MEDICAL MICROBIOLOGY

The Microbial Etiologies of Diarrhea in Hospitalized Patients from the Puerto Rico Medical Center Hospitals

MILDRED CARRER, MT(ASCP)‡; GUILLERMO J. VÁZQUEZ, MD*; RAFAEL I. LEBRÓN, MS*; XIOMARA MERCADO, MS*; IDALÍ MARTÍNEZ, PhD*; CARMEN O. VÁZQUEZ, PhD*; MARIA SANTÉ, MD†; IRAIDA E. ROBLEDO, PhD*

The development of diarrhea in hospitalized patients is a frequently encountered clinical problem, which may be due to infectious or non-infectious causes. The purpose of this study was to identify which common community enteric pathogens, if any, are responsible for diarrheal episodes in hospitalized patients. Stool samples from 76 consecutive, hospitalized patients were analyzed utilizing routine bacterial cultures, smears for identification of ova and parasites and Enzyme-Link Immunoadsorbent Assay (ELISA) for enteric bacteria, parasites and viruses. The results obtained demonstrated that the usual community enteric pathogens were not identified as a major cause of nosocomial diarrhea. In hospital-acquired diarrhea, *Clostridium difficile* toxins assay was the only clinically significant test in the evaluation of these patients. As a result of this study a guideline for the management of this condition in hospitalized patients is presented.

Key Words: Hospital-acquired diarrhea, Nosocomial diarrhea, Enteric pathogens, Clostridium difficile toxins

The public health burden of acute, community acquired diarrhea and gastroenteritis is substantial. Of the 250-350 million episodes that occur each year in United States, approximately 450,000 adults and 160,000 children are hospitalized with 4,000 estimated deaths. An etiologic agent is identified in less than 10% of the cases, as clinical laboratories test routinely only for *Salmonella*, *Shigella*, and *Yersinia* species; if requested by the physician, then *Campylobacter* species culture, detection of viral enteric pathogens and microscopic examination for ova and parasites are performed. The etiology of nosocomial diarrhea, however, may differ widely from community-acquired enteric infections. Since no data is available regarding the microbiological etiology of acute diarrhea in hospitalized patients in Puerto Rico, the aim of this study was to identify potential infectious agents in patients who develop diarrhea while hospitalized. A guideline for the evaluation of these patients is presented.

From the *Department of Microbiology and Medical Zoology, †Department of Pathology, School of Medicine, University of Puerto Rico and the ‡Department of Clinical Laboratory Sciences, College of Health Related Professions, University of Puerto Rico.

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Address correspondence to: Iraida E. Robledo, PhD, Department of Microbiology and Medical Zoology, School of Medicine, University of Puerto Rico. P.O Box 365067 San Juan, Puerto Rico, 00936-5067 Telephone: (787) 758-25252, ext.1311, Fax 787-758-4808; e-mail: irobledo@rcm.upr.edu

Materials and Methods

One hundred and one, consecutives, unique, stools samples from patients evaluated in the PR Medical Center (PRMC) Hospitals with a presumptive diagnosis of infectious diarrhea were examined at the PRMC Bacteriology Laboratory. The PRMC laboratory performed routine cultures for *Salmonella, Shigella,* and *Yersinia* species. Microbiological tests for the detection of *Campylobacter species, Clostridium difficile* toxins and stool smear for detection of ova parasites were done upon physician's request. The fecal samples and their microbiological results were sent to the Department of Microbiology, UPR - School of Medicine, Molecular Bacteriology and Virology laboratories where the following additional tests were performed: ELISA assays for the detection of *Campylobacter jejuni* (ProSpecT Campylobacter Microplate Assay, Alexon-Trend, Ramsey, MN), *Giardia lamblia* (ProSpecT *Giardia* Microplate

Assay, Alexon-Trend, Ramsey, MN), Rotavirus (IDEIA-Rotavirus Dako, Carpinteria, CA), Adenovirus (IDEIA-Adenovirus, Dako, Carpinteria, CA), and for the direct detection of shiga-toxin (VT1 and VT2) producing *E. coli* O157 and non-O157 serotypes in stool specimens (ProSpecT Shiga toxin *E. coli* Microplate Assay, Alexon-Trend, Ramsey, MN). Samples obtained utilizing rectal swabs were rejected, as the amount of stool was not sufficient to perform the ELISA assays.

For the epidemiological analysis, the age, sex, stool consistency and hospitalization status from the patients were obtained from their bacteriology report. Results was subjected to statistical analysis using Chi Square. A $P \leq 0.05$ was considered statistically significant.

Results

As demonstrated in Table 1, of the 101 stool samples, 76 (75.2%) were obtained from hospitalized patients. The gender distribution was equal among females and males (38/76 each). Figure 1 shows the age distribution of the 76 hospitalized patients. The mean age of female patients was 36.9 (ranged 9 days to 86 years), and 35.6 years (ranged 26 days to 89 years) for males. As shown in Figure 1, 62 patients (81.5%) were 15 years of age or older. In 96% of the stools samples their consistency was classified as either watery or unformed, as shown in Figure 2. The 23 samples obtained from ambulatory patients were not further analyzed.

Table 2 demonstrates the microbiological and ELISA assays results obtained from the fecal samples. None of the 76 routine cultures for *Salmonella spp., Shigella spp.* and *Yersinia spp.* were positive. *Campylobacter* cultures were performed in 4 stool samples and all were negatives. ELISA immunoassay testing showed that of the 76 tested stool samples, 1 (1.3%) was positive for *Campylobacter* species and one (1.3%) additional sample was positive for *Giardia*. No Shiga-like toxin (verotoxin 1 and 2) producing *E. coli* was identified. Two of the 76 (2.6%) tested samples were positive for Adenovirus and none for Rotavirus. Microscopic wet-mount examination of the stools for ova-parasites demonstrated that 1 out of 76 (1.3%) sample was positive for *Giardia lamblia* cysts. Three out of 29 (10.3%)

Table 1. General Epidemiological Data

Number of Stool Samples	101
Female participants	38
Male participants	38
Hospitalized patients	76/101
Ambulatory patients	23/101
Hospitalization status unknown	2/101

Table 2. Microbiological and ELISA Results in 76 Stools Samples from Hospitalized Patients.

Enteric pathogens	Number of Positive/ Total Tested and (%)			
	Culture	Toxin Assay	ELISA	Wet Mount
Salmonella spp.	0/76			
Shigella spp.	0/76			
Yersinia spp.	0/76			
Campylobacter spp	0/04		1/76 (1.3%)	
Shiga Toxin *E. coli* (VT1 and VT2)			0/76	
Clostridium difficile Toxins		3/29 (10.3%)		
Wet Mount for ova/parasite				1/76 (1.3%)*
Giardia ELISA Assay			1/76 (1.3%)	
Adenovirus			2/76 (2.6%)	
Rotavirus			0/76	

*The wet mount was positive for Giardia.

Figure 1. Age Range of Hospitalized Subjects

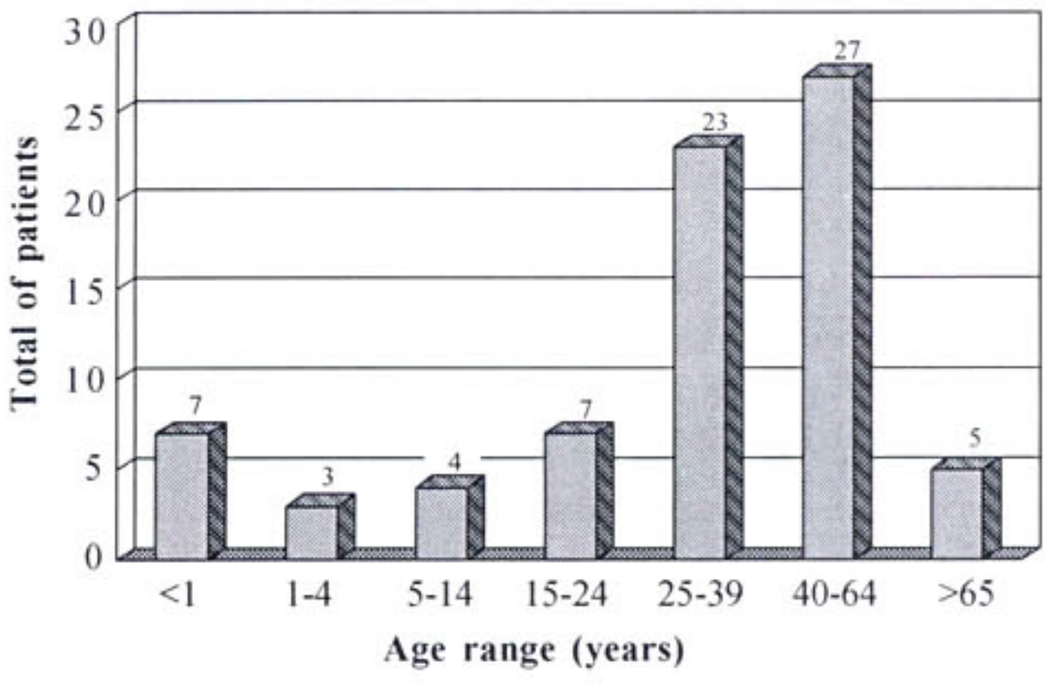
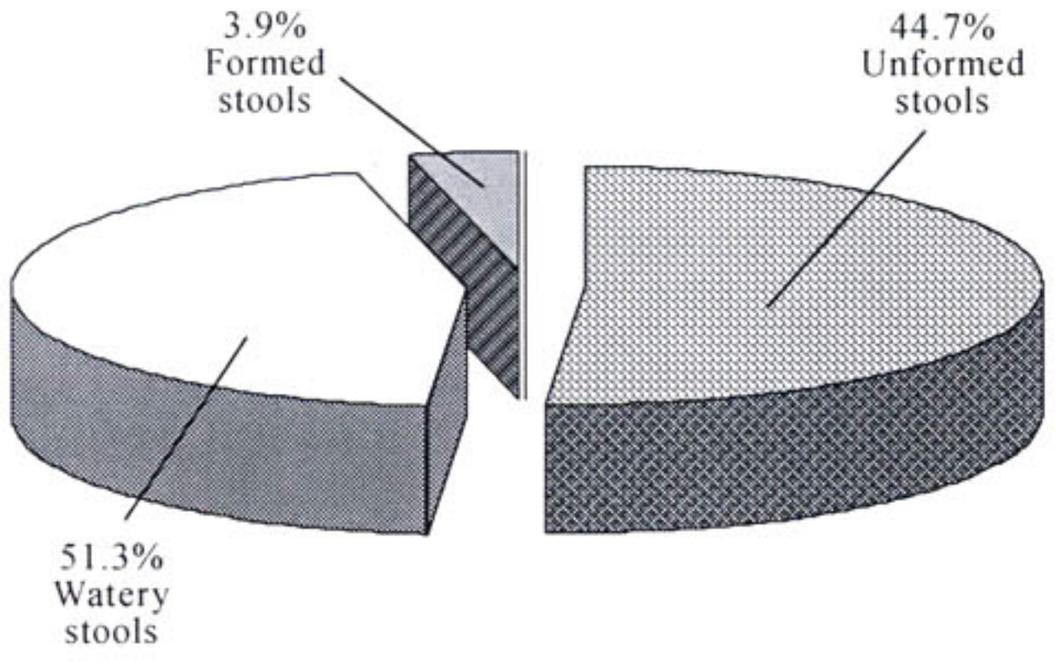


Figure 2. Consistency of Stools Samples



fecal samples were positive for the presence of *Clostridium difficile* toxins ($P \leq 0.019$).

Discussion and Conclusions

Gastrointestinal infections remain one of the most important diseases worldwide. Diarrhea is a very common complaint in both ambulatory and hospitalized patients. In Puerto Rico for the period between 1999 to 2002, the Department of Health reported a total of 3,641 identified community-acquired enteric infections of which: 3,087 were *Salmonella spp.*, 207 Shigella *spp.*, 167 *Giardia*, 119 *Campylobacter spp.*, 19 *E.coli* O157:H7, 17 *Yersinia spp.*, and 5 amebiasis; no enteric viral pathogens were reported (Personal communication, Puerto Rico Department of Health). There is, however, no data regarding the prevalence of enteric pathogens in patients who develop diarrhea while hospitalized. Nosocomial diarrheas are an important clinical problem and may be due to infectious or noninfectious causes.

The results of this pilot study suggest that the usual community acquired enteric pathogens are a very rare cause of diarrhea in hospitalized patients. All routine cultures were negative for the usual enteric bacterial pathogens. The use of ELISA immunoassays for the detection of enteric *Campylobacter,* enterohemorragic *E. coli* and *Giardia* did not improve the yield significantly. As for the enteric viruses, only two samples were positive for Adenovirus and none for Rotavirus. This low yield of viral detection may be explained by the fact that these two enteric viruses affect predominantly infants and children (1,8,10,11) and in our studied population, 81.5% of the patients were either adolescents or adults. *Clostridium difficile* toxins assay was the only clinically significant test in patients with nosocomial diarrhea (10.3%). The presence of these toxins has been correlated with exposure to antimicrobial agents or by the nosocomial transmission of the pathogen and it is an important cause of hospital-acquired diarrhea (4,6).

These results are in agreement with the published literature which suggest that the use of routine stool testing is not warranted in patients who develop diarrhea 3 days or more after hospitalization, with the exception of the detection of *Clostridium difficile* toxins in high risk patients (2,9,7,5,12). Bauer *et al.*, (3) estimated an annual savings of approximately $7,800.00 in a 355-bed institution, if routine stool examination is obtained only in selected cases. Complete routine fecal analysis should only be performed in the immunocompromised patients who develop nosocomial diarrhea, in nosocomial enteric pathogens outbreaks, or in patients older than 65 years with significant comorbidities (2). As previously mentioned, limiting routine stool testing for hospitalized patients would reduce hospitalization costs without interfering with the patient's clinical management.

We suggest the following guideline for the evaluation and management of patients with hospital-acquired diarrhea: a) for patients exposed to antimicrobial agents, testing for fecal leukocytes and *Clostridium difficile* toxins assay is recommended; b) for immunocompromised patients, in whom acute or chronic diarrheal illnesses are common complications, a complete microbiological fecal analysis is imperative to provide specific therapy; c) for the normal patient without any antibiotic exposure, correction of fluid and electrolytes imbalance, evaluation of the medications, diet or feedings and procedures which may be associated with diarrhea should be performed. Symptomatic treatment can be prescribed and if the diarrhea persists over 4-5 days, consider obtaining fecal leukocytes and *C. difficile* toxins assay, and d) a complete microbiological fecal analysis should be obtained in the presence of a nosocomial enteric outbreak.

Resumen

El desarrollo de diarreas en pacientes hospitalizados es un problema clínico frecuentemente observado que puede deberse tanto a causas infecciosas como no infecciosas. El propósito de este estudio fue identificar cuales patógenos entéricos comunes de la comunidad, si alguno, son responsables por la diarrea adquirida en el hospital. Muestras coprológicas consecutivas de 76 pacientes hospitalizados fueron analizadas utilizando técnicas rutinarias de cultivos bacterianos, frotis para la identificación de huevos y parásitos, y ensayos de ELISA para la detección de bacterias, parásitos y virus entéricos. Los resultados obtenidos demostraron que no se identificaron patógenos entéricos de la comunidad en los casos de diarrea nosocomial. En la diarrea adquirida en el hospital, el ensayo para la detección de toxinas de *Clostridium difficile* fue la única prueba clínicamente significativa en la evaluación de estos pacientes. Como resultado de este estudio se presenta una guía para el manejo de estas diarreas.

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