Medical Microbiology

Volume 1

edited by

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Preface

Medical microbiology has developed rapidly over the past decade. Immunological and biochemical techniques have been applied to the early diagnosis of infectious disease and to monitoring antimicrobial therapy. The importance of non-sporing anaerobes in causing infection has been recognized. There has been renewed interest in the beta-lactam antibiotics in which the basic penicillin and cephalosporin nucleus has been manipulated to increase both their spectrum of activity and resistance to beta lactamases. Effective antiviral chemotherapy now seems likely to become a reality. The theoretical background provided by cellular immunology is now being applied to the development of new improved vaccines. Medical microbiologists have, of course, not had things all their own way. Hospital-acquired infection, particularly in the immunocompromised patient, is an increasing problem and is often the limiting factor in the management of other diseases. Allied to this is the increase in antibiotic resistance not only in the hospital flora but in organisms such as *Neisseria gonorrhoeae* and *Haemophilus influenzae*. New infectious agents such as *Legionella pneumophila* and Lassa, Marburg and Ebola viruses have been described.

It is our aim in this open-ended series to include major review articles, not only by established authorities, but also by younger active research workers, which will reflect this diversity and be of interest to medical microbiologists and their veterinary colleagues. We plan two types of volume: the first consisting of subjects chosen for their topicality and general interest with no particular theme, and the second of a series of articles related to a common theme.

Volume 1 is of the first type. The dangers of colitis associated with particular antibiotics and the importance of *Clostridium difficile* and its toxin in this condition are discussed by Drs Bartlett and Taylor. Although its role in the pathogenesis of antibiotic-associated colitis is a recent finding, *C. difficile* is not a newly discovered organism. *Legionella pneumophila* and the bacterium causing contagious equine metritis are newly described bacterial pathogens. Their discovery, properties and the diseases they cause are discussed by Drs Reingold and Band and Platt and Taylor respectively. In contrast the problem of urinary tract infections, covered by Dr Gleckman, is one of the oldest and commonest in microbiology, but nevertheless still presents many difficulties. We have included two chapters on *Pseudomonas aeruginosa*. Drs Pavlokis and

Wretlind deal with extracellular toxins and their role in pathogenicity, Dr Homma with the exploitation of pseudomonal products as vaccine components. Four other chapters have an immunological flavour. Dr Finlayson covers the use of immunoglobulins as therapeutic agent. Drs Kohler, Wheat and White their use as diagnostic reagents. The type-specific antigens of group B streptococci, prime candidates for a vaccine, are discussed by Drs Kasper and Jennings. A rather different strategy, that of non-specific immunostimulation with propionibacteria (better known as *Corynebacterium parvum*), is covered by Dr Roszkowski and his colleagues. Finally, Dr Kato describes the use of staphylococcal alpha toxin as a biological probe for membrane studies.

We hope that this volume will be of interest to medical microbiologists. With the steady expansion of this discipline review articles are needed and there are very few review publications that deal exclusively with medical microbiology.

We should like to thank the authors for their contributions to this volume and we would appreciate any comments and suggestions for future volumes.

Janusz Jeljaszewicz *Charles Easmon*

August 1982

Contents

1 Antibiotic-associated colitis

JOHN G. BARTLETT and NANCY S. TAYLOR

Colitis is one of the most frequent and potentially severe adverse reactions associated with antimicrobial drug usage. A spectrum of pathological changes have been noted, the most characteristic lesion being pseudomembranous colitis. This lesion was described long before antimicrobial drugs were available and a variety of risk factors were noted at that time, but the vast majority of cases encountered currently are antibiotic associated. There has been marked progress during the past decade in our understanding of this complication. Initial studies made extensive use of endoscopy to provide extensive descriptions of pathology and the clinical features of the disease. In more recent years, *Clostridium difficile* has become the recognized pathogen in the vast majority of cases. The role of this microbe was initially detected in experiments utilizing an animal model of antibiotic-associated colitis in 1977. Clinical applications of these findings quickly followed, and by 1978 the culprit was defined, a sensitive diagnostic assay was described, and specific forms of treatment became readily available. There are few diseases in medicine in which this much progress was made over such short interval. There are also few medical conditions in which the lessons learned from animal experiments proved so directly applicable to the clinical setting. This chapter will review the topic of antibiotic-associated colitis with emphasis on these more recent developments.

I. HISTORICAL PERSPECTIVE

Studies of pseudomembranous lesions of the bowel may be divided into three periods (Table 1). The initial reports antedated the antibiotic era with the

Table 1 Observations with pseudomembranous enterocolitis during three study periods

		Antibiotic era	
	Preantibiotic era	1952–1965	1970–1980
Major risk factor	Intestinal surgery Others (see text)	Antibiotics Chloramphenicol Tetracycline	Antibiotics Ampicillin Clindamycin Cephalosporins
Anatomy			
Location of lesions	Small bowel and/or colon	Small bowel and/or colon	Primarily colon
Histology	←	Similar or identical	→
Suspected cause	Ischaemia(?)	*S. aureus*	*C. difficile*
Diagnosis			
Anatomical	Autopsy	Clinical features (often poorly established)	Endoscopy
Agent	—	Stool Gram stain and culture	Stool toxin assay
Treatment	Supportive only	Oral vancomycin	Oral vancomycin Cholestyramine

original description by Finney (1893) who noted "pseudodiphtheritic enteritis" in a patient who had undergone a gastro-enterostomy. Numerous reports followed in which a variety of risk factors were identified, primarily intestinal surgery complicated by hypotension, but also spinal fracture, intestinal obstruction, colonic carcinoma, uraemia, heavy metal poisoning, the haemolytic-uraemic syndrome and ischaemic cardiovascular disease (Bartlett and Gorbach, 1977). Many of these associated conditions suggested ischaemia of the bowel as an aetiological factor, although this was never proven. The disease at this time was relatively infrequent, but the mortality rate was high, possibly reflecting the fact that most cases were established only at autopsy examination (Penner and Bernheim, 1939; Pettet *et al.*, 1954).

The second period of study followed shortly after the availability of antimicrobials when the terms "antimicrobial-induced pseudomembranous enterocolitis", "post-operative enterocolitis" and "staphylococcal enteritis" were often used interchangeably. *Staphylococcus aureus* was the commonly accepted pathogen on the basis of Gram stains of stool showing clusters of Gram-positive cocci and stool cultures which yielded this microbe (Altemeier *et al.*, 1963; Azar and Drapanas, 1968; Hummel *et al.*, 1964; Wakefield and Sommers, 1953; Prohaska *et al.*, 1956). The majority of stool isolates produced an enterotoxin (Surgalla and Dacti, 1955) and the majority of typable strains were phage type 80/81, 53/77 or U-18 (Hummel *et al.*, 1963; Dearing and Needham, 1960). Oral administration of either the enterotoxin

or *S. aureus* isolates combined with antibiotics produced lethal enterocolitis in chinchillas (Wood *et al.*, 1956; Prohaska *et al.*, 1959; Tan *et al.*, 1959; Warren *et al.*, 1963). Many patients were treated with oral vancomycin with good results to provide further support for the etiological role of *S. aureus* (Kahn and Hall, 1966). "Staphylococcal enterocolitis" became a common diagnosis, especially in post-operative patients receiving antibiotics where reports of incidence were as high as 14% in one study (Azar and Drapanas, 1968) and 30% in another (Hummel *et al.*, 1964).

Critical analysis of the reports noted above cast doubt on the frequency of the complication and some now question the aetiological role of *S. aureus*. Investigators even at that time noted that most of the patients with this diagnosis who died of other causes had no demonstrable intestinal lesions at autopsy (Dearing *et al.*, 1960) and those who did have anatomically confirmed disease often had no evidence of staphylococci in their stool (Pettet *et al.*, 1954; Valberg and Truelove, 1961). Furthermore, *S. aureus* is found in the normal faecal flora of 15–30% of healthy adults (Finegold *et al.*, 1974; Hummel *et al.*, 1964), and colonization rates may exceed 90% in patients receiving some antibiotics (Hummel *et al.*, 1964). It is possible that the attention focussed on this organism reflected the widespread concern for staphylococci which was responsible for widespread epidemics of infections at the time. Although it is not possible to confirm or refute the conclusions of these prior studies, it does appear that even if *S. aureus* was once responsible for antibiotic-associated pseudomembranous colitis (PMC), it no longer represents an important agent of the disease.

There was a lull in reporting of antibiotic-associated PMC in the 1960s. However, this was followed by a flurry of reports in the 1970s which often emphasized the role of clindamycin in this complication. Initial work primarily concerned incidence data and anatomical descriptions made possible by the extensive use of endoscopy (Slagle and Boggs, 1976; Stroeghlein *et al.*, 1974; Scott *et al.*, 1973; Totten *et al.*, 1978; Tedesco *et al.*, 1974; LeFrock *et al.*, 1975). A striking feature in this work was that *S. aureus* was infrequently recovered (Keusch and Present, 1976) although extensive microbiological studies of the faecal flora failed to elucidate any alternative agent (Marr *et al.*, 1975; Allen *et al.*, 1977). This experience led to studies designed to demonstrate a transferable toxin in an animal model which eventually revealed the role of *C. difficile*.

II. CLINICAL AND PATHOLOGICAL OBSERVATIONS

A. Pathology

1. Pseudomembranous colitis (PMC)

The usual finding with gross inspection is multiple elevated yellowish-white plaques which vary in size from a few millimetres to 15–20 mm in diameter (Fig. 1). The intervening mucosa may appear normal or show hyperaemia and oedema. Occasionally, the pseudomembranes coalesce to involve large segments of the colonic mucosa. These may slough, leaving large, denuded areas of the mucosa. According to recent descriptions, PMC usually involves the distal colon and often involves the entire colon, but there may be rectal sparing. Small-bowel involvement appears to be infrequent.

Histological studies (Goulson and McGovern, 1965; Sumner and Tedesco, 1975) show the pseudomembrane arises from a point of superficial ulceration on an intact mucosa. There is an acute or chronic inflammatory infiltrate in the lamina propria with the submucosa showing oedema and vascular dilatation. The pseudomembrane is composed of fibrin, mucin, sloughed mucosal epithelial cells, and inflammatory cells. Price and Davies (1977) have classified the histological features of PMC into three categories which appear to be rather uniform in an individual patient. The earliest or most mild form consists of focal necrosis with polymorphonuclear cells and an eosinophilic exudate within the lamina propria. Splaying out from the necrotic focus is a collection of fibrin and polymorphonuclear cells which form the characteristic "summit lesion". The second category, representing more advanced disease, shows disrupted glands containing mucin and polymorphonuclear cells surmounted with typical pseudomembranes. Both types of lesions show areas of intervening normal mucosa and the inflammatory changes are limited to the superficial portion of the lamina propria, predominantly subepithelial in location. The third and most advanced form of the disease shows complete structural necrosis with extensive involvement of the lamina propria, which is overlaid by a thick confluent pseudomembrane.

Crypt abscesses are not a feature of PMC. There is one report showing fibrin thrombi in the mucosal capillaries, suggesting bowel ischaemia in the pathogenesis (Bogomoletz, 1976). However, this has not been a consistent finding by most observers; furthermore, the clinical presentation and other features of the pathological findings do not suggest ischaemia. There is no bacterial invasion of the bowel mucosa, and no typical bacterial morphotype is seen within the pseudomembrane.

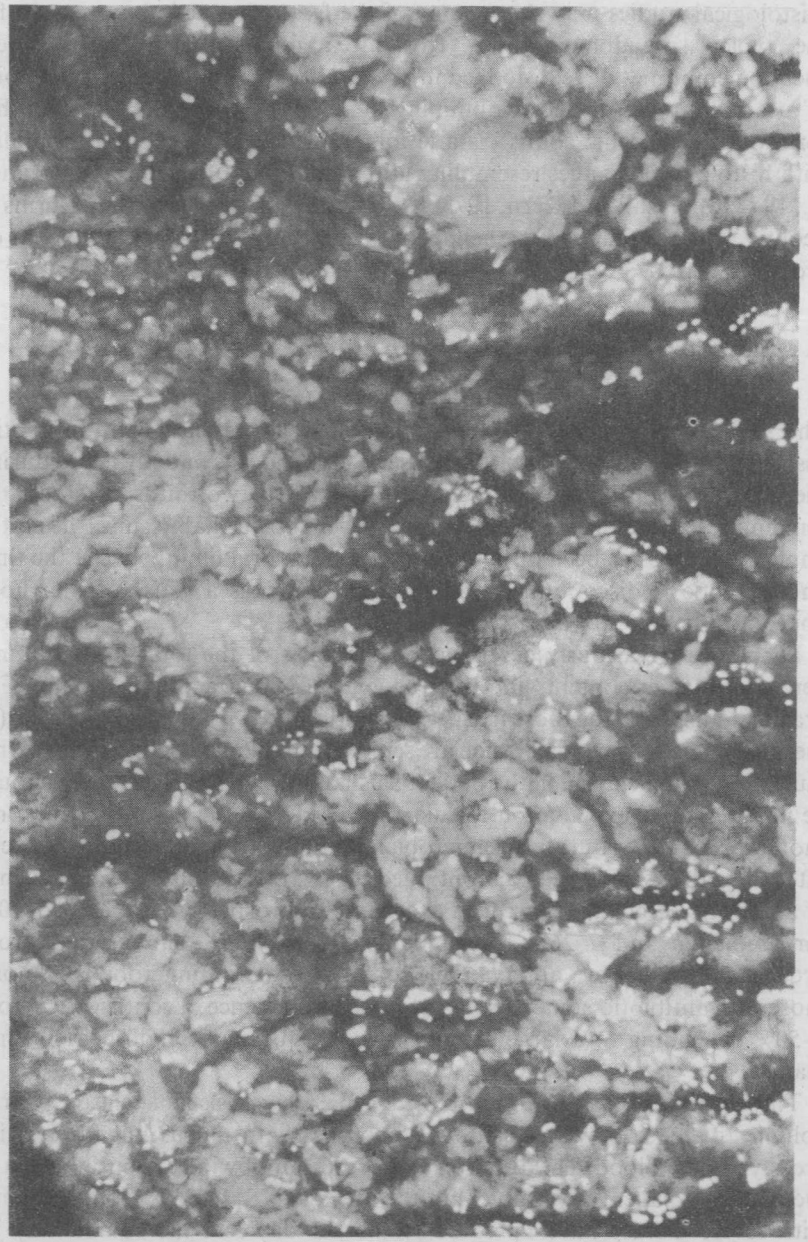

Fig. 1 Typical plaque lesions of pseudomembranous colitis.

2. *Colitis without pseudomembrane formation*

Histological studies may show many of the features noted above except for the typical pseudomembrane. This often represents situations where pseudomembranes were dislodged in preparation for endoscopy or the point of attachment was missed in obtaining the biopsy (Tedesco, 1976). Another form of "non-specific colitis" is a lesion showing granularity and friability with histological changes resembling idiopathic ulcerative colitis (Pittman *et al.*, 1974; Manashil and Kern, 1973; Koltz *et al.*, 1953). In less severe forms of "colitis" there is simply hyperaemia and oedema of the intestinal mucosa on gross inspection.

B. Signs and symptoms

1. *Antibiotic-associated diarrhoea*

The single symptom which is found in nearly all patients with antibiotic-associated colitis, is diarrhoea. The onset of diarrhoea is initially noted during the course of antibiotic treatment in one-half to two-thirds of cases; the remaining patients never detect a change in bowel habits until after the implicated drug has been discontinued. The temporal limit between the time an antibiotic is discontinued and its implication as a cause of diarrhoea appears to be 4–6 weeks.

Diarrhoea is variously described on the basis of the total stool volume, percentage water content, and the frequency and character of stooling. For practical purposes, the most frequent definition used in clinical studies is: (1) there are 2–5 stools per day which are semi-solid or liquid in character; (2) this must represent a change in the patient's usual bowel pattern; (3) there should be no alternative explanation for diarrhoea; and (4) the onset of symptoms should occur either during antimicrobial administration or within 4–6 weeks after these drugs have been discontinued. Using this definition, the incidence of antibiotic-associated diarrhoea according to prospective studies is 5–10% for ampicillin and 7–26% for clindamycin (Table 2). Similar data from prospective studies are not available for most other antimicrobials. Among those with antibiotic-associated diarrhoea, the incidence of colitis varies from 5–50% depending to a large extent on the frequency of endoscopic examination.

The diarrhoea ascribed to antibiotics generally consists of relatively large volumes of loose or watery stools, sometimes with mucus but rarely with grossly evident blood. The duration of diarrhoea following discontinuation of the implicated agent is variable, but the average is 8–12 days. Some patients with severe disease have up to 30 stools per day and the course may be protracted to 4 weeks or longer. Other patients have less severe symptoms which resolve rapidly. With clindamycin, which has been the most extensively

Table 2 Incidence of antibiotic-associated diarrhoea

	Cases	No. with diarrhoea	Percentage
Clindamycin			
Swartzberg *et al.* (1976)	1000	66	6.6
Neu *et al.* (1977)	200	27	13
Tedesco *et al.* (1974)	200	42	21
Gurwith *et al.* (1977)	343	61	18
Lusk *et al.* (1977)	62	16	26
Brause *et al.* (1980)	143	10	7
Leigh *et al.* (1980)	281	33	12
Ampicillin			
Tedesco *et al.* (1975)	200	9	4.5
Gurwith *et al.* (1977)	140	9	6
Lusk *et al.* (1977)	96	4	9
Brause *et al.* (1980)	318	16	5

studied, there may be two patterns with considerable overlap. One pattern is watery stools without colitis which occurs during antimicrobial administration, resolves promptly when the drug is discontinued, and may be dose related. A possible mechanism is a direct effect of the drug to cause altered intestinal water and electrolyte transport (Giannella *et al.*, 1981). The second pattern is diarrhoea, often with colitis, which is not dose related, is more likely to start after the drug has been discontinued, and often follows a protracted course. A major mechanism for this latter form is the toxin produced by *C. difficile* to be described below.

2. *Systemic symptoms*

Some patients with antibiotic-associated colitis have few symptoms other than diarrhoea. However, many individuals will experience abdominal cramps, abdominal tenderness, fever, and leucocytosis (Tedesco *et al.*, 1974; Mogg *et al.*, 1979). Fever is usually low grade, but may be as high as 106°F. Peripheral leucocyte counts are variable, often range from 10 000–20 000 mm^{-3} and may be 40 000 mm^{-3} or greater. Late and serious complications include severe dehydration, electrolyte imbalance, hypotension, hypo-albuminaemia with anasarca or toxic megacolon. Extra-intestinal symptoms appear to be extremely rare with antibiotic-associated colitis except for the complications which may be ascribed to fluid, electrolyte and albumin losses. However, there is an interesting case report of polyarthritis involving the shoulders, elbows, knees and ankles in a patient with clindamycin-associated colitis (Rollins and Moeller, 1975).

3. Prognosis

The prognosis for antibiotic-associated PMC without specific therapy is highly variable, depending to a large extent on the methods used to establish the diagnosis. In the report by Tedesco *et al.* (1974) where there was extensive use of endoscopy to detect this diagnosis even among patients with trivial symptoms, all patients with antibiotic-associated PMC recovered with simply supportive care. Nevertheless, many of these individuals suffered prolonged bouts of diarrhoea which often required hispitalization for extended periods. Other studies which focus attention on more severely ill patients indicate mortality rates as high as 20% (Mogg *et al.*, 1979). Mortality at the present time even for seriously ill patients is virtually nil reflecting the recognition of a microbial pathogen and the availability of specific forms of treatment to be discussed below.

C. Diagnosis

1. Endoscopy

The diagnosis of antibiotic-associated colitis should be suspected in any patient who has otherwise unexplained diarrhoea which occurs either during or up to 4–6 weeks following antibiotic administration. The favoured method for establishing the pathological changes noted in the colon is with endoscopy to detect typical mucosal plaque-like lesions (Sumner and Tedesco, 1975). There may be copious amounts of mucus, which must be removed with caution to avoid separation of the stalk attachment. Analogous precautions are necessary in the colon preparation prior to the procedure for the same reasons. Care must also be exercised to include the entire lesion in a biopsy, since the stalk attachment is necessary for microscopic confirmation. The distal colon is involved in the majority of cases so that sigmoidoscopy is generally adequate. However, occasional patients will have pseudomembranes restricted to the right colon necessitating the use of colonoscopy (Tedesco, 1980; Burbige and Radigan, 1981). Endoscopic observations in patients with antibiotic-associated diarrhoea without pseudomembranes include a normal mucosa, erythema and oedema, and friability, ulceration or haemorrhage. These latter findings may be very suggestive of idiopathic ulcerative colitis.

2. Radiology

Radiological findings may be helpful in establishing the diagnosis of PMC (Stanley *et al.*, 1974; Tully and Feinberg, 1974). Plain films in advanced disease often show a markedly oedematous colon, distorted haustral markings and distension of the entire colon. Occasionally, there are small irregularities which represent pseudomembranous plaques in profile. Barium

enema may show rounded filling defects which outline the pseudomembranous plaques. However, this examination is often non-diagnostic due to underpenetration of barium, excessive mucous secretions, confluence of the pseudomembrane or minimal involvement. Diagnostic accuracy is improved with air contrast studies, but this procedure must be performed with caution because of the potential complication of colonic perforation.

D. Antibiotics implicated

1. *Antibiotic-associated diarrhoea*

Nearly all antimicrobials with an antibacterial spectrum have been implicated in both diarrhoea and colitis. Exceptions are parenterally administered aminoglycosides and vancomycin which, to our knowledge, have not been associated with colitis. The most complete data for the incidence of diarrhoea and colitis based on prospective surveys are available for ampicillin and clindamycin as summarized in Table 2. The wide ranges noted in different studies presumably reflect vagaries in the definition of diarrhoea, the frequency of endoscopic examination, and epidemiological patterns. There is minimal variation in the incidence according to the route of drug administration so that parenteral usage confers the same risk noted with oral treatment. Most studies also show no good evidence for a dose relationship. Analysis of patients and their underlying diseases have failed to reveal any characteristics other than increasing age which is associated with an increased incidence of antibiotic-associated diarrhoea.

2. C. difficile-*induced diarrhoea and colitis*

A review of the antimicrobial agents implicated in 243 patients with *C. difficile*-induced diarrhoea or colitis showed the most frequent were ampicillin (82 patients), clindamycin (56), and cephalosporins (55) (Bartlett, 1981a). The cephalosporin group included virtually all compounds in this class which are currently marketed in the United States. Less frequent drugs which were implicated in 8 to 20 cases were penicillins other than ampicillin or amoxicillin, erythromycin, sulphamethoxazole-trimethoprim and sulphasalazine. It is of interest to note that there were only two cases associated with tetracycline and no cases which could be clearly ascribed to chloramphenicol or oral neomycin. This is emphasized due to the disparency noted with this series compared to the reports of PMC from the 1950s and 1960s showing chloramphenicol, tetracycline and oral neomycin to be the most frequently implicated drugs in antibiotic-associated PMC (Reiner *et al.*, 1952; Hale and Cosgriff, 1957; Altemeier *et al.*, 1963; Hummel *et al.*, 1964).

The results of our studies as well as those of others have shown that the only drugs which appear to be responsible for *C. difficile*-induced colitis are those