Effect of pH on an In Vitro Model of Gastric Microbiota in Enteral Nutrition Patients

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Patients with dysphagia due to oropharyngeal disease or cerebrovascular accident require long-term nutritional support via enteral feeding. Enteral nutrition (EN) is the preferred method of delivery; it is safer and more effective and maintains gut function more efficiently than parenteral nutrition, which can lead to bowel dysfunction. Percutaneous endoscopic gastrostomy (PEG) feeding, a variant of EN in which the tube passes through the abdominal wall into the gastric lumen, is increasingly being used for long-term patient support (21). Diarrhea is the most common complication of EN, although sepsis, aspiration pneumonia, and stoma infections also occur (1).

In health, the stomach is generally devoid of a significant microbiota, containing only low numbers of lactobacilli (ca. 102 CFU/ml of contents) and, in a subset of the human population, Helicobacter pylori (12, 28). The normal duodenum (27) mainly contains lactobacilli and streptococci (ca. 105 to 106 CFU/ml of contents). Microbial cell population densities increase along the jejunum and ileum; colonic contents harbor up to 1012 culturable bacteria/g, the majority of which are strict anaerobes. Bacteroides spp. and bifidobacteria are the main cultivable organisms in this part of the intestine (19), although molecular analyses indicate that clostridia, lactobacilli, and other gram-positive bacteria predominate in the microbiota (29, 30).

Low pH is generally considered to be important in preventing significant microbial colonization of the stomach (32). Indeed, a gastric pH of <4 is considered to be an effective barrier to microbial overgrowth (8). However, a number of potential pathogens have evolved multiple acid resistance mechanisms to increase their survival during gastric transit (2). Other innate defenses of the upper gastrointestinal (GI) tract include enterosalivary nitrate circulation (34) and peristalsis. Lack of normal mastication leads to reduced peristalsis and reduced production of gastric acid and saliva. Saliva contains nitrate concentrations typically in the region of 1 mM (3), around 30% of which is converted to nitrite by oral facultative anaerobes (20), including lactobacilli, on the tongue (33). When swallowed, nitrite is converted to nitric oxide and other compounds (20) and exerts an antimicrobial effect in the stomach (34). All of these mechanisms may break down in EN patients. The result is microbial overgrowth in the upper GI tract. Additionally, the feeding tube itself provides a conduit through which allochthonous microorganisms can gain access to the stomach and duodenum.

Microbial biofilms can be defined as matrix-enclosed microbial accretions which adhere to biological and nonbiological surfaces (14). They are implicated in the pathogenesis of many infectious conditions (4), particularly those involving indwelling medical devices (13). Such communities are particularly problematic because of their inherent recalcitrance to antimicrobial agents (7) and their abilities to act as reservoirs in which pathogens can survive during antibiotic therapy. Biofilms are known to form on PEG tubes. Such communities are composed of both bacteria and yeasts (6, 10, 11) and are known to be a cause of tube deterioration (9).

Previous work in our laboratory has demonstrated that patients receiving EN harbor an abnormal gastric microbiota,
comprised mainly of candidas, gram-positive facultative anaerobes, enterobacteria, and enterococci, with lower numbers of anaerobic genera such as bifidobacteria and clostridia. Similar profiles have been reported in other studies (5, 6, 9–11).

To model potential therapeutic interventions in EN patients, we developed a continuous culture-based model of the gastric microbiota which consisted of 11 microbial strains representative of those isolated most commonly in the endoscopy clinic. This system also facilitates quantitation and visualization of PEG tube biofilm communities. The aims of the present study were twofold: the first objective was to investigate the response of the microbiota in the model to changing pH, in terms of its composition and fermentation product output. The second aim was to assess the effect of pH on the structure and composition of microbial biofilms formed on PEG tube surfaces in the gastric simulator.

MATERIALS AND METHODS

Patient aspirates. Gastric and duodenal material was obtained from patients (n = 20) undergoing PEG tube placement at Ninewells Hospital, Dundee, United Kingdom. The Tayside Research Ethics Committee gave ethical permission for the work. All patients received nasogastric feeding prior to PEG tube insertion. Samples were aspirated at endoscopy from the stomach or duodenum, and analyzed immediately upon receipt in the laboratory. All endoscopes were sterilized, as per the manufacturer’s instructions, and were checked for sterility on a weekly basis. Duodenal fluid was aspirated into a sterile trap, and the endoscope was flushed with sterile water (20 ml) prior to aspiration of fluid from the stomach.

Samples were serially diluted to 10⁻³ in preruced half-strength peptone water, and aliquots (each, 100 μl) of each dilution from 10⁻¹ to 10⁻⁵ were spread onto agar plates. (i) For aerobic incubation, these were nutrient agar CM3 (for detection of aerobes and facultative anaerobes), MacConkey agar no. 2 (enterobacteria, and yeast and mould agar (yeasts and moulds). (ii) For anaerobic incubation, these were Wilkins-Chalgren agar (anaerobes/facultative anaerobes), MRS agar (lactic acid bacteria), Clostridium perfringens agar (C. perfringens and other clostridia), Rogosa agar (lactobacilli), blood agar (fastidious anaerobes/facultative anaerobes), brain heart infusion agar containing 5% (vol/vol) defibrinated horse blood (fastidious anaerobes/facultative anaerobes), azide blood agar (streptococci and enterococci), and Bacteroides mineral salts agar (Bacteroides spp.) (24).

Anaerobic incubation was done with a MACS MC-1000 Anaerobic Workstation (Don Whiteley Scientific, Ltd., Shipley, West Yorkshire, United Kingdom) under a 10% H₂–10% CO₂–80% N₂ atmosphere at 37°C for 72 h. Aerobic plates were incubated at 37°C, except those for yeasts, which were incubated at 30°C for 48 h. Following incubation, colony morphologies were recorded, and colonies representative of each type were subcultured onto appropriate solid media. Subcultures were transferred aseptically to 1.0 ml of cryogenic storage medium (comprising, per liter of distilled water, Wilkins-Chalgren anaerobe broth, 33 g; porcine gastric mucin [Sigma type III], 20 g; and glycerol, 100 ml) and stored at −85°C until required.

Bacterial isolates were identified by cellular fatty acid-methyl ester profiling using the MIDI system in combination with colonial and cellular morphology and reaction. Fatty acid methyl esters (FAMEs) were extracted from bacterial pellets obtained from approximately 40 ml of culture in anaerobic peptone yeast extract broth (17), supplemented with glucose (10 g/liter) for anaerobic isolates or from 40 mg of bacteria grown on BBL trypticase soy broth agar (Becton Dickinson, Ltd., Oxford, United Kingdom) for aerobic isolates by saponification, methylation, and extraction, as described previously (26). These FAMEs were comparing FAME profiles to known cultures in the MIDI aerobe and anaerobe 20 C AUX biochemical identification system (API, bioMerieux, Basingstoke, Hampshire, United Kingdom). The growth medium contained (per liter) porcine gastric mucin (Sigma type III), 0.1 g casein, 0.1 g peptone, 1.0 g yeast extract, 1.0 g tryptone soy broth, 0.1 g NaCl, 2.5 g KC1, 1.5 g KH₂PO₄, 1.0 g MgCl₂, 0.25 g CaCl₂, 0.15 g and hemin, 0.2 mg. The growth medium was set at pH 6.5 using 1.0 M HCl or 1.0 M NaOH, as required. Chemostat pH was controlled over a range of 6.0 to 3.0 (lower limit of the controller) by the addition of 1.0 M HCl using a New Brunswick Scientific (St. Albans, Herts, United Kingdom) pH 1000 pH system attached to a Thermo-Russell (Auchterarder, United Kingdom) CW711/EXT/250 pH electrode. The chemostats were maintained at 37°C by using a Haake B3 recirculating water bath and operated at a dilution rate of 0.2/h under putatively aerobic conditions. Growth medium was introduced into the fermentors 18 h after inoculation. Samples for microbiological and chemical analyses were taken after steady-state conditions had been achieved after at least nine culture turnovers. Lengths (ca. 12 cm) of silicon PEG tube were suspended in the chemostats to study biofilm formation. The PEG tubes were sampled twice daily for 4 days by aseptic excision of a 5-mm length of tube. Excess fluid was removed, and adherent microorganisms were sampled by scraping both the exterior and interior surfaces with a sterile scalpel.

Enumeration of microorganisms was done by serial dilution in half-strength peptone water and spread plating onto the following solid culture media: MacConkey agar no. 2 (E. coli and K. pneumoniae), rose Bengal chloramphenicol agar with 0.1 mg ml⁻¹ tetracycline (C. albicans and C. parapsilosis), nutrient agar with 8.0% (wt/vol) NaCl (S. aureus), Rogosa agar (L. casei and L. paracasei), Beersens agar (B. adolescentis), and kanamycin esculin azide agar (S. parasuans, S. intermedius, and S. agalactiae).

Biofilm visualization. Sections of PEG tube (each, 5 mm in length) were removed from the fermentors and cut into 1- by 1-mm squares with a sterile scalpel. Care was taken to minimize disturbance of the surface communities during this procedure. Each square was immersed in BacLight Live/Dead staining solution (Molecular Probes Europe BV, Leiden, The Netherlands) for 15 min. Sections were then examined by fluorescence microscopy with a Zeiss Axiovert fluorescence microscope connected to a Dell Optiplex GX110 PC, running C-Imaging Systems Simple-PCI imaging software (Compix, Inc., Cranberry Township, PA).

Analysis of microbial fermentation products. Samples were centrifuged (13,000 × g, 15 min) to remove microbial cells. Short-chain fatty acids (SCFA) were measured by GC after extraction into ether, as described previously (23), with the addition of an internal standard (50 mM tert-butyl acetate). SCFA were separated on a HP-INNOX wax cross-linked PEG (30 m by 0.25 mm) column (Agilent Technologies). Injector and detector temperatures were 250 and 300°C, respectively. The flow rate of the helium carrier gas was set at 1.8 liters/min. The oven temperature program was 120°C for 1 min, followed by 10°C min⁻¹ to 260°C, where it was maintained for 2 min. Lactate and succinate were measured by GC after extraction into chloroform (23), with the addition of an internal standard (100 mM oxalic acid), using the above GC settings.

Chemicals. Unless stated otherwise, all microbiological culture media were obtained from Oxoid, Ltd. (Basingstoke, Hants, United Kingdom). Other chemicals were purchased from the Sigma Chemical Co. (Poole, Dorset, United Kingdom).
TABLE 1. Composition of aspirate microflora and microbial cell population densities in relation to pH

<table>
<thead>
<tr>
<th>Genus</th>
<th>pH range</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0–2</td>
<td>3–6</td>
<td>&gt;6</td>
</tr>
<tr>
<td>Candida</td>
<td>4.0 ± 1.5 (5)</td>
<td>3.2 ± 0.2 (2)</td>
<td>4.7 ± 1.1 (4)</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>ND</td>
<td>4.2 ± 0.6 (3)</td>
<td>4.9 ± 1.5 (5)</td>
</tr>
<tr>
<td>Escherichia</td>
<td>3.1 ± 1.3 (2)</td>
<td>4.5 ± 1.5 (4)</td>
<td>5.4 ± 1.0 (4)</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>ND</td>
<td>4.9 ± 1.6 (3)</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>4.4 ± 1.3 (4)</td>
<td>4.1 ± 0.8 (2)</td>
<td>4.9 ± 1.1 (4)</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>ND</td>
<td>5.4 (1)</td>
<td>5.3 ± 1.8 (2)</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>5.9 ± 1.1 (5)</td>
<td>3.8 ± 0.5 (5)</td>
<td>5.4 ± 1.3 (5)</td>
</tr>
<tr>
<td>Total counts</td>
<td>4.5 ± 2.0 (13)</td>
<td>4.9 ± 1.4 (8)</td>
<td>5.8 ± 1.4 (9)</td>
</tr>
<tr>
<td>No. of genera</td>
<td>2.3 ± 1.2</td>
<td>4.3 ± 3.2</td>
<td>4.5 ± 2.7</td>
</tr>
</tbody>
</table>

* Data are expressed as log_{10} CFU/ml and means ± standard deviation (number of patients).

* ND, not detected.

* Mean number of genera per aspirate ± standard deviation.

RESULTS

Effect of pH on gastric and duodenal aspirate microflora. The effect of pH was investigated by examining gastric and duodenal aspirates together, because individually neither contained sufficient samples with a wide enough range of pH values to make a valid comparison. pH did not have a marked effect on total microbial counts of gastric and duodenal aspirates (Table 1). In contrast, the species composition of aspirates was affected by gastrointestinal pH. For example, bifidobacteria were only detected in aspirates with a pH of 3 or higher, as were staphylococci and klebsiellas. Streptococci, candidas, and lactobacilli were found throughout the pH range. Although *E. coli* was present in aspirates with pH values of 0 to 2, the organism was detected at a lower frequency and in fewer numbers than in aspirates of higher pH. The mean number of genera detected was similar between pH 3 to 5 and >6 (4.3 ± 3.2 and 4.5 ± 2.7, respectively), although it was lower in those aspirates with a pH of <3 (2.3 ± 1.2).

Effect of pH on the gastric microbiota in vitro. Chemostat pH varied from 6.0 to 3.0, and effects on the composition of planktonic microflora were assessed by traditional culturing techniques to determine microbial viabilities (Fig. 1). *Bifidobacterium adolescentis* was detected only at pH 6.0 (8.1 ± 0.2 CFU/ml). *Staphylococcus aureus* was found over a pH range of 6.0 to 5.0 (2.9 ± 0.1 and 3.6 ± 0.1 CFU/ml, respectively).

Viable counts of *E. coli* and *K. pneumoniae* decreased from ca. 10^9 CFU/ml to 10^7 CFU/ml as culture pH was reduced from 6.0 to 3.0 (Fig. 1A). In contrast, candidas occurred in the highest numbers at pH 4.0 and were present in higher numbers at pH 3.0 than at pH 6.0. Lactobacilli were also able to persist under acidic growth conditions, with maximum recoveries occurring at pH 4.0 and 5.0 (Fig. 1B). Streptococcal numbers at pH 6.0 and 5.0 were 8.2 ± 0.1 and 7.4 ± 0.1 CFU/ml (*S. parasanguis*) and 7.1 ± 0.2 and 6.6 ± 1.0 CFU/ml (*S. intermedius*), respectively. No streptococci were found at pH values of 3 or 4, due to overgrowth of lactobacilli on kanamycin esculin azide culture medium. *Streptococcus agalactiae* was never established in the chemostats.

The effect of pH on PEG tube biofilm communities was also assessed (Fig. 2). *Bifidobacterium adolescentis* and *S. aureus* were only found when culture pH was set at 6.0 (6.6 ± 0.5 and 0.9 ± 1.6 CFU/cm², respectively). In contrast, *E. coli* and *K. pneumoniae* were detected at all pH values in the biofilms. The highest numbers of *E. coli* and *K. pneumoniae* occurred at pH 5 (7.4 ± 0.3 and 7.3 ± 0.2 CFU/cm²). Candidas were recovered from PEG tube biofilms at all pH values, although the highest cell numbers were recorded at pH 3.0 (4.5 ± 0.1 and 4.6 ± 0.0 CFU/cm² for *C. albicans* and *C. famata*, respectively). Lactobacilli were also detected throughout the pH range, with maximum recovery at pH 5.0 (6.0 ± 0.6 and 6.9 ± 0.6 CFU/cm² for *L. paracasei* and *L. sharpeae*, respectively). *Streptococcus parasanguis* and *S. intermedius* were found in the biofilm at pH 6.0 and 5.0, with maximal recoveries of both taxa at pH 6.0 (7.5 ± 0.3 and 6.7 ± 0.3 CFU/cm²).

Analysis of microbial fermentation products. Low concentrations of lactate, succinate, and acetate were present in both
gastric and duodenal aspirates (Table 2), but propionate was only detected in duodenal aspirates. Other SCFA such as butyrate, isobutyrate, valerate, isovalerate, caproate, or isocaproate were not found. Lactate accumulated in chemostats at pH 3.0 and 4.0 (1.4 ± 1.6 and 2.3 ± 2.5 mM) but not at higher pH values (Table 3). Succinate was detected at low levels (>0.5 mM) from pH 4.0 to 6.0 but not at pH 3.0. Acetate concentrations were maximal at pH 5.0 (3.7 ± 4.1 mM), but this SCFA was not found at pH 3.0. Propionate was only detected in trace amounts at pH 5.0 and 6.0 (0.13 ± 0.01 and 0.18 ± 0.03 mM, respectively). No other SCFA or organic acids were present in the chemostats at any pH value.

**DISCUSSION**

Patients receiving EN have been reported to harbor an abnormal microbiota in their upper GI tract, which mainly consists of enterobacteria, streptococci, staphylococci, lactobacilli, and candidas (5, 6, 10). To investigate environmental factors affecting the growth of these organisms, we developed a continuous culture model of the EN patient upper GI tract microbiota to study the effects of pH on microbial community structure and metabolism.

Microbiological counts done of gastric and duodenal aspirates (Table 2), but propionate was only detected in duodenal aspirates. Other SCFA such as butyrate, isobutyrate, valerate, isovalerate, caproate, or isocaproate were not found. Lactate accumulated in chemostats at pH 3.0 and 4.0 (1.4 ± 1.6 and 2.3 ± 2.5 mM) but not at higher pH values (Table 3). Succinate was detected at low levels (>0.5 mM) from pH 4.0 to 6.0 but not at pH 3.0. Acetate concentrations were maximal at pH 5.0 (3.7 ± 4.1 mM), but this SCFA was not found at pH 3.0. Propionate was only detected in trace amounts at pH 5.0 and 6.0 (0.13 ± 0.01 and 0.18 ± 0.03 mM, respectively). No other SCFA or organic acids were present in the chemostats at any pH value.

**Visualization of PEG tube biofilms.** Live/Dead staining was used in conjunction with fluorescence microscopy to visualize the spatial organization of PEG tube biofilm communities (Fig. 3). Dead organisms were colored red, and live cells were colored green. The results showed substantial colonization of the PEG tube surfaces by bacteria and yeasts. At all pH values, surface growth was characterized by microcolonies of various sizes, interspersed with sparsely colonized regions (Fig. 3A to D). Microcolonies were generally composed of both bacterial and yeast cells. As pH was reduced in the fermentors, yeast cell mass increased. Additionally, at pH of ≤4, elongated yeast and bacterial cells were observed (Fig. 3C and D). Yeast pseudohyphae were frequently observed to pass through microcolonies that contained both bacteria and other yeast forms (Fig. 3C). This phenomenon was increasingly evident at low pH. Additionally, bacteria in the microcolony surrounding these intrusive filaments usually stained red, in contrast to their immediate neighbors, which were predominantly green (Fig. 3C).

**TABLE 2. Microbial fermentation products detected in gastric and duodenal aspirates**

<table>
<thead>
<tr>
<th>Aspirate</th>
<th>Fermentation product (mM)</th>
<th>Lactate</th>
<th>Succinate</th>
<th>Acetate</th>
<th>Propionate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric</td>
<td>0.70 ± 1.2 (13)</td>
<td>0.20 ± 0.2 (13)</td>
<td>0.20 ± 0.2 (21)</td>
<td>ND* (21)</td>
<td></td>
</tr>
<tr>
<td>Duodenal</td>
<td>0.52 ± 0.83 (14)</td>
<td>0.34 ± 1.27 (14)</td>
<td>0.39 ± 0.94 (14)</td>
<td>0.05 ± 0.19 (14)</td>
<td></td>
</tr>
</tbody>
</table>

*Data are means ± standard deviation (number of aspirates assayed). n varies because insufficient volume was available in some aspirates to undertake solvent extractions.

*ND, not detected.

**TABLE 3. Microbial fermentation products detected in chemostats run at different pH values**

<table>
<thead>
<tr>
<th>pH</th>
<th>Lactate</th>
<th>Succinate</th>
<th>Acetate</th>
<th>Propionate</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>1.37 ± 1.58</td>
<td>ND†</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4.0</td>
<td>ND</td>
<td>0.04 ± 0.06</td>
<td>0.17 ± 0.25</td>
<td>ND</td>
</tr>
<tr>
<td>5.0</td>
<td>ND</td>
<td>0.28 ± 0.09</td>
<td>3.68 ± 4.12</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>6.0</td>
<td>ND</td>
<td>0.25 ± 0.03</td>
<td>2.02 ± 0.22</td>
<td>0.18 ± 0.01</td>
</tr>
</tbody>
</table>

†Data are means ± standard deviations (n = 8).

*ND, not detected.
rates showed that pH had a marked effect on species composition and the number of microbial genera isolated per aspirate, but not on overall cell numbers in these ecosystems (Table 1). Acid suppression therapy is common in the treatment of EN patients, and these results suggest that its use will significantly affect the composition of microbial communities in the upper GI tract, for example, by encouraging the growth of candidas. The potential consequences of the ecological effects of acid suppression therapy should therefore be considered when it is used in EN patients, especially in those individuals who are immunocompromised and so particularly vulnerable to infection.

The effect of acidity on the upper GI microbiota was examined by varying the pH of steady-state chemostat cultures from 6.0 to 3.0 in incremental steps of 1 pH unit. The composition of planktonic and PEG tube biofilm microbiotas was assessed using traditional culturing techniques so that cell viability could be determined (Fig. 1 and 2). As pH was reduced, viable counts of *E. coli* and *K. pneumoniae* decreased, although both organisms were still present in significant numbers at pH 3.0. A similar effect was seen in the biofilm populations (Fig. 2A), showing that these clinical strains were tolerant of the levels of acidity found in the stomach of EN patients. *Staphylococcus aureus* and *B. adolescentis* were never detected in gastric or duodenal aspirates when the pH was <4.0 (data not shown), and these organisms could only be established at relatively high pH values in the chemostats. In contrast, acid tolerant lactobacilli and candidas were detected at all pH values in the aspirates and upper GI simulator (Fig. 2A and B), demonstrating that they were adapted to growth in the upper GI environment.

*Escherichia coli* is autochthonous to the lower GI tract, where it is present at levels of approximately $10^9$ CFU/g of gut contents, representing about 0.2% of the total microbiota as determined by fluorescent in situ hybridization (15). Like other colonic microorganisms, the bacterium must survive passage through gastric acid in the upper GI tract to reach the large bowel. For this reason, *E. coli* possesses a number of acid resistance mechanisms (2, 16, 25) that allow it to survive in environments with a pH of as low as 2 (32), although the minimum pH for it to be able to multiply has been reported to be 4.4 (22). Results obtained in this study show that in mixed culture, *E. coli* was able to multiply in both planktonic and biofilm environments where the pH was 3.0. This apparent ambiguity may be due to ecological or metabolic interactions with other microorganisms in the ecosystem. The more-acid-tolerant microorganisms within the microbiota (lactobacilli, candidas) may provide a protected niche within which less aciduric microorganisms (*E. coli* and *K. pneumoniae*) can grow. The existence of protected niches in biofilms is a well-recognized phenomenon (31), and similar mechanisms may occur in the planktonic population through the formation of bacterial aggregates. These data suggest that the typical levels of acidity found in the stomach of EN patients, together with the presence of an abnormal microbiota containing acid-tolerant microorganisms, is not a barrier to colonization by opportunistic pathogens such as *E. coli*. The presence of such a microorganism in the stomachs of a vulnerable group of patients may be a cause for concern and shows that there is a need for studies of interventions aimed at controlling microbial overgrowth in these individuals.

Previously, it has generally been accepted that bacterial overgrowth will not occur if gastric pH is <4. In vivo and in vitro studies have demonstrated that pH <4 results in killing of
99.9% of bacteria within about 90 min (8). Evidence obtained in this investigation suggests that this may not be the case. Data from both in vitro and in vivo studies showed that a variety of microorganisms, bacteria, and yeasts, including some potentially pathogenic taxa, are capable of multiplying in environments with pH values as low as 3. Future studies on the ability of potentially pathogenic microorganisms to overcome the gastric acid barrier should, therefore, take account of the likely ecologic and physiologic effects of gastric microorganisms in patients likely to be exposed to the pathogen.

Fluorescence microscopy demonstrated significant biofilm growth on PEG tube surfaces (Fig. 3). Mature surface growth occurred at all pH values in the form of discrete microcolonies surrounded by sparsely colonized interstitial voids. Microcolonies contained both live and dead rod and coccal forms, together with yeasts of various morphologies. As pH was reduced, consortia of potentially pathogenic microorganisms to overcome the gastrointestinal tract of EN patients. Neth. J. Med. 27:181–187.

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REFERENCES


