Thorax 1982;**37**:181-184

Microbial flora in carcinoma of oesophagus

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ABSTRACT The microbial flora associated with carcinoma of the oesophagus was studied in 12 patients. Oesophageal mucosa was biopsied at thoracotomy and cultured under both aerobic and anaerobic conditions. A heavy mixed growth of aerobic and anaerobic organisms was obtained in all patients. One-third of isolates were anaerobic. The flora was of oral origin. The most appropriate antibiotic combination in this study was ampicillin or penicillin with gentamicin and metronidazole.

Oesophageal resection for carcinoma is associated with a high mortality.[1] At least 50% of the deaths are attributable to anastomotic leakage and associated infection.[2] Despite the importance of sepsis in this situation, the microbial flora associated with carcinoma of oesophagus has not been previously investigated.

The aim of the present study, therefore, was to identify the flora associated with malignant strictures of the oesophagus and to establish the corresponding antibiotic sensitivities.

Patients and methods

Twelve consecutive patients undergoing oesophageal resection for carcinoma were included in the study. All patients had dysphagia (mean duration three months) and evidence of weight loss (mean 16 kg) before surgery. Seven patients had an adenocarcinoma. Five patients with squamous carcinomas received preoperative radiotherapy. A two-part "Ivor Lewis" oesophagectomy was performed on all patients with a gastro-oesophageal anastomosis fashioned in the thorax.

At thoracotomy, specimens of oesophageal mucosa were obtained from the proximal resection line after excision of the tumour. This precluded the possibility of contamination of specimens by oral secretions. These samples were immediately transported to the bacteriology laboratory in sterile containers and also in Robertson's cooked meat medium (RCM).

Agar plates were inoculated with portions of intact and ground mucosa. The media used were (1) 5% horseblood agar, (2) "chocolate" blood agar, (3) cysteine-lactose electrolyte deficient agar, (4) 5% horseblood agar containing 6 mg/l gentamicin and supplemented with yeast extract, cysteine hydrochloride, haemin and menadione, (5) McConkey agar. Plates containing media (1), (2), (3), and (5) were incubated at 37°C in CO_2 for a minimum of three days. Plates containing media (1) and (4) were incubated anaerobically (BBL GasPak H_2 + CO_2) at 37°C for a minimum of five days.

Portions of mucosa were also added to RCM and incubated at 37°C for five days. These were then subcultured to the above agar media and incubated anaerobically and also in CO_2 for a further five days. Agar media used for anaerobic incubation were prereduced in an anaerobic jar.

After incubation the plates were carefully examined and representative examples of each colonial type were selected for further study. Organisms were identified by the application of the following standard techniques: growth in air; growth in air + 5% CO_2 or in an anaerobic environment; colonial morphology; microscopic morphology; Gram reaction; haemolysis; catalase test; coagulase test; motility. Enterobacteriaceae were identified using the API 20E test kit. Strictly anaerobic gram-negative bacilli were regarded as bacteroides. *Bacteroides fragilis* was further identified by antibiogram and *Bacteroides melaninogenicus* by the black or grey colour of the colonies; other strains were called bacteroides species.

Antibiotic sensitivity testing was performed on each isolate by the disc diffusion technique using Isosensitest agar (Oxoid) (supplemented with 5% chocolated horseblood, haemin and menadione when necessary). *Escherichia coli* NCTC 10418 and *Staphylococcus aureus* NCTC 6571 were used as control organisms.

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Results

Eighty-five organisms were isolated from the 12 patients in the study. Fifty-seven (67%) organisms were aerobes and 28 (33%) were obligate anaerobes. Both aerobic and anaerobic organisms were isolated from all patients. The mean number of isolates per patient was seven (range 5-10).

The distribution of aerobic and anaerobic organisms isolated in each patient is shown in table 1. Alpha and non-haemolytic streptococci comprised 49% of the total aerobic growth. Coagulase negative staphylococci, lactobacilli, and corynebacterium species were frequent isolates. Coliforms were isolated in four patients.

Anaerobic Gram-negative bacilli were isolated from 11 patients. Of these, five were identified as *Bacteroides melaninogenicus* and two as *Bacteroides fragilis*. Anaerobic cocci were isolated from six patients.

The corresponding antibiotic sensitivities are shown in table 2. Ninety-one per cent of all strains were sensitive to ampicillin. Only 34% of aerobic strains were sensitive to gentamicin. All obligate anaerobes were sensitive to metronidazole.

Discussion

The microbial flora of the oesophagus has attracted little attention. When considering the normal body flora, authors have omitted to mention the organ or merely comment on the transient nature of the flora

Table 1 *Distribution of isolates in 12 patients*

Organisms (+ = *strain of organism*)	*Patients*												
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*Total strains isolated*
Aerobes													
α-Haemolytic Streptococcus	+	+	+ +		+ + +	+	+ +		+	+	+	+	14
Non-Haemolytic Streptococcus		+ + +	+		+	+ + +	+	+	+	+	+	+	14
Coagulase − ve Staphylococcus	+ +	+					+		+	+	+		7
Lactobacillus Sp	+	+		+		+	+		+		+		7
Corynebacterium Sp				+	+					+		+	4
Neisseria catarrhalis								+		+			2
E Coli				+			+			+			3
Proteus mirabilis	+												1
Branching Gram +ve Rod							+						1
Anaerobes													
Bacteroides melaninogenicus					+				+	+	+	+	5
Bacteroides fragilis	+			+									2
Other Bacteroides Sp		+			+	+	+	+	+				6
Anaerobic cocci		+	+			+ +		+			+	+	7
Clostridium Sp			+	+	+					+	+		5
Other Anaerobes		+				+			+				3
Yeasts	+	+	+					+					4
Total strains per patient	7	10	6	5	8	9	8	5	7	8	7	5	

Table 2 *Antibiotic sensitivity of species (yeast excluded)*

Aerobic organisms	*Number of strains*	*Antibiotic (% strain sensitive)*						
		Penicillin	*Ampicillin*	*Tetracycline*	*Erythromycin*	*Clindamycin*	*Gentamicin*	*Metronidazole*
α-Haem Streptococci	14	100	100	78	100	100	7	—
Non-Haem Streptococci	14	93	100	71	100	93	0	—
Staphylococci Sp	7	43	43	100	100	100	86	—
Lactobacilli Sp	7	100	100	71	100	83	29	—
Corynebacterium Sp	4	100	100	75	75	50	75	—
Coliforms	4	0	75	50	0	0	100	—
Others	3	100	100	100	100	100	67	—
Aerobic sensitivity total	53	83	91	77	90	83	34	—
Anaerobes								
Bacteroides Sp	13	85	85	85	85	100	—	100
Anaerobic Cocci	7	100	100	57	100	100	—	100
Others	8	100	100	75	63	63	—	100
Anaerobic sensitivity total	28	93	93	75	81	83	—	100
Combined aerobic + anaerobic sensitivity	81	86	91	77	83	83	22	35

which reflects the episodic passage of swallowed material.[3 4] This study has demonstrated that the dilated oesophagus proximal to a malignant stricture harbours an extensive aerobic and anaerobic flora. Although the number of patients sampled is small, the flora demonstrated in each case is similar and therefore we doubt whether inclusion of further patients would alter the results significantly.

The anaerobes were isolated by carefully employed standard bacteriological techniques. It may be that improved anaerobic laboratory facilities might increase the yield of fastidious strains. Although no formal quantitative analysis was performed, direct plating of material yielded a profuse growth of most organisms.

The spectrum of organisms was similar to that found in the oral cavity suggesting that the flora is primarily of oral origin.[5] There was no possibility of contamination of specimens by oral secretions or by a nasogastric tube since all the samples were obtained at thoracotomy. It would appear that the proximal oesophagus acts as a reservoir for oral secretions in these patients. The presence of small numbers of *E coli*, *Proteus mirabilis*, and *Bacteroides fragilis* does not preclude the probability of an oral origin since these strains may be isolated from the saliva of both hospitalised patients and those receiving antibiotic therapy.[6 7]

The pathogenicity and stability of this flora remains speculative. The contribution, however, from swallowed saliva to the ecology of this particular microhabitat must be regular and presumably significant. Furthermore specimens were obtained from the proximal oesophageal resection edge, which forms part of the gastro-oesophageal anastomosis and hence these organisms may be available for implantation into the thorax in the event of anastomotic leakage. Pathogenicity of individual members of a flora, when considered in isolation, may not be impressive (lactobacilli, coagulase negative staphylococci, corynebacterium species), but the infectivity of a mixed inocula often exceeds the sum of the contributions of the individual components of the inocula.

It is notable that most of these organisms have been previously isolated as pathogens in thoracic empyemas. The anaerobes may be of particular importance in this respect. Bartlett *et al*,[8] in a review of 83 cases of empyema, recovered anaerobic bacteria in 76%. Bacteroides melaninogenicus, previously considered to be an oral commensal, was the second most frequently isolated anaerobic pathogen. Furthermore the spectrum of isolates in Bartlett's study correlates very closely with the flora described above. The reduction in apparently "sterile" empyemas since the introduction of adequate anaerobic culture techniques provides further evidence for the role of anaerobes in thoracic sepsis.[9]

In the present study most aerobic organisms were sensitive to ampicillin. Although gentamicin was effective against only one-third of aerobes, it was highly effective against the small number of ampicillin-resistant coliforms. Metronidazole was effective against all anaerobes. The presence of penicillin-resistant *Bacteroides fragilis* and the emergence of penicillin-resistant *Bacteroides melaninogenicus*,[10] underlines the necessity to include metronidazole in any therapeutic regime directed against the anaerobic component of sepsis associated with oesophageal surgery.

The role of antibiotic prophylaxis in oesophageal surgery has not been clearly defined. In the present study, the most appropriate antibiotic combination for prophylaxis would appear to be ampicillin or penicillin with gentamicin and metronidazole. It is of interest to note that Little *et al* have demonstrated that prophylactic cephamandole in patients undergoing oesophageal resection for carcinoma significantly reduced the incidence of wound infection.[11]

In the management of an established oesophageal anastomotic leak, surgical elimination of the septic focus, as advocated by Belsey, is of primary importance.[12] In the absence of specific bacteriological information and until further data regarding the relative pathogenicity of individual components of the flora are available, we would recommend the use of the same combination of ampicillin or penicillin with gentamicin and metronidazole in anastomotic leakage.

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