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Case Study Open Access

The Contribution of Pathogenic Bacteria to GI Symptoms in Parasite-Free Patients

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Abstract

At the Parasitology Center, Inc. (PCI), Scottsdale, Arizona, we come across a number of patients with GI symptoms suggestive of parasitic infections that turn out to be free of parasites. Tests for pathogenic bacteria using swab culture tests showed that practically all these patients were infected with pathogenic bacteria that produce symptoms similar to those known in classical parasitic infections. Swabs from a random sample of 60 patients (21 males, 39 females between 2 and 87 yr old) with overt GI symptoms that tested negative for parasite infections during the second half of 2010 were cultured. All cultures proved to be positive for 2 or 3 of 5 species of pathogenic bacteria (Entrobacteriaceae), including, Escherechia coli (prevalence of 100%), *Klebsiella* sp. (72%), *Proteus vulgaris* (33%), *Citrobacter freundii* (25%), Pse*udomonas aeruginosa* (7%), and 1 fungus species, *Candida* sp. (5%). Epidemiological aspects of these infections are discussed and plausible explanation of the symptomology associated with bacterial infections in the absence of parasites is provided.

Keywords: Pathogenic bacteria; GI symptoms; Parasites

Introduction

In observations of many PCI (Parasitology Center, Inc.) patients over the years, we noted that many experienced GI symptoms but no parasites were detected from fecal samples provided. These cases were explained as possibly relating to "other pathogenic organisms, ex., pathogenic bacteria, that can cause symptoms comparable to those produced by typical parasites [1]. However, no actual bacteriological testing was done for verification.

In a cross-sectional study of 5,792 fecal specimens from 2,896 patients in 48 states and the District of Columbia, 32% were found positive for protozoan and helminth parasites during the year 2000 [2]. The most common parasites, in order of prevalence, were *Blastocystis hominis*, *Cryptosporidium parvum*, and *Entamoeba* spp. The first two species were subsequently studied in more detail by Amin [3, 4, 5, 6]. A sizable proportion of patients without infections, nevertheless, exhibited GI symptoms, including but not limited to diarrhea, constipation, and abdominal cramps, similar to those observed in parasite infected patients. Those patients remained unaccounted for in terms of causation.

Our present results verify the original assumption [1], document the identity of bacterial agents involved in the GI symptomology in patients proven to have no intestinal parasites, and provides the results of sensitivity and resistance tests for treatment purposes. The GI symptoms in those parasite-free patients can now be explained by the pathogenic bacteria documented for each case. A more recent study [7] shows that IBS associated with abdominal pain, bloating, and diarrhea is caused by intestinal bacteria.

Materials and Methods

Patients with GI symptoms who initially submit fecal specimens for Comprehensive Stool Analysis (CSA) only are encouraged to follow up with bacterial testing by inserting the following statement in their CSA diagnostic report: "Patients with symptoms but with no detected parasites are highly recommended to do the PCI swab culture test for pathogenic bacteria that cause GI symptoms similar to those caused by parasite infections. Swab kits are available by calling PCI at 480-767-2522".

The study population constituted 60 patients (21 males and 39 females) between the ages of 2 and 87 yr who experienced GI symptoms, tested negatively for parasites using the PCI CSA, and tested for pathogenic bacteria at the same time, between August and December, 2010.

The CSA was performed [2] as follows. The specimens were collected and fixed in SAF, processed and stained in CONSED™ according to manufacturer's (Alpha-Tec Systems, Inc., Vancouver, WA) directions. Fixed specimens were filtered, mixed with CONSED™ and ethyl acetate, vortexed, and centrifuged. All but the fecal plug was decanted, and mixed with CONSED™ diluting reagent. The plug was then transferred to and mounted on a microscope slide for light microscopy examination. All microscopic evaluations and identification were made by the same observer(s) blinded to patient information, e.g., symptoms, travel, etc. Positive results were quantified (number of organisms per high-power field on a scale of 1 to 4) from duplicate samples from the same patient.

Swabbed fecal specimens were collected using sterile transporter swabs manufactured in Italy by Copan for Healthlink, McKesson, Richmond, VA. Specimens were deposited in selective Platin Medium (MacConkey, XLD, SS agar) and incubated for 24-48 hr at 37°C. Colony morphology was observed after Gram-staining. Suspected bacterial pathogens were then tube-tested using BIOQUIMICS test in Tse, Lia, Ornitin, Indol, and Simmon citrate, and incubated for 24 hr at 37°C or tested using packed BIOQUIMICS. Specimens were then identified using criteria of colony morphology of common enteric bacteria on differential and selective plating media, e.g., MacConkey agar with crystal violet, XLD agar, SS agar, DCA, and HEA [8].

Sensitivity results were obtained by culturing identified specimens in Mueller-Hinton agar. Antibiotic discs for Gram-positive and Gram-negative bacteria were then placed on the culture and sensitivity and

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resistance results were read. The disc antibiotic concentrations varied between 30.0 μg, 10.0 μg, 5.0 μg, and 1.0 μg for various antibiotics tested.

Results

Five species of gram negative pathogenic bacteria and 1 fungal species were cultured from fecal swabs of a random sample of 60 symptomatic, but parasite-free, patients tested between August and December, 2010. These are *Escherechia coli* (Migula, 1895) Castellani and Chalmers, 1919, *Klebsiella* sp., *Proteus vulgaris* Hauser, 1885, *Citrobacter freundii* (Braak 1928) Werkman and Gillen 1932, *Pseudomonas aeruginosa* (Schröter, 1872) Migula, 1900, and the fungus *Candida* sp. Most patients were concurrently infected with 2 or 3 species of bacteria (Table 1). The prevalence rate of the above cultures was 100 % in *E. coli*, 72% in *Klebsiella* sp., 33% in *P. vulgaris*, 25% in *C. freundii*, 7% in *P. aerruginosa*, and 5% in *Candida* sp. Of a total of 145 positive findings, *E. coli* made up 41% of total infections, *Klebsiella* sp. 30%, *P. vulgaris* 14%, *C. freundii* 10%, *P. aeruginosa* 3%, and *Candida* sp. 2%.

Diagnostic test results of patient cultures are supplemented with sensitivity and resistance test results derived independently for each individual patient's sample. Sensitivity test results are provided in 4 major categories with the most efficient antibiotics included in category no. 1 and the least efficient in category no. 4. A selection of antibiotics from categories 1 and 2 is recommended. Antibiotics in all categories are individually designed for the treatment of concurrent infections that the patient may have, and will not be the same from patient to patient even with the same infections. Antibiotics to which cultured species are resistant also varied from patient to patient.

Discussion

The frequency distribution of Enterobacteriaceae associated with bactermia in the USA (courtesy of Barnes Hospital, St. Louis) was 45% for *E. coli*, 24% for *Klebsiella*, 9% for *Proteus*, and 4% for *Citrobacter*, among others [11]. Our results were very similar for *E. coli* (41%) but slightly higher for *Klebsiella* sp. (30%) and *P. vulgaris* (14%), and *C. freundii* (10%). The Barnes Hospital survey included 2 other pathogens that were not found in our survey, Enterobacter sp. (13%), and *Serratia* sp. (4%) [11] compared to our finding of *P. aerruginosa* (3%) and *Candida* sp. (2%) made up the difference in the overall prevalence.

Most of our patients were infected with 2 or 3 species of bacteria. Their symptoms, thus, express the composite effect of their over all infections and cannot be assigned to single species of bacteria alone.

Escherechia coli

Over 700 antigenic serotypes of *E. coli* are recognized based on O, H, and K antigens. Most human beings have more than 1 strain of E. coli at the same time [9]. Most strains of *E. coli* live in the intestine of humans and other mammals without causing any pathology [10]. Pathogenic strains of E. coli, however, are responsible for 3 types of infections in humans: urinary tract infections, neonatal meningitis, and intestinal diseases. The latter includes (1) ETEC (Enterotoxigenic *E. coli*) causing diarrhea in infants and travelers, (2) EIEC (Enteroinvasive *E. coli*) causing dysentery-like diarrhea with fever, (3) EPEC (Enteropathogenic *E. coli*) causing watery, sometimes bloody, diarrhea especially in children, and (4) EHEC (Enterohemorrhagic *E. coli*) causing hemorrhagic diarrhea and/or food poisoning which may develop into hemolytic uremic syndrome (HUS) and includes the invasive 0157:H7 strain making up 80% of the EHEC serotypes producing the verotoxin or Shiga toxin [9,11]. We do not know which strain(s) of *E. coli* did our patients test positive for. Strain identification requires molecular techniques not readily available in most diagnostic laboratories. Our study population comprised patients of all age groups (2-87 yr old) and both sexes. Judging by symptoms alone, patient no.17 may have been experiencing an infection with an EPEC or EHEC strain.

Klebsiella sp.

Pathogenic varieties of *Klebsiella* are grouped in 2 antigenic groups: the O antigen with 9 varieties and the K antigen with over 80 varieties. Klebsiella is increasingly reported as a nosocomial infection second only to *E. coli* in urinary tract infections in women [12]. *Klebsiella pneumoniae* is an opportunistic infection in older patients with weakened immune system which also causes nosocomial pneumonia, intraabdominal infections and intestinal pathology. It is a resident of the intestinal track in about 40% of man and animals [13]. *Klebsiella* sp. was the second most commonly cultured bacteria in our study. It was not possible to assign any symptomology specific to *Klebsiella* alone since all *Klebsiella* infected patients in our study group were also concurrently infected with *E. coli*.

Proteus vulgaris

Proteus vulgaris inhabits the intestinal tract of humans and animals. It is also found in the soil, water, putrefied meat, and fecal matter and is associated with long-term care facilities and hospitals where it is also known to colonize the skin and oral mucosa of patients and hospital personnel alike. It is an opportunistic pathogen in humans where it is also known to cause urinary tract (UT) and wound infections [14,15]. While *Proteus* spp. are not the most common sources of bacterial infections in humans, *P. vulgaris* holds yet a smaller role in the pathology caused by this group [16]. *Proteus* species most frequently cause UT infections, with P*roteus mirabilis* Hauser, 1885 producing 90% of the cases [14,15]. In our study population of 60 patients, *P. vulgaris* infected mostly 19-58 yr old females; only 7 males were infected. It is, herein, suggested that the prevalence of *P. vulgaris* infections in the intestinal tract of females (Table 1) may be related to cross contamination from UT infections not tested for in the same patients. In our study population, it infected 7 males and 13 females.

Citrobacter fruendi

Citrobacter is found in the human intestine and almost everywhere else including water, waste water, soil, etc. It is an indicator of a potential source of contamination but is rarely a source of illness [17]. *Citrobacter freundi*, however, is often the cause of opportunistic infections mostly causing abnormal inflammatory changes in the intestinal tract [17] and affecting biliary, urinary, and respiratory tracts, and blood of patients with weak immune system [18]. It has been "suspected to cause diarrhea and possibly extraintestinal infections including peritonitis [19]. Of 38 hospitalized patients in 2 community teaching hospitals in the Detroit Medical Center, *Citrobacter* bacteremia developed in elderly patients (65%) and was hospital acquired (77%) with initial sites of infection including the UT (39%), intestinal tract (27%), wound (10%), and unknown (13%) [17]. In our patient population, it infected 5 males and 10 females.

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a free-living organism commonly found in soil and water as well as on the surfaces of plants and animals. It is an emerging opportunistic and nosocomial pathogen infecting only compromised tissues and causing pathology in the gastrointestinal tract, heart, blood, respiratory system, central nervous system, ear, eye, bone and joint, UT, skin, and soft tissues. In the intestinal tract, it causes pathology from the oropharynx to the rectum including perirectal infections, pediatric diarrhea, typical gastroenteritis, and necrotizing enterocolitis. The GI tract is also an important portal on entry in

No.	Name	Age	Sex	State	Bacteria species cultured*						Symptoms
					EC	KL	PV	CF	PA	C	
1	MW	45	F	IL	X	X					Loose stools, gas, severe food allergies, poor digestion, stressed immune system
2	RB	76	F	VA	X	X					Discomfort in stomach area
3	KB	19	F	VA	X	X	X				Intermittent abdominal cramps, diarrhea, nausea, gluten sensitivities
4	LF	17	F	VT	X	X	X				IBS, fatigue
5	RN	?	M	AZ	X	X		X			Anal itching
6	SG	57	F	VA	X	X				X	No apetite, extreme fatigue, headaches, insomnia, brain fog
7	GH	37	F	CA	X	X	X		X		Bloating, gas, passing mucus w/t stool, constipation, water retention, insomnia, cramps
8	BS	28	F	NV	X	X	X				Bloating, fatigue, cramping, upset, hardening of stomach
9	BT	23	F	IN	X	X		X			Cramping, vomiting, diarrhea, blood in stool, anal itching
10	CF	9	F	VA	X	X					Food allergies, not responding to acupuncture
11	KM	38	M	CA	X	X		X			Lower GI cramps, gas, bloating
12	DS	48	F	VA	X	X		X			Acidic taste in mouth, stomach acid reflex
13	BP	7	M	VT	X	X					Flatulence, anal itching, hyperactivity, bed wetting, teeth grinding
14	TM	36	F	FL	X	X		X			IBS, constipation, diarrhea, malabsorption, fatigue, bloating, foul gas, acne, insomnia, frequent urination, low immune, acne, hormonal dysfunction
15	LD	47	F	AZ	X	X	X				Inflamed intestine, ulcers, vomiting, abdominal pain
16	LP	46	F	NY	X	X	X				Yellow odorous stools, diarrhea for 2 yr, elevated liver enzymes, enlarged spleen & liver, fluid retention, hair loss, acne, rashes
17	GB	46	M	CA	X	X					Anal itching, bleeding
18	CB	80	M	AZ	X	X					Chronic diarrhea
19	LP	45	M	VT	X	X		X			Constant flatulence, bumps on scalp, stomachaches, insomnia, fungus on toe nail
20	SH	60	M	VA	X	X		X			GI problems, fatigue, skin cancer, adrenal & thyroid problems, heart palpitations
21	AS	31	F	VA	X	X					Abdominal pain, gas, fatigue, brain fog, headaches, joint pain, hair loss, sinus congestion, body aches, night sweats
22	MB	43	F	VA	X	X					Digestive problems, rash on ankle & arm, Staph. Infection on same ankle
23	MD	2	F	NY	X	X		X			Bloating, stomach discomfort, chest pain, hunger
24	PT	4	M	VA	X	X					Food allergies
25	SW	42	F	KY	X	X					Stmach & intestine “blubbing up,” can’t digest, rectal proplapse, reflex, insomnia, nervous
26	HP	5	F	VT	X	X					Flatulence, stomachache, always hungry, bloating, mood swings, bed wetting
27	TT	50	F	CA	X	X		X			Digestive distress. Gluten sensitive
28	RB	58	M	NM	X	X	X				Bowel trouble, diarrhea, difficulty in digestion, swollen abdomen
29	MW	68	F	AZ	X	X					Diarrhea, vomiting
30	LB	31	M	PA	X	X		X			Constipation, fatigue, underweight, hypotension
31	KS	54	F	CA	X	X	X				Diarrhea over a yr, stomachaches. Spasms, soar throat, lung congestion, hair breaking, depression, heart palpitation
32	AC	45	F	TX	X	X	X				Bloating, fatigue, itching, gas, shingles, caugh, skin infections, allergies, headaches
33	AN	33	F	VA	X	X		X			Loose stools, digestive issues, fatigue
34	AC	65	M	PA	X	X					IBS, prostatitis
35	JH	48	F	CA	X	X	X				Diarrhea/Indonesia, Nausea/Hong Kong, loose stools/Jamaica
36	AP	30	F	CA	X	X					Bloating, constipation, abdominal & general pain, burning, nausea, teeth grinding, lethargic, toxic, twitching
37	PM	42	F	FL	X	X				X	Bloating, constipation, diarrhea, spots on back & chest, , flu-like symptoms
38	DR	47	F	NM	X	X		X			Pain in stomach, intestine, muscle & joints, headaches, earaches, itching, weight loss
39	BG	52	F	NY	X	X	X				Stomachache, allergies
40	MS	46	M	PA	X	X					Soft bowels, bloating, fatigue, hair loss since 6/08
41	AV	20	M	AZ	X	X	X				Vomiting, nausea, diarrhea, abdominal pain, Loss of apetite, weight loss
42	GL	55	M	CA	X	X	X				Gas, diarrhea, carbohydrate intolerance starting 1 year ago
43	KM	64	F	VA	X	X					Bloating, gas, IBS, allergies, rectal itching
44	SF	36	F	VA	X	X	X				Lower abdominal pain, stomach upset after eating, loud bowel sounds
45	RS	29	M	WY	X	X	X		X		Constipation, gas always, acid reflex, belching pain, bloating especially in lower colon, mood swings, depression, anxiety, rashes in hands, low sex drive
46	RO	29	F	IL	X	X			X		Colitis, abdominal pain, fibromyalgia, anal & nose itching
47	JB	58	F	AZ	X	X					Gas, bloating, diarrhea, constipation
48	PJ	63	F	VT	X	X		X			Very sofy stools, diarrhea starting 2/08
49	KT	48	F	GA	X	X		X			Chronic diarrhea
50	BM	59	F	CA	X	X	X		X		Digestive tract inflamed, bloating, constipation, diarrhea, body aches, brain fog, anxiety, heart palpitation, fatigue
51	HV	61	M	CA	X	X	X				Bloody stool, gas, headaches, brain fog, asthma, fatigue, swelling on lips
52	YM	28	M	IL	X	X					Constipation, gas, bloating, allergies, teeth grinding, fatigue, sleep disorder, joint & muscle aches, nervousness
53	SD	20	M	NV	X	X					malabsorption, gluten intolerance
54	BJ	36	F	CA	X	X					Diarrhea, cramps, nausea, stomach pains
55	JW	15	M	AZ	X	X	X				Chronic constipation, severe gas spasms, GI distress, odorous flatulence, low stamina, excellent apetite
56	LO	43	F	OH	X	X	X				GI distress, body pains, headaches, dry eyes & mouth keep him up at night
57	AG	30	M	NY	X	X	X			X	Bloating, intestinal dysbiosis
58	JH	64	M	TX	X	X	X				Diarrhea, erratic bowels, not digesting food, extreme bloating
59	JW	47	F	VT	X	X		X			Constipation, stomach pain, tenderness, used to eat raw sushi
60	BR	87	F	FL	X	X					Uncontrolled thin watery stool, sometimes gas
Total					60	43	20	15	4	3	
Prevalence (%)					100	72	33	25	7	5	

*Species positively cultured are marked with X. EC: E. coli, KL: Klebsiella sp., PV: P. vulgaris, CF: C. freundii, PA: P.

Table 1: Prevalence of infections with pathogenic bacteria in fecal specimens of 60 symptomatic patients free of between August and December, 2010 intestinal parasites sampled.

Pseudomonas septicemia and bacteremia. It has been isolated from the throat (5%) and stool (3%) of nonhospitalized patients. In some studies, gastrointestinal carriage rates increased in hospitalized patients to 20% within 72 hr of admission [9].

Four patients, (7%) of our study population of 60 were infected with *P. aeruginosa*. All were concurrently infected with *E. coli* and *P. vulgaris* and experienced mixed GI symptoms. Four other patients of 25 who were not tested for parasites (16%) were also infected with *P. aeruginosa* but their symptoms were not known in the absence of CSA test results.

Candida sp.

Fecal specimens of only 3 patients cultured positively for *Candida* concurrently with other infections (Table 1). Those patients were also positive for *Candida* using microscopical CSA. Diagnostic microscopical examination of fecal specimens of 40 other patients of the same study group of 60, using CSA, were positive for *Candida* at levels of 1 or 2 out of 4 possible infection intensities. This suggests that *Candida*, a fungus, does not readily grow in swab cultures and that microscopy provides a better detections in fecal specimens. The cyclical nature of *Candida* presence affected by diet and time of sampling after a compromising meal may be related.

Concurrent infections

As indicated earlier, most patients were concurrently infected with 2 or 3 species of bacteria (Table 1). It is clear that patients' symptoms are related to the cumulative effect of their composite infections that cannot be attributed to single bacterial species alone. IBS is one such situation [7] where the phenomenon of multiple causation applies.

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