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Effect of pH and Antibiotics on Microbial Overgrowth in the Stomachs and Duodena of Patients Undergoing Percutaneous Endoscopic Gastrostomy Feeding

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Enteral nutrition via a percutaneous endoscopic gastrostomy (PEG) tube is often part of management in patients with dysphagia due to neurological or oropharyngeal disease. Gastrostomy placement can affect normal innate defense mechanisms in the upper gut, resulting in bacterial overgrowth. In this study microbiological investigations were done with gastric and duodenal aspirates from 20 patients undergoing PEG tube placement and PEG tubes from 10 patients undergoing tube replacement. Aspirate and PEG tube microbiotas were assessed by using viable counts and selective solid media followed by aerobic and anaerobic incubation to assess cell viabilities. The antibiotic susceptibility profiles of the isolates were determined by the disk diffusion method, and gas chromatography was used to study the bacterial metabolic products in the aspirates. The aspirates and PEG tubes contained mainly streptococci, staphylococci, lactobacilli, yeasts, and enterobacteria. Enterococci were detected only in PEG tube biofilms and not in aspirates. Gastric pH affected the composition of the aspirate microbiotas but not the total microbial counts. Staphylococci, *Escherichia coli*, and *Candida* spp. were isolated only from antibiotic-treated patients, despite the sensitivities of the bacteria to the agents used. Antibiotic treatment had no effect on the incidence of infection or the length of hospital stay in these patients.

Patients with dysphagia due to neurological or oropharyngeal disease require long-term nutritional support. Enteral nutrition (EN) is the preferred route because it is safer and more physiologically relevant in that it preserves the barrier (19, 41) and absorptive (4) functions of the gut. Percutaneous endoscopic gastrostomy (PEG) tube feeding involves delivery of nutrients via a silicone tube directly into the stomach and is usually done after patients have been receiving EN nasogastrically (NG). EN of either type bypasses many of the mechanisms that prevent microbial colonization of the upper gut, and the feeding tube itself acts as a conduit through which allochthonous microorganisms can migrate into the stomach from the external environment. Common complications of EN include diarrhea, aspiration pneumonia, and infections of the stoma.

Normally, the upper gastrointestinal (GI) tract is sparsely colonized by microorganisms. The stomach is generally devoid of a significant microbiota other than *Helicobacter pylori* and some lactobacilli, which are present in low numbers (ca. 10^1 to 10^3 CFU ml contents^{-1}) (15, 32). In contrast, the duodenum contains a resident microbiota from which lactobacilli and streptococci are the main species culturable at cell population densities of approximately 10^2 to 10^4 CFU ml contents^{-1} (29). Microbial density increases along the small bowel, and colonic contents contain up to 10^{12} CFU per gram (18).

Low gastric pH is thought to be a major factor that suppresses microbial colonization of the stomach (40), but some enteric bacteria possess acid resistance mechanisms (5) that may confer protection in the GI tract. However, many innate defense mechanisms break down in PEG tube patients, because the lack of sensory stimuli associated with food intake inhibits saliva production and peristalsis, while reduced swallowing increases the pH and reduces gastric nitrite concentrations. The net effect is greater susceptibility to microbial overgrowth in the stomach and duodenum, which often results in diarrhea, although more serious complications such as malabsorption and sepsis can also occur (3). The formation of microbial biofilms on PEG tubes is an unavoidable consequence of bacterial overgrowth, and they are difficult to eradicate with antimicrobial agents (35, 38). Moreover, biofilms can harbor pathogens (1) and/or microorganisms that carry antibiotic resistance genes (30) and often cause problems with indwelling devices (31).

Candida spp. are known to colonize PEG tubes (12, 13), a phenomenon that may also lead to tube deterioration (11). Enterococci, staphylococci, *Escherichia coli*, lactobacilli, candidas, pseudomonads, and bacilli have been isolated from pediatric PEG tube patients (7), while other studies found that bacilli, enterococci, enterobacteria, candidas, pseudomonads, and staphylococci predominated in PEG tube biofilms (26).

Despite these investigations, little is known of the microcosms associated with EN or the factors that affect their development. The aims of this investigation were to examine the planktonic and surface-associated microbial populations in patients receiving EN and to study the effects of gastric and

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TABLE 1. Clinical details of pre-PEG tube patients

Characteristic	Patient group		
	Total	Antibiotic treated	Non-antibiotic treated
Age (yr)[d]	69.4 ± 14.5	71.4 ± 14.8	61.8 ± 12.4
No. of males:no. of females	15:9	12:6	2:3[c]
Indications for PEG (% of patients)			
CVA[a]	47.8	50.0	40.0[c]
Dysphagia	21.7	22.2	20.0
Weight loss	8.7	11.1	0.0
OP[b] carcinoma	8.7	5.6	20.0[c]
Other	13.0	11.1	20.0
Duration of EN (days)[d]	12.9 ± 10.9	15.6 ± 11.0	4.2 ± 4.0[c]
Antacid treatment (% of patients)	34.8	44.4	0.0[c]

[a] CvA, cerebrovascular accident.
[b] OP, oropharyngeal carcinoma.
[c] Significant difference between the antibiotic-treated and non-antibiotic groups-treated ($P < 0.05$). Antibiotic-treated group, $n = 18$; non-antibiotic-treated group, $n = 5$.
[d] Data are presented as mean values ± standard deviations.

duodenal pH, together with antibiotic therapy, on these microbiotas.

MATERIALS AND METHODS

Patients. Gastric and duodenal aspirates ($n = 20$) were obtained from patients undergoing PEG tube placement (pre-PEG), as well as PEG tubes from patients ($n = 10$) undergoing tube replacement procedures at Ninewells Hospital, Dundee, United Kingdom. Pre-PEG tube patients received NG feeding prior to PEG tube insertion, and individuals from whom PEG tubes were obtained received PEG feeding for at least 4 weeks before samples were taken. Approval for this research was obtained from the Tayside Medical Research Ethics Committee, Ninewells Hospital.

TABLE 2. Effect of pre-PEG antibiotic treatment on patient outcomes

Characteristic	Patient group		
	Total	Antibiotics	No antibiotics
Infection rate (% of patients)			
UTI[a]	13.0	16.7	
RTI[b]	8.7	11.1	
PEG stoma	17.4	11.1	20.0
AP[c]	26.1	22.2	20.0
Other	13.0	16.7	
Multiple	13.0	16.7	
Total	60.9	60.0	40.0
Mortality rate (% of patients)			
One mo	8.7	11.1	0.0
Three mo	4.3	5.6	0.0
Six mo	39.1	33.3	60.0[e]
Length of stay[d]	54.0 ± 55.7	59.2 ± 59.5	35.6 ± 38.9

[a] UTI, urinary tract infection.
[b] RTI, respiratory tract infection.
[c] AP, aspiration pneumonia.
[d] Number of days post-PEG insertion.
[e] Significant difference between the antibiotic-treated and non-antibiotic-treated groups ($P < 0.05$). Antibiotic-treated group, $n = 18$; non-antibiotic-treated group, $n = 5$. Data are expressed as mean values ± standard deviations.

Analysis of gastric and duodenal microbiotas. Samples from the gastrum or duodenum were aspirated at endoscopy and were analyzed within 1 h. Prior to use all endoscopes (Keymed EVIS GIF-XK240 gastroscopes; KeyMed Ltd., Southend-on-Sea, United Kingdom) underwent a full sterilization process (gluteraldehyde, 2.0% [vol/vol]; 20 min), according to the manufacturer's instructions. The sterility of the endoscopes was tested weekly by the clinical microbiology laboratories at Ninewells Hospital by the use of culturing methodologies. Gastric and duodenal fluid was aspirated into a disposable sterile trap, and the

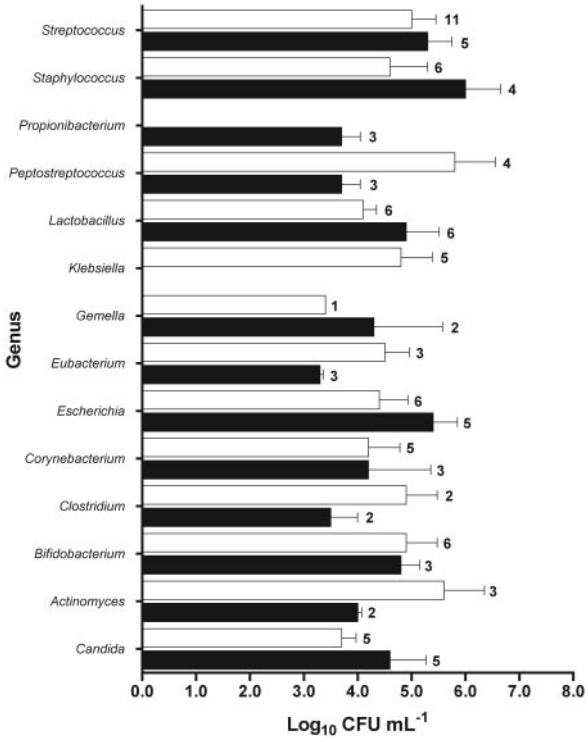

FIG. 1. Characterization of the microorganisms isolated from gastric (■) and duodenal (□) aspirates. The values at the ends of the bars indicate the number of patients from whom the organisms were isolated. The results are expressed as means ± standard deviations. The data are for a total of 20 patients.

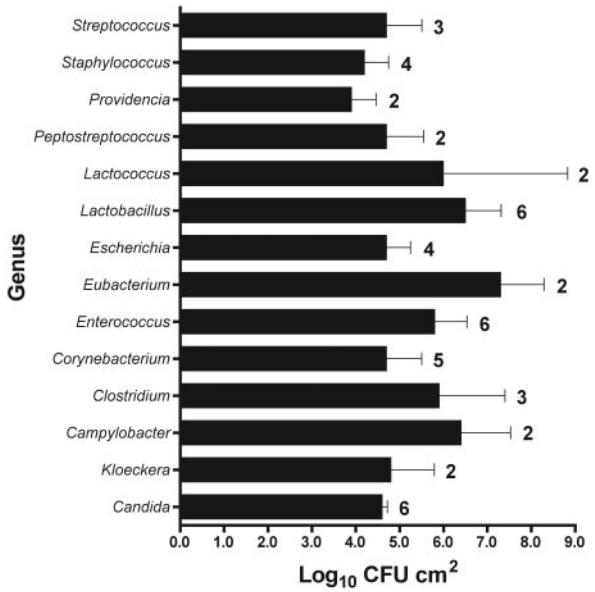

FIG. 2. Characterization of the microorganisms isolated from PEG tubes. The values at the ends of the bars indicate the number of patients from whom the organisms were isolated. The data are expressed as means ± standard deviations. The data are for a total of 10 patients.

endoscope was flushed with sterile water (20 ml) prior to aspiration of fluid from the stomach.

Aspirate pH was determined with an Hanna Instruments pH 210 pH meter (Hanna Instruments Inc., Woonsocket, RI). Samples were serially diluted to 10^{-5} in prereduced half-strength peptone water. Aliquots (100 μl) of each dilution from 10^{-1} to 10^{-5} were spread onto agar plates. These were as follows: (i) for aerobic incubation, nutrient agar CM3, MacConkey agar No. 2, and yeast and mold agar; (ii) for anaerobic incubation, Wilkins-Chalgren agar; de Man, Rogosa, Sharpe agar; *Clostridium perfringens* agar; Rogosa agar; blood agar; brain heart infusion agar containing 5% (vol/vol) defibrinated horse blood; azide blood agar; and *Bacteroides* mineral salts agar (24).

Aerobic incubation was carried out at 37°C without CO_2. Anaerobic incubation was done in a MACS MC-1000 Anaerobic Workstation (Don Whitley Scientific Ltd., Shipley, United Kingdom) under a 10% H_2, 10% CO_2, 80% N_2 atmosphere at 37°C for 72 h. Aerobic plates were incubated at 37°C (yeast and mold agar, however, was incubated at 30°C) for 48 h. Subcultures were transferred aseptically to 1.0 ml cryogenic storage medium comprising the following per liter of distilled water: Wilkens-Chalgren anaerobe broth, 33 g; porcine gastric mucin (type III; Sigma), 20 g; glycerol, 100 ml. The subcultures were stored at −85°C until they were required.

PEG tubes were taken from patients and immediately placed into sterile bags. A length of tubing (5 mm) from the distal end of the tube was excised aseptically, and excess fluid was removed. Microorganisms were removed by vortex mixing for 1 min in prereduced half-strength peptone water, followed by repeated aspiration through a sterile 25-gauge needle. This procedure was continued until no visible clumps remained. Microbiological analyses were done as described above.

Bacteria were identified by cellular fatty acid-methyl ester profiling by using a MIDI system, as described previously (18), in combination with colonial and cellular morphology and a Gram stain reaction. Yeasts were identified by using an API 20C AUX biochemical identification system (Bio-Merieux, Basingstoke, United Kingdom).

Antibiotic susceptibilities. Antibiotic susceptibilities were determined by the disk diffusion method. Antibiotic disks (ciprofloxacin, 5 μg; erythromycin, 10 μg; cefuroxime, 30 μg; amoxicillin-clavulanic acid, 30 μg; metronidazole, 5 μg; tetracycline, 10 μg; doxycycline, 30 μg; amoxicillin, 10 μg; ampicillin, 10 μg; penicillin G, 1 U; neomycin, 30 μg; vancomycin, 30 μg; co-trimoxazole, 25 μg) were obtained from Oxoid Ltd. (Basingstoke, United Kingdom).

Isolates from the clinical samples that belonged to the major genera, together with species identified as potentially pathogenic but not part of the major genera, were spread onto the surface of a Wilkins-Chalgren agar plate to achieve complete coverage. Antibiotic disks were then placed aseptically onto the plates by using an Oxoid ST 6090 disk dispenser. The plates were incubated anaerobically at 37°C for 24 to 48 h.

TABLE 3. Effect of pH on incidence and cell population sizes of individual genera and total counts of gastric and duodenal aspirates

Genus	Population size[a] at the following aspirate pH:		
	0–2	3–5	>6
Candida	4.0 ± 1.5 (5)	3.2 ± 0.2 (2)	4.7 ± 1.1 (4)
Bifidobacterium	ND[b]	4.2 ± 0.6 (3)	4.9 ± 1.5 (5)
Escherichia	3.1 ± 1.3 (2)	4.5 ± 1.5 (4)	5.4 ± 1.0 (4)
Klebsiella	ND	4.9 ± 1.6 (3)	4.4 (1)
Lactobacillus	4.4 ± 1.3 (4)	4.1 ± 0.8 (2)	4.9 ± 1.1 (4)
Staphylococcus	ND	5.4 (1)	5.3 ± 1.8 (2)
Streptococcus	5.9 ± 1.1 (5)	3.8 ± 0.5 (5)	5.4 ± 1.3 (5)
Total count[c]	4.5 ± 2.0 (13)	4.9 ± 1.4 (8)	5.8 ± 1.4 (9)

[a] Data are expressed as mean $\log_{10}$ CFU ml^{-1} ± standard deviations (number of patients).
[b] ND, not detected.
[c] All genera.

Fermentation product analysis. Samples were centrifuged (13,000 × *g*, 15 min) to remove bacteria. Short-chain fatty acids were measured by gas chromatography (GC) after extraction into ether, as described by Macfarlane and Englyst (23), with the addition of an internal standard (50 mM *tert*-butyl acetic acid). Short-chain fatty acids were separated on a HP-INNOwax cross-linked polyethylene glycol (30 m by 0.25 μm) column (Agilent Technologies). The injector and detector temperatures were 300 and 250°C, respectively. The flow rate of the helium carrier gas was set at 1.8 ml min^{-1}. 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, with the addition of an internal standard (100 mM oxalic acid) by using the GC settings indicated above.

Chemicals. Unless stated otherwise, all microbiological culture media were obtained from Oxoid. Other chemicals were purchased from the Sigma Chemical Co. (Poole, Dorset, United Kingdom).

Statistical analysis. Numerical data were analyzed by the independent *t* test if they were normally distributed and the Mann-Whitney U test otherwise. Proportion data were analyzed by the χ^2 test. A *P* value of less than 0.05 was taken as a significant difference.

RESULTS

Patients. Gastric and duodenal aspirates were obtained from a total of 20 pre-PEG tube patients and PEG tubes from 10 individuals. Of these, 23 and 8, respectively, had medical notes available at the time of follow-up. Of the pre-PEG tube patients, 18 (78%) had received antibiotics, while 5 (22%) had not. The most commonly prescribed antibiotic was the combination amoxicillin-clavulanic acid (15 of 18 patients). Four received ciprofloxacin, while three were given clarithromycin, gentamicin, flucoxacillin, metronidazole, and nystatin. Eleven patients received multiple antibiotics. The ages of the patients in the antibiotic- and the non-antibiotic-treated groups were similar (Table 1). The most common indication for PEG insertion was cerebrovascular accident (48% of all patients) in both the antibiotic-treated (50%) and the non-antibiotic-treated (40%) groups ($P = 0.292$). In contrast, however, patients given antibiotics had been receiving EN for a significantly shorter time than those individuals who did not receive antimicrobial therapy (4.2 ± 4.0 and 15.6 ± 11.0 days, respectively; $P = 0.013$). Almost half (44%) of patients in the antibiotic-treated group, although none of the patients in the non-

TABLE 4. Effect of antibiotic treatment on incidence and cell population sizes of individual genera and total counts of gastric and duodenal aspirates

Genus	Population size[a] with the following treatment:	
	Antibiotics	No antibiotics
Candida	4.2 ± 1.0 (6)	ND[b]
Bifidobacterium	4.9 ± 0.5 (3)	4.3 ± 0.8 (3)[c]
Escherichia	4.8 ± 1.0 (9)	ND[c]
Klebsiella	5.3 ± 0.9 (3)	3.2 (1)
Lactobacillus	4.9 ± 1.1 (8)	3.0 (1)[c]
Staphylococcus	5.1 ± 1.5 (8)	ND[c]
Streptococcus	5.1 ± 1.1 (7)	3.7 ± 0.7 (4)[c]
Total count[d]	5.3 ± 2.0 (22)	3.4 ± 1.8 (6)

[a] A total of 22 and 6 patients receiving and not receiving antibiotics, respectively. Data are expressed as mean $\log_{10}$ CFU ml^{-1} ± standard deviations (number of patients).
[b] ND not detected.
[c] Significant difference between the antibiotic-treated and non-antibiotic-treated groups ($P < 0.05$). Aspirates were obtained from antibiotic-treated and untreated patients, respectively.
[d] All genera.

antibiotic-treated group, had received acid suppression therapy prior to PEG insertion ($P = 0.001$). The results show that antibiotics did not affect the rates of future infections in pre-PEG tube patients (Table 2) and that the duration of hospital stay was not significantly affected by antibiotic treatment. Ten of the 23 patients survived until the medical notes were examined between 6 months and 2 years later. Two patients died within 30 days of PEG insertion, one died within 3 months, and nine died within 6 months (Table 2). Patients who did not receive antibiotics had a significantly greater 6-month mortality rate than those individuals who did (60% and 33%, respectively; $P = 0.005$).

Microbiological analysis of EN patients. The microbiotas of the pre-PEG tube patients' gastric and duodenal aspirates ($n = 20$) and the compositions of PEG tube surface-attached communities ($n = 10$) were investigated. Aspirate pH and the antibiotic susceptibilities of the isolates were also determined. The mean pH of the gastric aspirates was 3.4 ± 2.0, while the duodenal aspirates were less acidic (5.7 ± 2.0), although this difference was not significant ($P = 0.205$). Three and two of 20 gastric and duodenal aspirates, respectively, were culture negative. In general, however, aspirates contained significant numbers of microorganisms (Fig. 1); the mean total counts were 4.5 ± 2.3 and 4.8 ± 2.2 $\log_{10}$ CFU ml^{-1} for the gastric and duodenal aspirates, respectively. Yeasts and facultative anaerobes, particularly lactic acid bacteria such as lactobacilli and streptococci, together with enterobacteria, were the most commonly isolated microorganisms. The microbiological profiles of the gastric and duodenal aspirates were similar with respect to cell numbers and generic distribution. Two exceptions were *Klebsiella* spp. and *Propionibacterium* spp., which were detected only in the duodenal and gastric aspirates, respectively (Fig. 1). The profiles of the microorganisms isolated from PEG tubes were similar to those isolated from the aspirates (Fig. 2). All tubes were culture positive (mean total count, 6.3 ± 1.7 $\log_{10}$ CFU/cm^2).

Effects of pH and antimicrobial therapy. pH had a marked effect on the microfloras of the gastric and duodenal aspirates of pre-PEG tube patients (Table 3). *Candida* spp., *Streptococcus* spp., and *Lactobacillus* spp. were found at all pH values in relatively constant numbers. In contrast, *E. coli* was present at lower cell densities and in fewer people at lower pH values (pH 0 to 2). Staphylococci, bifidobacteria, and klebsiellas were detected only in aspirates with a pH greater than 3.

Antimicrobial therapy also had a marked effect on the microbiotas of the gastric and duodenal aspirates of the pre-PEG tube patients (Table 4); and the mean total counts were higher in aspirates from patients receiving antibiotics than in those from patients not receiving antibiotics (5.3 ± 2.0 and 3.4 ± 1.8 $\log_{10}$ CFU ml^{-1}, respectively), although this difference was not significant ($P = 0.244$). Candidas, staphylococci, and *E. coli* were isolated only in aspirates from patients who had received antibiotics. These microorganisms were detected in 6 (27%), 9 (41%), and 8 (36%) of 22 aspirates from these patients, respectively. Lactobacilli were isolated at a significantly higher frequency from patients who had received antibiotics than from patients who had not received antibiotics (36% and 17%, respectively; $P = 0.009$). In contrast, species belonging to the genera *Bifidobacterium* and *Streptococcus* were isolated at significantly higher frequencies in aspirates from patients who had not received antibiotics (67% and 32%, respectively; $P = 0.001$) than from patients who had received antibiotics (50% and 14%, respectively; $P = 0.001$).

Analysis of fermentation products. Lactate was the major fermentation product in the aspirates, followed by acetate, succinate, and propionate (Table 5). Propionate was detected only in duodenal aspirates. Other than propionate, no significant differences between gastric and duodenal samples were found.

Antibiotic susceptibilities. The antimicrobial sensitivities of isolates from the genera *Staphylococcus*, *Escherichia*, *Lactobacillus*, *Klebsiella*, *Streptococcus*, and *Enterococcus* to penicillin G, ciprofloxacin, vancomycin, tetracycline, metronidazole, doxycycline, amoxicillin-clavulanic acid, erythromycin, sulfamethoxazole, cefuroxime, and neomycin are shown in Fig. 3. The majority of staphylococci were insensitive to ciprofloxacin,

TABLE 5. Microbial fermentation products detected in gastric and duodenal aspirates

Aspirate	Fermentation product concn (mM)[a]							
	Lactate		Succinate		Acetate		Propionate	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Gastric	0.0–3.6	0.7 ± 0.3	0.0–2.4	0.2 ± 0.2	0.0–3.6	0.2 ± 0.2	0.0–0.0	0.0 ± 0.0
Duodenal	0.0–2.5	0.5 ± 0.2	0.0–4.7	0.3 ± 0.3	0.0–3.3	0.4 ± 0.2	0.0–0.7	0.1 ± 0.1

[a] Data are expressed as the range and the mean ± standard error of the mean ($n = 20$).

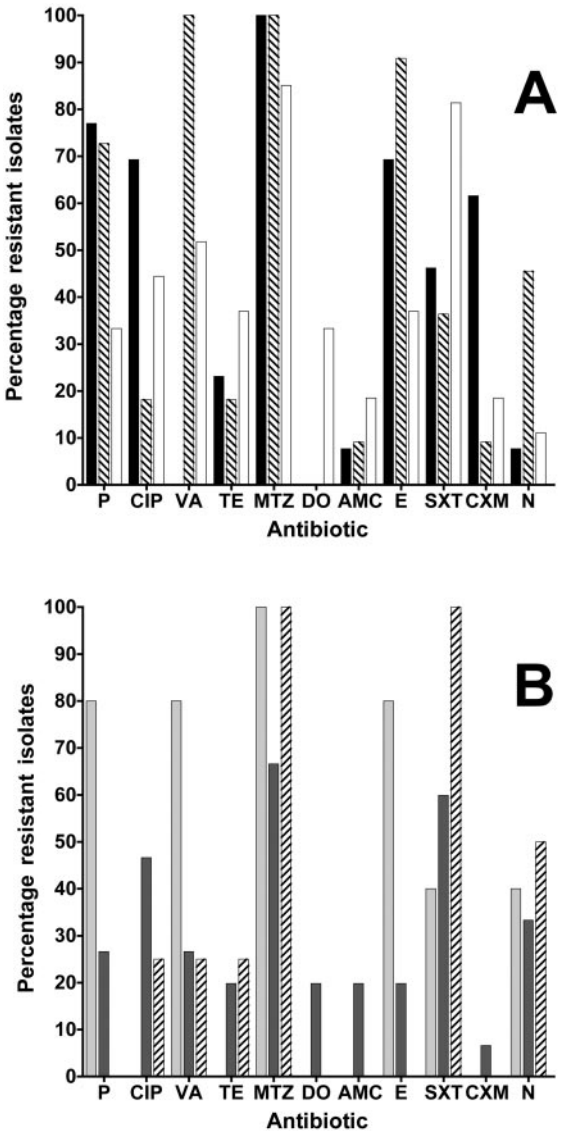

FIG. 3. Percentage of microorganisms resistant to penicillin G (P), ciprofloxacin (CIP), vancomycin (VA), tetracycline (TE), metronidazole (MTZ), doxycycline (DO), amoxicillin-clavulanic acid (AMC), erythromycin (E), sulfamethoxazole-trimethoprim (SXT), cefuroxime (CXM), and neomycin (N). (A) Dark bars, *Staphylococcus*; slashed bars, *Escherichia*; white bars, *Lactobacillus*; (B) light grey bars, *Klebsiella*; slashed bars, *Streptococcus*; dark grey bars, *Enterococcus*.

while the reverse was true for amoxicillin-clavulanic acid. Most *E. coli* isolates were susceptible to ciprofloxacin and amoxicillin-clavulanic acid, while all staphylococci and three of four enterococcal isolates were vancomycin sensitive (Fig. 3).

DISCUSSION

EN bypasses innate defense mechanisms in the upper GI tract, the result of which is microbial overgrowth. This study therefore aimed to characterize the nature and the extent of colonization in the upper gut in patients with PEG tubes to examine the effects of antibiotic usage and GI pH and to investigate the composition of PEG tube biofilms.

In health, the stomach generally contains small numbers of autochthonous microorganisms (15). In contrast, the results from this study show that pre-PEG tube patients who have previously undergone EN via an NG tube possess abnormal gastric microbiotas that mainly comprise yeasts and facultatively anaerobic bacteria, predominantly streptococci, staphylococci, lactobacilli, and enterobacteria. The microbiological profiles of gastric aspirates broadly agreed with those from previous studies in which the gastric pH was raised during chemotherapy (17, 20, 34). Duodenal aspirates in these individuals contained similar numbers of microorganisms. Total microbial numbers and some microbial species were similar to those in the normal duodenum, such as streptococci, bifidobacteria, and lactobacilli (2). However, other species (*E. coli*, candidas) were also detected (Fig. 1), showing that prior to PEG tube placement these patients harbored abnormal upper GI microbiotas, probably resulting from EN. Moreover, similar types and numbers of organisms were isolated from both kinds of aspirates, with the exception of klebsiellas, which occurred only in the duodenum, and propionibacteria, which were detected only in the stomach. It therefore seems that EN results in a loss of physiologic distinction between the stomach and duodenum, at least in terms of their microbiotas.

The effect of pH on the gastric and duodenal microbiotas of pre-PEG tube patients was also assessed in this investigation (Table 3). Aspirates were divided into three groups: (i) those with a pH of 0 to 2, (ii) those with a pH of 3 to 5, and (iii) those with a pH of 6 and over. The total counts in these groups were similar, suggesting that a low pH in the stomach does not a priori prevent microbial overgrowth. This stands in contradiction to the paradigm that microbial overgrowth will not occur at a pH of less than 4 (10).

The microorganisms isolated in this study were predominantly yeasts (particularly *Candida* spp.), gram-positive facultative anaerobes, and enterobacteria. More acid-tolerant microorganisms were found to be unaffected by low pH, in terms of either their incidence or their numbers. In contrast, those organisms known to be acid sensitive were not isolated from aspirates at low pH. For example, bifidobacteria are reported to grow at pH 4 (8), but acidity greater than this is rapidly lethal (14, 25), while *Klebsiella pneumoniae* is rapidly killed by a pH of less than 3 (27). These data suggest, therefore, that although low pH does not affect the overall cell numbers in gastric and duodenal aspirates, it does affect the species composition. This is important, particularly in immunocompromised individuals, because acid suppression therapy is commonly used in EN patients. These data suggest that use of acid suppression can result in a change in the resident microbiota in EN patients with an increase in potential pathogens such as *Staphylococcus* spp. or *Candida albicans*.

The species in the PEG tube biofilms (Fig. 2) were generally similar to the planktonic organisms isolated from aspirates. The extent of colonization of PEG tubes was in the region of 6.3 $\log_{10}$ CFU/cm^2. Enterococci were isolated from PEG tubes but not from aspirates, while the opposite was true for bifidobacteria. Enterococci are potentially pathogenic and form biofilms (21, 33). Their presence on PEG tubes may be a problem for EN patients, although no evidence of vancomycin resistance was detected in these bacteria (Fig. 3B). This may be important because (i) high levels of vancomycin resistance

occur in these organisms (9) and (ii) vancomycin-resistant enterococci cause problems in hospitals, both as pathogens (37) and as vectors for the spread of vancomycin resistance to staphylococci (6, 39). However, none of the staphylococci isolated from PEG tube patients were vancomycin resistant (Fig. 3A).

Cerebrovascular accident patients with dysphagia are at risk of aspiration pneumonia. Similarly, many EN patients suffer urinary tract infections secondary to catheterization (36). Those patients who had received antibiotic treatment stayed in hospital for an average of 59.2 ± 59.5 days, whereas untreated patients stayed in hospital for an average of 35.6 ± 38.9 days, although this difference was not significant (Table 2). Additionally, it is possible that the clinical conditions of the patients receiving antibiotic treatment were worse than those of individuals who were not treated. This is supported by the fact that the mortality rate in non-antibiotic-treated patients was significantly higher from 3 to 6 months and that a higher proportion of these individuals were suffering oropharyngeal carcinoma (Table 1). Individuals receiving antibiotic therapy had been undergoing EN for a significantly longer period than those not receiving antibiotics (15.6 ± 11.0 and 4.2 ± 4.0 days, respectively, $P = 0.013$) (Table 1).

When the effects of antibiotics on the gastric and duodenal microbiotas of pre-PEG tube patients were investigated, total microbial counts were higher, although not significantly so, in the antibiotic-treated group (5.3 ± 2.0 and 3.4 ± 1.8 $\log_{10}$ CFU ml^{-1}, respectively; $P = 0.625$) (Table 4). *Candida* spp., *E. coli*, and *Staphylococcus* spp. were detected only in antibiotic-treated patients. This may have been caused either by the antibiotics themselves or by the fact that the antibiotic-treated patients had been undergoing EN for longer durations (Table 1). The duration of EN may, therefore, determine the composition of upper GI tract microbiotas in these patients, an effect that could be exacerbated by antibiotic usage. Further work is needed to be sure whether EN duration, antibiotic administration, the apparently worse clinical condition of patients receiving antibiotic therapy, or a combination of one or more of these factors is responsible for the altered microflora.

Amoxicillin-clavulanic acid (15 of 23 patients) and ciprofloxacin (4 of 23 patients) were the most commonly used antibiotics in this patient group. Most *E. coli* isolates were sensitive to both amoxicillin-clavulanic acid (91%) and ciprofloxacin (81.2%), while staphylococci were usually amoxicillin-clavulanic acid sensitive and were predominantly insensitive to ciprofloxacin (Fig. 3). However, these bacteria were detected only in patients who had received antibiotics (Table 4). Therefore, administration of antibiotics to which these putatively pathogenic organisms were sensitive did not result in their elimination from the upper gut. A possible explanation for this finding is that the antibiotic-induced changes in microbial communities in the stomach and duodenum provided new protected niches for staphylococci and *E. coli*. Similar phenomena have been described in microbial biofilms (16).

Low concentrations of lactate, succinate, and acetate were detected in aspirates (Table 5). The prevalence of lactic acid is unsurprising, given the predominance of lactic acid bacteria in the upper gut (Fig. 1); but acetate and, to a lesser degree, propionate are known to have immunomodulatory properties, such as leukocyte activation via modulation of intracellular calcium levels (22, 28). Therefore, it is possible that their presence in the stomach, where they are not normally found, may affect local host immune system reactivity. Additionally, the levels of fermentation products present in the stomach and duodenum were almost identical, again raising the possibility that in EN patients the stomach and duodenum can no longer be viewed as microbiologically distinct compartments.

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