are inhibited. It can be used with or without a cefixime–tellurite (CT) supplement to increase its selectivity for Enterobacteriaceae.

More than 300 bacterial and yeast strains were isolated on O157:H7 ID with or without CT and MacConkey agar plates.

Fertility is equivalent on O157:H7 ID and MacConkey agar plates. After 20 h of incubation, most *E. coli* O157:H7 colonies are larger on O157:H7 ID, with or without CT supplement, than on MacConkey agar plates. Selectivity for Gram-positive bacteria and yeasts is 100%. When the medium is used with CT supplement, most Gram-negative bacteria are inhibited. In this case, selectivity increases from 30% to 55%. Sensitivity of *E. coli* O157:H7 detection is 100% between 20 h and 48 h of incubation. Specificity on O157:1-17 ID without CT supplement is 95% but increases to 98% when CT supplement is added. Only *E. vulneris* strains are false positive but on O157:H7 ID with the CT supplement they are inhibited.

The detection of more than one enzymatic activity, with well-contrasted colors, combined with the possibility of using a CT-selective supplement, provides excellent sensitivity and high specificity. Consequently, O157:H7 ID is easy to read and interpret and appears to be very well adapted to the microbiological food control and clinical detection of *E. coli* O157:H7.

P678 Prevalence of *E. coli* O157:H7 during the past year (1997) in Madrid, Spain

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Objectives: To determine the prevalence of *E. coli* O157:H7 in our area.

Methods: 1800 stool specimens were screened for *Escherichia coli* O157:H7 between January and December 1997. All samples were screened by inoculation onto sorbitol-McConkey agar at 37°C. The sorbitol-negative colonies were agglutinated with latex particles covered by *E. coli* O157:H7 antiserum (*E. coli* O157:H7 Test kit, Oxoid). The positive isolates for the agglutination were subcultured onto 5% sheep blood agar, followed by a confirmatory conventional biochemical set. The specimens were also inoculated onto routine media to investigate other enteropathogens (*Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Aeromonas* spp., and *Campylobacter* spp., parasites using the common techniques, and rotavirus and adenovirus antigens.

Results: No *E. coli* O157:H7 strains were isolated among all the specimens analyzed. The recovered enteropathogen rate was 33% with the following distribution:

	N° isolates	(%)
Campylobacter spp.	201	31,4
Salmonella spp.	167	26,1
Cryptosporidium spp	15	2,3
rotavirus antigen	177	27,6
adenovirus 40:41 antigen	12	1,9
Yersinia enterocolitica 0:3	12	1,9
Giardia intestinalis	8	1,2
Shigella spp	2	0,3
Aeromonas spp	2	0,3

Conclusions: According to the results, we do not recommend routine screening for *E. coli* O157:H7 in our area.

P679 *Escherichia coli* O157:H7 incidence in a Spanish hospital

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Objectives: To determine the incidence in our population of *E. coli* O157:H7 in diarrhea during 6 months.

Methods: 512 stools from 263 adult patients were studied. All were tested to discard usual enteropathogens by standard methods including Sorbitol-MacConkey agar (Oxoid). Sorbitol-negative colonies were identified as *E. coli* using conventional methods. These ones were tested by a commercial latex agglutination test (*E. coli* 0157 Latex Test: Oxoid) to detect *E. coli* O157:H7. Confirmation was done by detection of VT I, VT2 and *eae* genes.

Results: 38 (7.4%) sorbitol-negative *E. coli* were identified and only 1 (0.38%) agglutinated in the latex test and was confirmed as *E. coli* O157:H7 by molecular methods. The strain came from an HIV patient and he had no data of epidemiologic interest. Three samples from the same patient were examined, all of them showing *E. coli* O157:H7.

Conclusions: There was a low incidence (0.3%) of *E. coli* O157:H7 in our community, as in other data published. The case reported was an HIV-infected patient. Further studies are necessary to determine the role of this enteropathogen in this group.

P680 Bacterial causes of infectious diarrhea with emphasis on *E. coli*

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Objectives: To determine the most important causes of bacterial diarrhea in a Danish population.

Methods: Stools from 3880 episodes of diarrhea were examined for *Salmonella*, *Campylobacter*, *Yersinia*, *Shigella* and vibrios using standard methods. A fraction were also examined for *E. coli* by colony hybridization of virulence genes (2720 episodes for ETEC, EIEC and VTEC, and 538 children below 2 years for EPEC). The episodes were grouped by the requesting physician as acute diarrhea acquired in Denmark (AD), persistent diarrhea (PD) or travelers' diarrhea (TD).

Results: Overall, a bacterial cause was found in every 4th case of AD or TD but in only 7.3% of PD. *Salmonella* was the most commonly isolated pathogen in all three groups (3.0–13.6%), followed by *Campylobacter* (1.9–8.1%) and *E. coli* (1.5–5.7%). *Yersinia* was found in 0.3–1.2% and *Shigella* in 0–2.6% of the episodes. Among the *E. coli* types, EPEC and VTEC dominated in the AD and PD groups, and ETEC in TD. For children below 2 years of age, *E. coli* was the most common pathogen in AD and PD (AD: *E. coli* 5.1%, *Salmonella* 4.3%, *Campylobacter* 4.0%; PD: *E. coli* 7.3%, *Salmonella* 2.4%, *Campylobacter* 1.8%). In the AD group, EPEC and VTEC were equally common. In the PD group, there were twice as many isolations of EPEC as VTEC.

Conclusions: *E. coli*, especially EPEC and VTEC, are common causes of diarrhea in at least young children.

P681 The efficiency of *Campylobacter jejuni/coli* identification in the conditions of atypical strain circulation

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Objectives: The determination of efficiency of *Campylobacter jejuni/coli* identification and the quota of circulating atypical strains.

Methods: 56 Campylobacter strains, isolated from 309 diarrheic children, were studied. The differentiation of *C. jejuni* species was done by hippurate hydrolysis test supplemented by assimilation of D-malate, propionate and gamma-glutamyltranspeptidase (gamma-GTP) tests. The strains were concomitantly identified by API-Campy systems (BioMérieux) and by PCR in which VERSUS 15/VERSUS 16 for *C. jejuni* and CSF/CSR for *C. coli* primers were used (Stonnet, Guesdon, 1993, 1995).

Results: The hippurate hydrolysis test identified 66.9% of strains belonging to the *C. jejuni* species, and 27.1% to *C. coli*; another 6% of strains remained undefined. The API-Campy test systems and PCR identically identified 75.5% strains as *C. jejuni* and 24.5% as *C. coli*. The analysis of comparative results showed that the quota of correctly identified strains using the hippurate hydrolysis test constituted only 80.8%. The supplementation of the hippurate hydrolysis test with gamma-GTP assimilation of D-malate and propionate tests allowed an increase in the efficiency of correctly defined species to 100%. The errors of the hippurate test were conditioned by an incomplete hydrolysis of substratum, manifested by false-negative, false-positive and dubious results (12.3%) and by atypical strains. Among them, 3.4% of strains were defined as *Campylobacter* resistant to nalidixic acid and 10.3% of strains as hippurate-negative *C. jejuni*. The results of PCR and API-Campy systems had not been influenced by the presence of atypical strains.

Conclusions: The low efficiency of the hippurate hydrolysis test, the high quota of atypical *Campylobacter* strains, and the significant prevalence of *C. jejuni* strains, make necessary the supplementation of that test with the above-mentioned differential enzymatic tests or application of API-Campy systems or PCR in *Campylobacter* identification.

P682 Clinical microbiology of campylobacter enteritis in our experience

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Objectives: To point out the importance of *Campylobacter jejuni/coli* in community enteritis and to remark on the diagnosis and antibiotic resistances.

Methods: During 1996–97, stool specimens were processed for enteric pathogens. To isolate *Campylobacter* we used filter-membrane technique and specific selective media. Incubations and identifications were obtained by the conventional methods. Antimicrobial susceptibility tests were performed by a modified agar diffusion method.

Results: 95 strains were isolated. We identified 67 strains of *C. jejuni* (71%) and 28 of *C. coli* (29%). Seven were isolated among children (81%), particularly 2–6-year-olds (62%). Liquid feces were observed in 51%, abdominal pain in 61%, and fever in 56%; fecal white cells were present in 55%, and fecal blood in 36%. Resistances were: to erythromycin 11.6%, to morfloxacin 42.1%, to tetracycline 54.7%, and to amoxycillin–clavulanate13.7%.

Conclusions: *Campylobacter* may play a severe role in acute enteritis. The resistances to quinolones are increased.

P683 Isolation of *Campylobacter jejuni* and *Campylobacter coli* from patients with enterocolitis in Sofia, Bulgaria

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Interest in the micro-aerophilic spiral-to-curve-shaped bacteria belonging to the genus *Campylobacter* has greatly increased in recent years. *C. jejuni* (Cj) and *C. coli* (Co) are important causative agents of enterocolitis. Infection with these microorganisms is widespread and they can be isolated in 4–30% of fecal specimens of patients with acute diarrheal diseases.

The aim of this study was to clarify the role of Cj and Co in the etiology of diarrheal diseases in Sofia for the period 1987–97. It included different groups of ages from 0 to 60 years.

A total of 30 033 fecal specimens were screened for *Campylobacter*. Cj and Co were detected in 4.55%, with the highest percentage in 1988 (7.51%) and the lowest in 1994 (1.66%, $p<0.01$). Campylobacteriosis was mostly spread in wet spring and summer months (April, May and June, respectively, 6.78%, 6.98% and 6.35%) and most rarely in January (1.83%, $p<0.011$).

The most affected groups were among the children from 1 to 3 years old (30.56%), followed by 4–7 (26.98%), 8 –14 (14.19%) and 0–1 (11.49%).

Campylobacter takes first place (4.53%) among the agents of enterocolitis—*Shigella* 3.84%, *Salmonella* 2.74%, and *E. coli* 2.60%—in the period 1989–97 and it is considered an important agent of enterocolitis in Bulgaria.

P684 Use of arbitrarily primed PCR (AP-PCR) in molecular typing of *Salmonella enteritidis* food poisoning outbreak in a tertiary care hospital

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Objectives: AP-PCR was used to evaluate a salmonella gastroenteritis outbreak that occurred among hospital staff members of a tertiary care university hospital between 29 September and 10 October 1997.

Methods: The hospital staff members who developed diarrhea and high fever between 26 September and 10 October 1998 were included in this evaluation. A questionnaire was applied and stool samples were obtained from all 131 symptomatic patients and kitchen staff.

Results: A total of 131 hospital staff who ate regularly at staff cafeteria were affected. It was found that they all had consumed the same lunch that was served at those cafeterias on 26 Septembe. From their stool cultures, 43 strains of *Salmonella* were isolated and serotyped as *Salmonella enteritidis*. All the isolates showed identical antibiotic resistance and AP-PCR patterns. Surprisingly, cultures from all kitchen personnel were negative.

Conclusions: From these results, *Salmonella enteritidis* seems to be the infecting agent of this outbreak. Although we could not obtain any cultures from the leftovers of that meal, since every item was washed and discarded, this outbreak was probably caused by the 26 September lunch that was mainly prepared with eggs that were contaminated with *Salmonella*.

P685 An outbreak of *Salmonella blockley* infections associated with an atypical food vehicle, Germany 1998

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Objectives: In June 1998, 10 cases of *S. blockley* infection were reported from one German state (Mecklenburg-Vorpommern (MV)). Because *Salmonella blockley* is extremely uncommon in Germany, a case-control study was performed in order to find the source.

Methods: Cases were defined as persons with positive stool culture for *S. blockley* who had spent time in MV from 26 May to 8 June 1998. Three additional cases were identified by reviewing laboratory data. Twenty-one neighborhood controls were nominated by the cases or, if none was nominated, were chosen from the telephone book. Telephone interviews using standardized questionnaires gathered information about demographics, symptoms and food consumed.

Results: Nine of 12 cases and 2 of 21 controls with food consumption histories reported eating smoked eel (OR 24; 95% CI 3.9–235.3). The consumed eel came from five different local smokeries, but could be traced back to two wholesalers. Two fish farms in Italy (province Veneto) delivered eel to both wholesalers in May/June 1998. About 40% of all Italian *S. blockley* isolates are usually identified from Veneto.

Conclusions: Eel imported from Italy was the most likely cause of this outbreak. *S. blockley* is common from the area of origin of the eel; however, the means of eel contamination is unknown. Smoked eel has not yet been described as a vehicle for salmonella infections. This outbreak suggests that the smoking process may not eliminate bacterial contamination from raw fish.

P686 The isolation of *Salmonella* with a new culture medium (SMID) in comparison with SS agar

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Objectives: To evaluate a new chromogenic culture medium (SMID, bioMerieux) in the isolation of *Salmonella* spp. from fecal specimens in comparison with SS agar.

Methods: The study was divided into two parts. In the first part, 112 fecal specimens were examined with direct culture on SS agar and SMID medium. The second part included 115 fecal specimens which were cultured on SS agar and SMID medium after enrichment in selenite broth. The identification of *Salmonella* strains was performed with the Vitek GNI card.

Results: From the 112 fecal samples in the first part of the study, 13 *Salmonella* strains were isolated, 13 on SMID medium and 11 on SS agar. The SMID medium showed better sensitivity, and the isolation rate for this medium was 12%, and for SS agar 10%. Of the strains isolated, 11 were *S. enteritidis* and 2 *S. typhimurium*. In the second part, 20 *Salmonella* strains were isolated, 20 on SMID medium and 15 on SS agar. The isolation rate was 17% for SMID medium and 13% for SS agar. Of the *Salmonella* strains isolated, 18 were *S. enteritidis*, 1 *S. typhimurium* and 1 *S. blockley*.

Conclusions: The SMID medium in comparison with the SS agar has shown better results in isolation and preidentification of *Salmonella* from fecal specimens. For better results, this medium must be used for culture after enrichment in a selective broth.

P687 Clinical and laboratory study of salmonella gastroenteritis in adults (1995–97)

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Objectives: The clinical and laboratory study of *Salmonella* spp. gastroenteritis in adults.

Methods: During a 3-year period (1995–97), 43 patients with *Salmonella* spp. gastroenteritis were admitted to our hospital because of the severity of their symptoms. Their age ranged from 15 to 87 years (median 46). The identification of *Salmonella* was performed by conventional methods and API (bioMerieux) and susceptibility testing by the Kirby–Bauer method.

Results: *S. enteritidis* was isolated most frequently (65%), followed by S. *typhimurium* (27.9%). Of the patients, 67.4% had watery diarrheal stools (10 loose stools per day), 53.5% fever 38.5°C, 58.1% abdominal pain, 62.8% vomiting, 16.3% bloody stools and 7% myalgias and arthralgias. The duration of hospitalization was 1–15 days (mean 5.5 days). Of the patients, 46.5% had leukocytosis, 100% had neutrophils and 48.8% had erythrocytes in their feces. Serum CPK was examined in 25 patients, in 6 of whom it ranged from 1000 to 8500 U/L, while CPK-MB was normal. Of *Salmonella* spp. isolates, 55.8% were resistant to ampicillin, 11.6% to trimethoprim/sulfamethoxazole and 0% to ciprofloxacin. Antibiotics were administered in 25 patients. The outcome of the infection was recovery in all patients.

Conclusions: The most frequently isolated serotype was S. *enteritidis*, and 58.1% of the patients were administered antibiotics because of the severity of the infection.

P688 Etiologic structure of non-typhoidal salmonellosis and antimicrobial resistance of *Salmonella* spp. isolated in the town of Plovdiv (1996–98)

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Objectives: To investigate the dynamics of the etiology of non-typhoidal salmonellosis and the trend of resistance of NTS spp. isolated over the last 3 years in the Clinic of Infectious Diseases, Plovdiv.

Methods: 210 inpatients with culture-confirmed salmonellosis were studied. NTS spp. were obtained from feces (209), urine (3) and blood (1). Bacteria were identified by standard methods and serotype (Hauffmann–White scheme). The antibiotic sensitivity pattern was assessed by the disk diffusion method (Bauer–Kurby).

Results: The 9 serotypes of NTS identified belong to 5 serogroups: D, B, C_1, C_2 and E. The predominant etiologic agent was *S. enteritidis* (70.71%), followed by *S. typhimurium* (20.36%); in a few cases, other serotypes were isolated. Most of the cases were sporadic (80.66%). Nosocomially acquired salmonella infection was observed in 28 patients (13.33%). Poultry, eggs of poultry and related products were responsible for 26% of all cases, but for 38.33% of *S. enteritidis* infection. Susceptibility to antibiotics of the different serotypes was variable. Gentamicin inhibited 81.99% and nalidixic acid 68.42% of the NTS studied. The cefa-third generation and amikacin inhibited more than 90% of the strains.

Conclusions: The antibiotic sensitivity pattern showed a gradual increase in resistance over the years and even resistance to the new quinolones and cefa-third generation has emerged. Antimicrobials with little demonstrated resistance should be considered for patients with complicated illness and at high risk of resistant infection.

P689 Analysis of shigella outbreak typing with molecular and classical methods

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Objectives: *Shigella sonnei* is a major cause of diarrheal diseases in Turkey. There was an outbreak of shigella gastroenteritis in Samsun, Turkey from 8 to 13 November 1996. The aim of the study is to identify the type of *Shigella* isolates using molecular and classical methods.

Methods: 628 people had gastroenteritis and 27 of them were hospitalized at our infectious disease clinic. 342 stool cultures were obtained from patients. *S. sonnei* had been isolated from stool cultures of 94 patients. Since *S. sonnei* has a single serotype, different methods were tried to refine the epidemiologic analysis. 25 *S. sonnei* isolates were analyzed with arbitrarily primed polymerase chain reaction (AP-PCR), plasmid DNA analysis, resistance typing and biotyping (API 32E (Bio Merieux, France)).

Results: The strains displayed four patterns with biotyping. 25 isolates had the same antibiotic resistance pattern, with one exception. Two different plasmid profiles were identified during this outbreak. All isolates had the same clone with AP-PCR.

Conclusions: The results of this study indicate that AP-PCR fingerprinting has more power for discrimination of epidemiologically related isolates than plasmid profile analysis, resistance typing and biotyping.

P690 In vitro susceptibility to antimicrobial agents in *Shigella* spp.

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Objectives: To review the antimicrobial susceptibility patterns of *Shigella* spp. isolated in Pleven, Bulgaria from 1994 to 1997.

Methods: Ninety-one *S. flexneri* and 35 *S. sonnei* isolates were tested for their susceptibilities to 11 antimicrobial agents. MICs for ampicillin (AMP), ampicillin/sulbactam (SAM), ceftriaxone (CRO), cefoperazone (CFP), gentamicin (GEN), amikacin (AMK), kanamycin (KAN), ciprofloxacin (CIP), norfloxacin (NOR), sulfamethoxazole (SMZ) and trimethoprim (TMP) were determined by an agar dilution method.

Results: The *S. flexneri* and the *S. sonnei* isolates had high frequencies of resistance to AMP, SAM, SIVIZ and TMP. Eighty-two (90%) of the tested *S. flexneri* strains were resistant to AMP (MIC range 64–1024 μg/mL). Only nine (10%) *S. flexneri* isolates were susceptible to AMP, with MIC range 0.5 - 2 μg/mL. About 24% of *S. flexneri* strains were resistant to TMP (MIC range 4–256 μg/mL). In the *S. sonnei* isolates, high frequencies of resistance were also seen for some agents. The *S. sonnei* strains had significantly lower percentages of resistance to AMP than *S. flexneri* (74% resistance in *S. sonnei* with MIC range 64–512 μg/mL). No isolates were resistant to AMK, KAN, CRO, CFP, CIP and NOR, and most isolates showed high susceptibility.

Conclusions: These results show high frequencies of resistance in *S. flexneri* and *S. sonnei* to many of the first-line antimicrobial agents. Resistance trends regarding ampicillin and TMP/SMZ, agents typically used to treat shigellosis, mandate the consideration of other therapeutic options.

P691 Dynamics of shigellosis morbidity in the Republic of Belarus

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Objectives: To study the manifestations of epidemic processes in the Republic of Belarus.

Methods: In our work we used the reports of local centers of hygiene and epidemiology (CHE) from the last 10 years. We performed a retrospective analysis of morbidity, seasonal prevalence and etiologic structure of shigellosis.

Results: In the past 10 years the morbidity rose from 36.02 to 139.65 cases per 100 000 population. Cyclical peaks were found every 2–3 years. The cases of shigellosis ranged from 29.5% (for the period of cyclical decrease) to 48.67% (for the years of maximal rise of morbidity) of acute intestinal disease totals. *Shigella sonnei* predominated in the etiologic structure (up to 82.19%). Since 1994 *Shigella flexneri* morbidity has tended to rise. The morbidity of shigellosis caused by *Shigella flexneri* in 1997 was two times as high as in 1996 and amounted to 29.2 per 100 000 population. *Shigella flexneri*'s share in the morbidity caused by shigellosis increased to 38.35% in 1997. The decreased intensity of seasonal rises is a feature of shigellosis caused by *Shigella flexneri* (from 11.63% to 49.12%).

Conclusions: A rise of morbidity caused by *Shigella flexneri* has been noted from 1994. *Shigella flexneri* is characterized by a lower intensity of seasonal rises and higher levels of non-seasonal spread.

P692 Detection of ShET-1 and ShET-2 of *Shigella* strains isolated from patients with traveler's diarrhea

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Objectives: *Shigella* spp. are known primarily for causing a bacillary dysentery; however, a sizeable number of cases exhibit in the first phase a watery diarrhea that could or not be followed by dysentery. Therefore, new virulence factors associated with the species of *Shigella* have recently been described. These are enterotoxins 1 and 2 of *Shigella* (ShET-1 and ShET-2). The objective of this study was to determine the prevalence of ShET-1 and ShET-2 in species of *Shigella* isolated from patients with traveler's diarrhea (TD).

Methods: During 1993–8, stool samples from 500 travelers with diarrhea were cultured for the isolation of *Shigella* spp. and other enteropathogens. The detection of ShET-1 and T-2 was done by a PCR technique, using specific primers.

Results: Of a total of 51 strains of *Shigella* spp. isolated in this period (22 *S. flexneri*, 26 *S. sonnei*, and 3 *S. dysenteriae*), in 31 (60.78%) at least one enterotoxin was detected: 2 (9.09%) only produced ShET-1 (all *S. flexneri*), while 21 (41.17%) produced ShET-2 (3 *S. flexneri*, 15 *S. sonnei*, and 3 *S. dysenteriae*). Furthermore, 8 (15.69%) out of 22 *Shigella flexneri* strains presented both enterotoxins.

Conclusions: The prevalence of ShET-1 and ShET-2 is highly significant in the species of *Shigella*.

P693 *Clostridium perfringens* enterotoxin and *Clostridium difficile* toxins can be detected with similar frequency in stool samples of patients with antibiotic-associated diarrhea

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Objectives: To investigate the prevalence of *Clostridium perfringens* enterotoxin (CPE) and *Clostridium difficile* toxins in stool samples of patients with antibiotic-associated diarrhea (AAD).

Background: *Clostridium perfringens* (*C. perfringens*) is a well-known cause of food poisoning. Sporadic cases of AAD caused by CPE have been reported. Whereas *C. difficile* is the main causative agent in patients with AAD, the prevalence of CPE in stool samples of these patients remains to be investigated.

Methods: Between March and June 1998, 242 consecutive stool samples obtained from 157 patients with suspected AAD were tested for the presence of CPE and *C. difficile* toxins by commercially available ELISA. Culture and identification of *C. perfringens* and *C. difficile* were performed using standard methods. Review of patient charts was performed to correlate clinical information with bacteriologic results.

Results: CPE was detected in 10 (4.1%) *C. difficile* toxins in 12 (5.0%) samples obtained from 10 (6.4%) patients each. One sample was positive for both CPE and *C. difficile* toxins. *C. perfringens* was isolated from 10 (4.1%) and *C. difficile* from 4 (1.7%) samples.

Conclusions: These results indicate that *C. perfringens* enterotoxin may be an important cause of AAD. The frequency of detection suggests a similar prevalence for CPE and *C. difficile* toxins in stool samples of patients with AAD.

P694 IMMULITE *C. difficile* for detection of toxin A

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Objectives: We have developed an assay for the detection of *C. difficile* toxin A in stool samples for the IMMULITE automated chemiluminescent immunoassay analyzer. The purpose of this study was to evaluate the performance of the IMMULITE *C .difficile* toxin A against another EIA assay.

Methods: Enclosed within the proprietary IMMULITE test unit is a polystyrene bead coated with a specific anti-toxin A monoclonal antibody. Patient specimen and buffer reagent are simultaneously introduced automatically by the IMMULITE into the test unit and incubated for 30 min. After a spin-wash step, sample, excess reagent and wash solution are captured in a coaxial waste chamber. An alkaline phosphatase-labeled polyclonal antibody specific for toxin A is introduced and another 30-min incubation follows. The bead is then washed again and enzyme label is measured with a chemiluminescent substrate, a phosphate ester of admantyl dioxetane. A qualitative result is obtained by comparing the patient result to an established cut-off. A collection of 105 specimens was tested by both the IMMULITE and Premier *C. difficile* toxin A (Meridian Diagnostics, Inc.) assays.

Results: The relative sensitivity and specificity values for the IMMULITE assay were both 84% versus the Meridian kit, with 86% agreement between the two assays.

Conclusions: IMMULITE is the first continuous random access, chemiluminescent enzyme immunoassay system to offer an assay for detection of *C. difficile* toxin A.

P695 Distribution of *Clostridium difficile* diarrhea in La Paz Hospital, Madrid, Spain

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Objectives: The aim of our study is to analyze the distribution of diarrhea caused by *C. difficile* in the different departments of our hospital.

Materials and methods: The diarrheal infection caused by *C. difficile* was diagnosed by the detection of 'A toxin' in the stools using an enzyme immunoassay (Premier *C. difficile* Toxin A, Meridian Diagnostics, Inc.). A retrospective study from 1994 was done, including a total amount of 31 724 samples. The A toxin of *C. difficile* was found in 1399 of them. The distribution of the positive samples, within the different departments of the hospital, was analyzed, as well as their percentage with respect to the total number of stool cultures and A toxin assay prescribed.

Results: The percentage of tests positive for A toxin of *C. difficile* among all stool cultures prescribed was 0.84%. The percentage of positives of all the A-toxin *C. difficile* prescriptions was 19.8%.

	1994	1995	1996	1997	TOTAL
Intern Medic	20(38.4%)	18(33.9%)	38(38.3%)	18(30%)	95(35.5%)
Neurology	14(26.9%)	4 (7.14%)	27(27.29%)	9(15%)	54(20.2%)
Others	18(34.6%)	33(58.92%)	34(34.34%)	33(55%)	118(44.19%

Conclusions: Neurology and internal medicine were the services with the highest incidence of *C. difficile* infection. The wide use of antimicrobial agents in these departments, as well as the use of clindamycin in neurology to treat pneumonia by aspiration, explain our results.

P696 Comparison of *Clostridium difficile* isolates from colonized asymptomatic individuals on admission and from symptomatic patients

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Objectives: To determine the relatedness of *C. difficile* (CD) isolates from asymptomatic, colonized community patients with those causing hospital-acquired infection.

Methods: Over 1 year, stool swabs or specimens were collected from asymptomatic elderly patients, on admission to one of two elderly care medicine wards. Specimens were cultured anaerobically on cycloserine–cefoxitin egg yolk (CCEY) agar for 48 h and also in pre-reduced Robertson's cooked meat broth, with subculture to CCEY. Colonial morphology and odor were used to detect suspected CD colonies, which were then tested for toxin B production. Non-toxigenic strains were identified using the RapID ANAII identification kit. Isolates were fingerprinted using PCR amplification of 16S–23S ribosomal spacer DNA, and compared with strains recovered from inpatients with CD diarrhea.

Results: Samples were obtained from 88 patients and 10 yielded CD (11.4%), of which 8 were toxigenic (80%). DNA fingerprints of these toxigenic isolates were very similar to those from symptomatic inpatients (3 or fewer bands difference). 2/10 (20%) versus 7/78 (9%), respectively, of CD-colonized and non-colonized patients (on admission) subsequently developed toxin-positive diarrhea.

Conclusions: Asymptomatic patients admitted from the community appear to carry similar CD strains to those causing disease in hospital. This may be due to a widespread virulent clone, or to acquisition during prior hospital visits. Alternatively, it may suggest that patient factors rather than bacterial virulence are more important in the pathogenesis of CD disease. We were unable to confirm earlier

observations that CD carriage on admission protects against subsequent hospital-acquired diarrhea.

P697 Predominant *Staphylococcus aureus* in stools is responsible for postantibiotic diarrhea and produces LukE/LukD and enterotoxin A

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This study concerned the clinical significance and the toxin production of *Staphylococcus aureus* isolated in predominant or pure culture from stools of hospitalized patients.

During a 2-year period (July 1996 to June 1998), *S. aureus* was isolated from 64 patients in predominant or pure cultures, while 247 patients were affected by *Clostridium difficile* among the 19 062 coprocultures considered. Among the 64 patients, 60 had postantibiotic diarrhea elapsing from 3 to 70 days with a critical prognosis. These patients were aged (mean 59.2±23 years) and were generally treated with fluoroquinolones and/or aminoglycosides or ß-lactams. Diarrhea disappeared with prompt oral vancomycin or discontinuation of antibiotic therapy.

Most *S. aureus* isolates were resistant to methicillin, which may suggest a nosocomial origin. PFG typing of these isolates showed 21 different *Sma*I pulsotypes previously observed for resident strains. The same pulsotypes were recorded for isolates from stools and from bacteremia for given patients. By using a commercial test for enterotoxins and a radial gel immunoprecipitation for bicomponent leukotoxins, it appeared that 90% of isolates produced LukE/LukD, 76% produced enterotoxin A, and 70% the two toxins. These distributions were significantly different ($p<0.01$) from those observed for random *S.aureus* strains. No genetic link exists between genes encoding the two toxins.

In conclusion, *S. aureus* is of clinical relevance for PAD, which constitutes a serious source of bacterial spread in hospital. Specific strains are responsible for such infections. LukE/LukD, a bicomponent leukotoxin and enterotoxin A may combine to produce the clinical expression of the disease.

P698 The fast method for indication of microecological changes in the intestine

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The ability to resist colonization by an invasion of opportunistic pathogens is dependent on a healthy uncompromised intestine microflora and mucosal immune system. We estimated the qualitative and quantitative composition of fecal microflora and the level of slgA (by ELISA) of primates under conditions of stress. We found that the development of dysbacterioses is followed by an increase in the excretion of slgA with feces. There is a negative correlation between the levels of slgA and the defensive groups of microorganisms: for bacteroids $r=-0.80$, and for lactobacilli $r=-0.50$, for $n=50$ and p 0.01.There was no correlation between the levels of slgA and other groups of intestine microorganisms. We estimated the intestine microflora and the level of slgA in the feces of astronauts and people employed in ground-based industrial processes of an airport (increased noise, vibrations, fuel fumes, oil and lead). In the group of astronauts the coefficients of correlation (r) of the levels of slgA and bifidobacteria and lactobacilli were -0.64 and -0.37 respectively ($n=20$ and $p=0.01$). There was no correlation between the levels of slgA and other groups of intestine microorganisms. Also, in the group of airport workers, the development of dysbacterioses I–IV degree of manifestation was followed by an increase in the level of sIgA up to 1010±131 mg/g feces ($n=25$). We suggest that the sharp increase in the level of slgA in feces is a sign of the steep shifts of intestine microflora and may be used as a diagnostic indicator.

P699 Laboratory diagnosis of Whipple's disease

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Whipple's disease is a rare multisystemic bacterial infection that may involve any organ system in the body. The gastrointestinal tract is the most frequently involved site, with symptoms such as malabsorption, persistent diarrhea, and weight loss. However, patients with arthralgias, low-grade fever, and cardiac and central nervous system involvement are not uncommon. These heterogeneous manifestations make the clinical diagnosis very difficult. In the absence of reproducible cultures, the traditional diagnosis was based on the demonstration of periodic acid Schiff-positive rod-shaped inclusions in infected tissues and/or bacteria with a trilamellar membrane shown by electron microscopy. Recently, the causative organism, *Tropheryma whippelii*, has been characterized at the molecular level by PCR using universal bacterial primers for highly conserved regions of the 16S ribosomal RNA gene. From the 16S ribosomal RNA gene sequence, species-specific primers were derived for the detection of *T. whippelii* in clinical specimens. This technique has been shown to be more sensitive than histopathologic analysis; however, *T. whippelii* DNA has also been found in persons without clinical or histologic evidence of Whipple's disease. This suggests the existence of an asymptomatic carrier state which may be associated with as yet unidentified host factors and/or the presence of virulence factors in some strains of *T. whippelii*. To understand more about this enigmatic disease we need to know more about the genome of *T. whippelii*.

P700 Isolation of *Yersinia enterocolitica* in cases of acute appendicitis in Tripoli Medical Center

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Objectives: To investigate whether the occurrence of pathogenic *Yersinia enterocolitica* in cases of operated appendectomy was the primary cause of acute abdominal pain.

Methods: A total of 70 appendiceal specimens were analyzed for detection of *Yersinia enterocolitica*. The operative specimens were collected aseptically from patients hospitalized with a clinical diagnosis of acute appendicitis in the Tripoli Medical Center (TMC). One appendiceal division was examined bacteriologically and the other histopathologicly for each case.

Results: The results obtained showed that both *Y. enterocolitica* and *Citrobacter freundii* were recovered in appendiceal specimens (17.1% and 8.6% respectively). The evaluation of three selective broths used for detecting *Y. enterocolitica* can be summarized as follows: thioglycollate medium was more selective and productive (11.4%) compared to tryptone soya broth (TSB) supplemented with polymyxin (20 000 µ/L) and 10 mg/L irgasan (4.3%) and phosphate-buffered saline (2.8%) in appendiceal specimens. Other pathogenic bacteria which were isolated from operative specimens were *Klebsiella pneumoniae* (5.7%), *Salmonella* sp., *Shigella* sp., and *Pseudomonas aeruginosa* (each 2.9%). Those patients with positive bacterial culture in each histopathologic diagnostic category, as in two cases, were diagnosed as chronic appendicitis infected with *Y. enterocolitica* and the other as pathogenic bacteria.

Conclusions: *Y. enterocolitica* constituted the majority of causative agents of acute appendicitis 11/25 (44%). The recommended drugs for *Y. enterocolitica* infection were chloramphenicol, gentamicin, tetracycline and trimethoprim–sulfamethoxazole.

P701 The microflora of the intestinal tract as a reservoir for microbial translocation—is it similar in young children of Sweden and Estonia?

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Objectives: The imbalance of the normal microflora serves as direct precondition for translocation of opportunistic pathogens to lymph nodes and blood from mucosa. Experimentally, the close correlation between the high numbers of opportunistic microbes of gut and their translocation has been revealed. The aim of our study was to find out whether there are any differences in intestinal microflora composition of Swedish and Estonian 2-year-old children.

Methods: Altogether, 30 Estonian and 36 Swedish 2-year-old children born in 1995 in Tartu (Estonia) and Linköping (Sweden) were recruited in the study. In their fecal samples, 13 groups of aerobic, micro-aerophilic and anaerobic bacteria were quantified (log CFU/g feces) by serial dilutions on 10 different media. Non-parametric Mann–Whitney test was applied for statistical evaluation of data.

Results: Concerning opportunistic pathogens, the Swedish 2-year-old children were more often and in greater numbers colonized with *Staphylococcus aureus* as compared to Estonian children ($p<0.001$). At the same time, the latter showed more frequent prevalence and higher counts of *Candida* spp. than Swedes ($p<0.001$). Some differences were also revealed in the intestinal indigenous microbiota: in Sweden, there was more intensive colonization with both bifidobacteria and bacteroides ($p<0.01$), while in Estonians colonization by lactic acid-producing streptococci ($p<0.01$) could be revealed.

Conclusions: The intestinal microflora of 2-year-old Swedish and Estonian children were not similar and it should be kept in mind that there is a possibility of clinical infections by pathogenic staphylococci in Swedes and by *Candida* in Estonians.

Respiratory tract infections

P702 The most frequent bacterial agents in respiratory infections

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Follow-up of the isolates of materials from the respiratory tract over a period of 6 months is necessary in every hospital for lung diseases.

Our aim was to evaluate a group of 480 children from the department for intensive care and the department for chronic lung diseases who were subjected to drainage secretions and bronchial aspirate.

We obtained these results:

Bacterial agents	Intensive care	Chronic diseases
Streptococcus pneum.	16 (06.15%)	19 (08.6%)
Haemophilus spp.	8 (0.76%)	16 (07.2%)
Moraxela cat.	2 (0.76%)	7 (03.18%)
Staph. Aureus	25 (09.6%)	3 (01.36%)
Staph.coagul.neg.	9 (03.45%)	/
Escherichia coli	18 (06.9%)	7 (03.18%)
Klebsiela spp.	5 (01.92%)	1 (0.45%)
Pseudomonas aerugenosa	6 (02.3%)	9 (04.09%)
Proteus mirab.	9 (03.46%)	3 (01.36%)
Enterobacter spp.	1 (0.38%)	/
Acinetobacter spp.	2 (0.76%)	/
Streptococcus beta haem.	/	1 (0.45%)
Candida spp.	19 (7.3%)	8 (03.6%)

On the basis of these results we can conclude that in the drainage secretions of all children Gram negative flora was dominant. Because of this, it is necessary to follow up the isolates for adequate and timely treatment with antibiotics, and to be aware of the epidemiologic situation in the department, patients' social status and excessive use of antibiotics.

P703 Characteristics of *N. meningitidis, H. influenzae* and *S. pneumoniae* strains isolated from upper respiratory tracts of healthy people

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N. meningitidis, H. influenzae and *S. pneumoniae* strains are among the main bacterial pathogens of the respiratory and central nervous systems. These infections begin with nasopharynx bacterial colonization. The carrier status of particular pathogens is correlated with human age. Nasopharyngeal swabs were taken from over 100 children 1–5 years old in order to establish *H. influenzae* carrier status and from 6-year-old and 14-year-old children to look into *S. pneumoniae* and *N. meningitidis* colonization respectively. Almost 150 military recruits were assayed to detect the above-mentioned microorganisms. A longitudinal study (2 months) of *N. meningitidis* carrier status was also performed. All obtained isolates were phenotypically characterized, and MICs of selected antimicrobial agents were estimated.

A non-meningococcal strain was isolated from children. Over 50 phenotypically differerent *N. meningitidis* isolates were obtained from recruits' swabs. The dominant meningococcal phenotypes were NG21P1.7 and B21P1.7. All isolates were susceptible to penicillin, cefotaxime, ciprofloxacin, chloramphenicol, tetracycline, and sulfonamides. *H. influenzae* strains were isolated from 28% of healthy young children and from 11% of recruits. All obtained strains were nongroupable except two serogroup b strains obtained from children. Biotypes 1, and then V and II, were the most common. 6% and 20% of isolates were penicillin and sulfamethoxazole resistant, respectively. Pneumococci were recovered from 9% of children and 19% of recruits. All isolates were sensitive to penicillin.

P704 *Chlamydia pneumoniae* (Cp) detection in oropharynx by direct immunofluorescence (DIF) test

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Objectives: Cp is a pathogen of lower and upper respiratory tract infection (RTI) but has also been found in retropharyngeal swabs of healthy individuals. The aim of this study was to evaluate the frequency of Cp colonization in oropharynx.