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DIARRHEA DIGEST

DIARRHEA DIGEST is an irregular publication of TECHLAB®, Inc. dedicated to the etiology, diagnosis, and therapy of diarrheal diseases and related aspects of intestinal ecology

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C. difficile spores … Basics 101

Clostridium difficile is the most common cause of nosocomial diarrhea. The growth of *C. difficile* is inhibited by the normal gut flora, and colonization by *C. difficile* happens when the normal flora is disrupted by treatment with antibiotics. Patients treated with antibiotics such as cephalosporins, clindamycin or fluoroquinolones and patients with compromised immune systems, such as elderly patients, are at high risk of developing *C. difficile* infection.

A study in mice showed that asymptomatic carrier mice became "supper shedders" of *C. difficile* spores when treated with clindamycin [1]. The spore count went up by 10^6 fold as the result of clindamycin treatment. These spores also facilitate the transmission of the disease among humans. In hospitals and nursing homes, *C. difficile* is transmitted easily among patients and even health care workers. The easy transmission of *C. difficile* is due to its resistance to harsh environmental conditions and the extremely high amount of spores shed by symptomatic patients.

The spores are highly resistant to heat and chemicals and can survive outside the human body for months. Ethanol is not effective in killing *C. difficile* spores. To prevent transmission, health care workers should not use water-free hand sanitizers, but use soap instead. The efficacy of disinfectants against the spores varies. Quaternary ammonium and phenol are only effective at killing vegetative cells but not spores. The CDC-recommended disinfectant for cleaning rooms of *C. difficile* infected patient is 1:10 diluted sodium hypochlorite (bleach) [2]. The bleach based disinfectant should be made fresh because the effectiveness of 1:10 diluted sodium hypochlorite is reduced by 50% after being stored at room temperature for 30 days. Besides bleach, electrochemically activated solutions (ECAS) were recently shown to reduce the number of spores to below the detection limit within 20 seconds of contact. The low cost of production and environmental compatibility make ECAS promising sporacides [3].

The *DIARRHEA DIGEST* is now green. Just like previous paper issues, the green version will be an irregular publication and it will be available on our website. The green version may not be as easy to take to the bathroom, but by saving trees, the green version will help make sure that you don't run out of toilet paper.

Typically, *C. difficile* is mainly transmitted through contact. Symptomatic patients shed a large amount of the spores in their stools, contaminating their hands, clothing, bedding and nearby surfaces. Other patients and health care professionals in the same hospital may acquire spores from direct contact with the contaminated surfaces. Recent studies revealed the aerial dissemination of *C. difficile* spores. Using a portable cyclone, spores were found in the air in a UK hospital near an elderly care bay [4]. In another study, spores were isolated from the air surrounding 7 out of 10 patients tested [5]. Although the airborne spore count was low, considering the low infectious inoculum needed to cause disease [6], even a low number of spores might still pose a threat to antibiotic treated or immunocompromised patients.

The dormancy of spores renders them resistant to antibiotics unlike vegetative cells that are sensitive. The structure of *C. difficile* spores provides protection against harsh environmental conditions. The outer layers of

exosporium, spore coat, and cortex protect the internal cytoplasm. Sporulation is triggered by the limitation of nutrients and is regulated by both the transcription factor Spo0A [7] and sporulation specific sigma factors of RNA polymerase [8]. The increased sporulation ability of outbreak strain 027 may contribute to its increased virulence [9].

The germination of the spore stage is also a tightly regulated process. Infection with *C. difficile* often results from the ingestion of spores. The spores resist the acidity of the stomach and start to germinate in the intestines. Germination is induced by exposure to glycine, bile salts such as taurocholate, cholate [10] and high level of inorganic phosphate [11], but inhibited by bile salt chenodeoxycholate [12]. The environmental signals are sensed by germinant receptors located on the inner membrane of the spores. Germination is a series of well regulated biological processes including the release of monovalent cations and dipicolinic acid followed by the hydrolysis of the peptidoglycan cortex and the hydration of the core [13]. It was speculated that cholate and chenodeoxycholate might compete for binding to the germinant receptors. The higher absorption rate of chenodeoxycholate compared to the absorption rate of cholate by the large intestine insures that the germination does not happen until the spores reach the cholate rich environment in the large intestine [14].

The link between spores and the virulence and transmissibility of *C. difficile* calls for effective decontamination of spores in hospitals and nursing homes. Research on the germination mechanism has lead to the development of several strategies for reducing spore contamination. For example, the treatment with a solution containing taurocholate to induce germination resulted in enhanced killing of *C. diff* spores by UV radiation and heat [15]. The inhibition of germination by chenodeoxycholic acid analogs was also successfully demonstrated [16]. The increased understanding of the sporulation and germination processes will lead to more effective sanitation against *C. difficile* spores and reduction of infection by this organism.

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References

1. Lawley, T.D., et al., *Antibiotic treatment of Clostridium difficile carrier mice triggers a supershedder state, spore-mediated transmission, and severe disease in immunocompromised hosts.* Infect Immun, 2009. **77**(9): p. 3661-9.
2. Mayfield, J.L., et al., *Environmental control to reduce transmission of Clostridium difficile.* Clin Infect Dis, 2000. **31**(4): p. 995-1000.
3. Robinson, G.M., et al., *Evaluation of the efficacy of electrochemically activated solutions against nosocomial pathogens and bacterial endospores.* Lett Appl Microbiol, 2010. **50**(3): p. 289-94.
4. Roberts, K., et al., *Aerial dissemination of Clostridium difficile spores.* BMC Infect Dis, 2008. **8**: p. 7.
5. Best, E.L., et al., *The potential for airborne dispersal of Clostridium difficile from symptomatic patients.* Clin Infect Dis, 2010. **50**(11): p. 1450-7.
6. Merrigan, M., et al., *Susceptibility of hamsters to human pathogenic Clostridium difficile strain B1 following clindamycin, ampicillin or ceftriaxone administration.* Anaerobe, 2003. **9**(2): p. 91-5.
7. Underwood, S., et al., *Characterization of the sporulation initiation pathway of Clostridium difficile and its role in toxin production.* J Bacteriol, 2009. **191**(23): p. 7296-305.
8. Haraldsen, J.D. and A.L. Sonenshein, *Efficient sporulation in Clostridium difficile requires disruption of the sigmaK gene.* Mol Microbiol, 2003. **48**(3): p. 811-21.
9. Merrigan, M., et al., *Human hypervirulent Clostridium difficile strains exhibit increased sporulation as well as robust toxin production.* J Bacteriol, 2010. **192**(19): p. 4904-11.
10. Howerton, A., N. Ramirez, and E. Abel-Santos, *Mapping interactions between germinants and Clostridium difficile spores.* J Bacteriol, 2011. **193**(1): p. 274-82.
11. Paredes-Sabja, D., et al., *Germination of spores of Clostridium difficile strains, including isolates from a hospital outbreak of Clostridium difficile-associated disease (CDAD).* Microbiology, 2008. **154**(Pt 8): p. 2241-50.
12. Sorg, J.A. and A.L. Sonenshein, *Chenodeoxycholate is an inhibitor of*

Clostridium difficile spore germination. J Bacteriol, 2009. **191**(3): p. 1115-7.
13. Paredes-Sabja, D., P. Setlow, and M.R. Sarker, *Germination of spores of Bacillales and Clostridiales species: mechanisms and proteins involved.* Trends Microbiol, 2011. **19**(2): p. 85-94.
14. Mekhjian, H.S., S.F. Phillips, and A.F. Hofmann, *Colonic absorption of unconjugated bile acids: perfusion studies in man.* Dig Dis Sci, 1979. **24**(7): p. 545-50.
15. Nerandzic, M.M. and C.J. Donskey, *Triggering germination represents a novel strategy to enhance killing of Clostridium difficile spores.* PLoS One, 2010. **5**(8): p. e12285.
16. Sorg, J.A. and A.L. Sonenshein, *Inhibiting the initiation of Clostridium difficile spore germination using analogs of chenodeoxycholic acid, a bile acid.* J Bacteriol, 2010. **192**(19): p. 4983-90.

C. difficile continues to spread

- The first recognized case of ribotype 027-related *C. difficile* disease has been seen in Australia. Med J Aust. 2011 Apr 4:194:369-371.
- *C. difficile* ribotype 002 is emerging in Hong Kong as a predominant clone. Eur J Clin Microbiol Infect Dis. 2011 Apr 6 (Epub ahead of print).

Can you shine the light on that again?

Recent advances in optics technology will translate into more efficient endoscopy. These advances result in doubling of the light input so that gastroenterologists can see whatever it is they see with more confidence. The increased light does create more heat, so an improved integrated fan is part of the system. The light intensity can be controlled between 0 and 100% --- not so much for "mood" lighting since we can imagine what kind of mood you're in from the prepping that you've gone through, but rather for proper illumination of the mucosa.

Colon photosensitizers

Collaborative efforts are now underway at Liverpool John Moores University, Massachusetts General Hospital, and Harvard Medical School to develop methods that deliver active photosensitizers to the colon. The concept involves the use of "photodynamic therapy" to destroy pathogens in our gut. Part of this effort is the delivery of tetrachlorodecaoxide, which might boost oxygen levels in the intestine. Although *Enterococcus faecalis* and *Bacteroides fragilis* were initially targeted by investigators Wainwright, Dai, and Hamblin, they go on to mention that *Clostridium difficile* is one pathogen that should be targeted. This is a new approach and many challenges remain, particularly the general effect of photosensitizers on our protective flora. (Photochem Photobio. 2011 Mar 19. doi: 10.111/j.1751-1097.2011.00925.x)

Rectalling

Yes, it's a real word and yes, it can be used as a verb --- kind of like, "here we go a wassailing" or "here we go a caroling", but instead it's "here we go a rectalling". The word has been around a while, and yes, it is what you think it is. But for scientific purposes, the term is used more commonly when talking about rectal simulators. Veterinary students are now being trained on the Breed'n Betsy trainer, in which they get a chance to learn about cow anatomy through rectalling by using these rectal simulators (you can go online to see a picture of the machine --- just google rectal simulators). One of the key things learned is how to diagnose pregnancy. Importantly, students learn how to "get inside and back out" of a cow as quickly as

possible by first training with the machine --- which makes both the student and the cow happy.

Rain or shine but not uniform

A postal worker recently was fed up with her neighbor, or more specifically, her neighbor's dog, who kept using the bathroom in her yard. The postal worker collected the droppings in a box, and deposited them on her neighbor's porch with a note to quit using her yard as a bathroom. The neighbor, in turn, called the U.S. Postal Service, and complained by telling the Postal Service what one of their employees was delivering, and what were they going to do about it?

The U.S. Postal Service called the postal worker in and asked if this was true, to which the postal worker replied "yes". Then the Postal Service asked the postal worker if she was wearing her uniform at the time. She said, "no", she was not wearing her uniform. So to clear up the matter, the US Postal Service said that the employee was not allowed to wear her uniform any time she was delivering a box of dog poop to her neighbor.

Ever been guilty of looking at others your own age and thinking, surely I can't look that old? Read the following story.

I was sitting in the waiting room for my first appointment with a new gastro-enterologist. I noticed his diploma, which bore his full name. Suddenly I remembered a tall, handsome, dark haired boy with the same name had been in my secondary school class some 30-odd years ago. Could he be the same guy that I had a secret crush on, way back then?

Upon seeing him, however, I quickly discarded any such thought. This balding, gray haired man with the deeply lined face was far too old to have been my classmate. After he examined me, I asked him if he had attended Morgan Park Secondary School. "Yes, yes I did. I'm a Morganner!" he beamed with pride. "When did you leave to go to college?" I asked. He answered "In 1965. Why do you ask?" "You were in my class!" I exclaimed. He looked at me closely. Then that ugly, old, bald, wrinkled, fat arsed, grey haired, decrepit, bastard asked … "What did you teach?"

Dysentery in the Military - the hidden enemy that always gets you in the end!

Diarrheal infections, especially dysentery, have plagued the world since before ancient times. The first known recorded mention of diarrhea is from the ancient Egyptian Ebers Papyrus, circa 1550 BC. The ancient medical document prescribes acacia, bayberry, henna, myrrh, and sandalwood as cures for the malady. The ancient Greeks knew that drinking unclean water could result in dysentery (2). Nonetheless, over 3,000 years later, both diarrhea and dysentery continue to plague modern man, especially during times of civil unrest.

Unhygienic conditions, lack of sanitary facilities, poor personal hygiene, and crowded living conditions are optimal conditions for the spread of communicable disease, and are commonplace in impromptu military barracks. Furthermore, many military campaigns occur in countries and/or locales with less than ideal sanitation. As a result, many troops are incapacitated to some degree by severe diarrhea acquired during combat operations. This is a large enough problem that in 1949 the U.S. Army formed a Commission on Enteric Infections due to the fact that "diarrheal diseases have accounted for rates of morbidity and mortality equal to those of enemy action"(3).

Throughout history, diarrhea has been as common as swords and guns on the battlefields of the world. In 1779, while

England was preoccupied with the American Revolution, the French were planning an invasion of England, which was in part thwarted by an outbreak of dysentery among the French military ranks (4). Diarrhea also played a part in Napoleon's failed campaign in Russia, as diseases such as dysentery claimed the lives of over 200,000 French soldiers (5). Dysentery was commonplace during the American Civil War as well - more soldiers were killed by diarrheal diseases than injuries acquired during battle (6). Some Civil War buffs wonder if the "War of Northern Aggression" would have ended differently had General Robert E. Lee not been incapacitated with a case of the "Tennessee Trots" during the Battle of Gettysburg (7). During World War II, the Allied victory at El Alamein, a pivotal turning point in the Great War, was perhaps a bit easier, as dysentery had sickened more than half of the German troops stationed there (8).

Recent conflicts are not exempt from the scourge of diarrhea – in a recent survey, over ¾ of troops deployed in Iraq and over half of troops deployed in Afghanistan reported at least one diarrheal episode. The average number of reported episodes was 5, each lasting an average of 4 days, and more than half were serious enough to require professional medical attention (9). By comparison, the average American citizen deals with diarrhea only once per year (10). Last year during an insurgent uprising, training was suspended for the Syrian Army due to epidemic outbreaks of diarrhea at several army bases (11).

How different might a world map look today, had diarrhea not had such an impact on the battlefields? Would travelers need a passport when crossing the Mason-Dixon Line? Would the British and Russians speak French? Would the Cold War have been between the United States and France? Would Germany occupy most of Europe?

Jeremy Boone

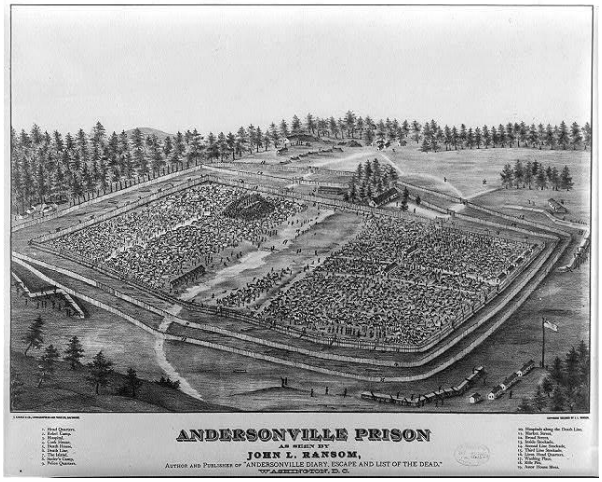
References

1. Ebers Papyrus. Toxipedia. http://toxipedia.org/display/toxipedia/Ebers+Papyrus
2. Alton, G. History of Dysentery. eHow. http://www.ehow.com/about_5370448_history-dysentery.html
3. Wood, M.J. and R.B. Hornick. U. S. Army Medical Department Office of Medical History. History of the Commission on Enteric Infections http://history.amedd.army.mil/booksdocs/historiesofcomsn/section5.html
4. Williams, B. 1997. Infectious Diseases in History. Urban Rim. http://www.urbanrim.org.uk/diseases.htm
5. Burnham, R. Why did Napoleon Fail in Russia in 1812? The Napoleon Series. http://www.napoleon-series.org/faq/c_russia.html
6. Military Medicine. http://www.faqs.org/health/topics/45/Military-Medicine.html
7. Symonds, C.L. and W.J. Clipson .1992. Gettysburg: A Battlefield Atlas. Nautical & Aviation Publishing Company of America. Baltimore, Maryland.
8. DuPont, H.L. and R. Steffen. 2008. Travelers' Diarrhea. B.C. Decker, Inc. Hamilton, Ontario.
9. Sanders J.W., S.D. Putnam , C. Frankart, R.W. Frenck, M.R. Monteville, M.S. Riddle, D.M. Rockabrand, T.W. Sharp, and D.R. Tribble. 2005. Impact of Illness and Non-Combat Injury During Operations Iraqi Freedom and Enduring Freedom (Afghanistan). Am J Trop Med Hyg. 73(4):713-719.
10. DuPont, H.L. 1997. Guidelines on Acute Infectious Diarrhea in Adults. Am J Gastroenterol. 92(11):1962–1975.
11. London. 2010. Syrian Troops Overwhelmed by Diarrhea Epidemic, Heat Wave, Budget Shortfall. WorldTribune.com. http://www.worldtribune.com/worldtribune/WTARC/2010/me_syria0629_07_08.asp

Life as a prisoner during the Civil War

Disease at Civil War prison camps was rampant. A birds' eye view of the Andersonville prison, located in Georgia from 1863-1865, is shown in the following sketch. This camp was built in 1863 to hold 10,000 prisoners, but the inmate population swelled quickly to >30,000. Over the 14 months the prison was in operation, more than 45,000 prisoners were housed there, with 13,000 dying, many from diseases such as dysentery, gangrene, and diarrhea. The prison had a creek, called the "Stockade

Branch", running through it. This creek, which supplied most of the water for the prison, was used for drinking, washing, and removing excrement. The prisoners could not be properly taken care of simply because the Confederacy did not have the money, supplies, or facilities.



FROM: File: Andersonville birdseye ransom.jpg – Wikipedia, the free encyclopedia. Notice the creek, which was the primary water source, running through the middle of the prison.

Our protective gut flora does more than simply outcompete pathogens

Because of our work in studying *C. difficile* disease, we tend to think that *C. difficile* rules the intestine in its ability to infect the patient after wiping out the normal flora with antibiotics. However, other pathogens can do this as well once we kill our gut flora. In a recent study reported in ScienceNOW (March 11, 2011), researchers at the University of British Columbia, Vancouver, expanded what we know about how our intestinal flora actually may be protecting us. *Citrobacter rodentium* (previously referred to as *C. freundii*) has virulence factors similar to enterohemorrhagic and enteropathogenic *E. coli*. Researchers have shown that an infection with *Citrobacter rodentium* is more severe (i.e., diarrhea and possibly death) in certain mouse strains than others. Although some evidence possibly linked this to genetic differences, it doesn't appear to be solely because of genetics. In the study, when susceptible mice had their protective flora killed with antibiotics and then were given fecal material from a less susceptible strain, the "once-susceptible" mice were protected against *C. rodentium*. In this case, it appears that normal flora may be affecting or influencing levels of certain cytokines, which in turn, may affect susceptibility to this pathogen.

Your Gut: The future of personalized medicine

Have you ever had a tailored suit - one that has been made specifically for your body, style, and comfort? Imagine if medicine could be customized to your specific needs. This idea is not far off, and is likely to become a reality in the next decade. Personalized medicine has the ability to find the best way to "fix" an individual's illness - whether it be a rare cancer, a weight problem, or even the common cold. The potential for personalized medicine allows the doctor to treat an illness with a specific drug and dosage that allow for the *least* amount of side effects and the *most* amount of healing. It can also allow insight into disease prevention where a person can modify their diet and - to some extent - environment to prevent future illness. Finally, personalized medicine could allow people to know *exactly* - down to their DNA - what is causing an ailment. All this can be made possible through a variety of methods such as sequencing a patient's genome, determining potential mutations, or even looking at the microbes that live in the gut. The "bugs" that live in your gut can tell you an amazing amount of information about your body and your health.

A person has over a thousand varieties of microorganisms living in their gut, and each of these has their own, distinct genome. While some of these can be harmful, such as *Clostridium difficile,* many play a necessary and beneficial role. Microbes can help metabolize food to allow us to absorb essential vitamins or ward off pathogens. They can also assist in drug metabolism, meaning that doctors of the future may be able to determine which type of treatment to give a person based on the bacteria that live in their intestines. Probably one of the most important roles that these bugs play involves

working with our own immune system. Microbes have been shown to be very telling in studies involving stress, obesity, allergies, autoimmune diseases, cancers, inflammatory bowel disease, and even autism.

Researchers have found that a person's microbiome - the make up of microorganisms in the human body - changes with their environment. For instance, babies that are born vaginally have a very different microbe make-up compared to those delivered by cesarean section. Those born vaginally have essentially been "colonized" with vaginal and fecal bacteria from the mother, where those born through cesarean section often show bacteria that are more common in a hospital setting. Additionally, the well-known hygiene hypothesis - where children raised in a sterile environment are more likely to develop allergies, asthma, and autoimmune diseases compared to those raised in a "dirty" environment - has further been proven when comparing the microbiome of children raised in developed countries compared to those in underdeveloped countries. Personalized medicine can take advantage of the knowledge gained in these types of studies to prevent problems caused at such a young age. Perhaps in the future babies will receive cultured yogurt in addition to infant vaccination.

The gut also is home to a number of biomarkers - molecules that are easily detectable (through blood, saliva, urine, feces, etc.) and are up- or down-regulated in particular cases of disease. Some of these biomarkers are microbes and others can be molecules produced by the host. Personalized medicine would have the option to test patient samples for a panel of biomarkers that could point doctors in the right direction for diagnosis and treatment. They could be used as a means of preventative care if, for instance, a panel was run as part of an annual doctor's checkup. Many companies are already using biomarkers in their diagnostic kits (e.g., lactoferrin tests from TECHLAB). Even more exciting, preventative care is encouraged under the new Patient Protection and Affordable Care Act meaning that the use of biomarkers and early diagnostic platforms could increase in the near future.

Personalized medicine still has many hurdles to overcome. Price is a major concern: the first human genome for about 500 million dollars, more recently the price has dropped closer to $20,000 but researchers hope that this will eventually drop to about $1000 in the next five years. Perhaps more practical and cost effective, protein microarrays are some of the currently available diagnostics using a panel of biomarkers that have received FDA clearance; autoimmune and infectious disease arrays are on the market now. Gastroenterology has advanced greatly in finding useable biomarkers for diseases such as IBD. In many cases though, much more has to be learned. For instance, researchers have determined that NOD2/CARD15 could be a biomarker of Crohn's disease, however this is only true in *some* cases – possibly indicating the important role that environment can have on a person's health.

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References

Phillips, M. Gut Reaction: Environmental Effects on the Human Microbiota. *Environmental Health Perspectives*. 2009. 117: A198-A205.

Patel, K. and Babyatsky M. Medical Education: A Key Partner in Realizing Personalized Medicine in Gastroenterology. *Gastroenterology.* 2008. 134: 656-661.

Nicholson, JK. Global systems biology, personalized medicine and molecular epidemiology. *Molecular Systems Biology.* 2006.

Yu, X. Schneiderhan-Marra, N., and Joos T. Protein Microarrays for Personalized Medicine. *Clinical Chemistry.* 2010. 56: 376-387.

http://med.stanford.edu/ism/2010/september/relman.html

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