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Volume 9 No 1 March 1988

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Campylobacter pylori — Isolation, characterization and association with chronic gastritis and ulcers

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In 1906 Krienitz reported the presence of spiral bacteria in the human stomach.[1] Similar observations were made sporadically over the next few years, but the organisms were never cultured. In the early 1980's work in Perth, Australia by Drs B Marshall and R Warren led to the identification of a new species of *Campylobacter*, initially called *C.pyloridis*. This bacterium, which was frequently present in the gastric mucosa of patients undergoing biopsies for chronic gastritis and ulcers,[2,3] could be demonstrated histologically and cultured under microaerophilic conditions. This year Marshall and Goodwin revised the original nomenclature and renamed the organism *C.pylori*.[4] They proposed that this organism is a common and important pathogen in the aetiology of gastrointestinal disease.[3] Moreover, Marshall after ingesting a pure culture of *C.pylori* experienced a mild gastrointestinal illness accompanied by histological evidence of gastritis.[5] Since then the isolation of *C.pylori* from gastric mucosa has been confirmed in studies throughout the world. In a study undertaken at the University of Alberta Hospital, *C.pylori* was isolated from 43 per cent of patients undergoing gastric biopsy. Moreover, there was a strong association (95.5 per cent) between *C.pylori* in the gastric mucosa and histologically defined gastritis.[6] There was no obvious association between *C.pylori* and ulcers. However, our study was small and only a few patients with ulcers were included.

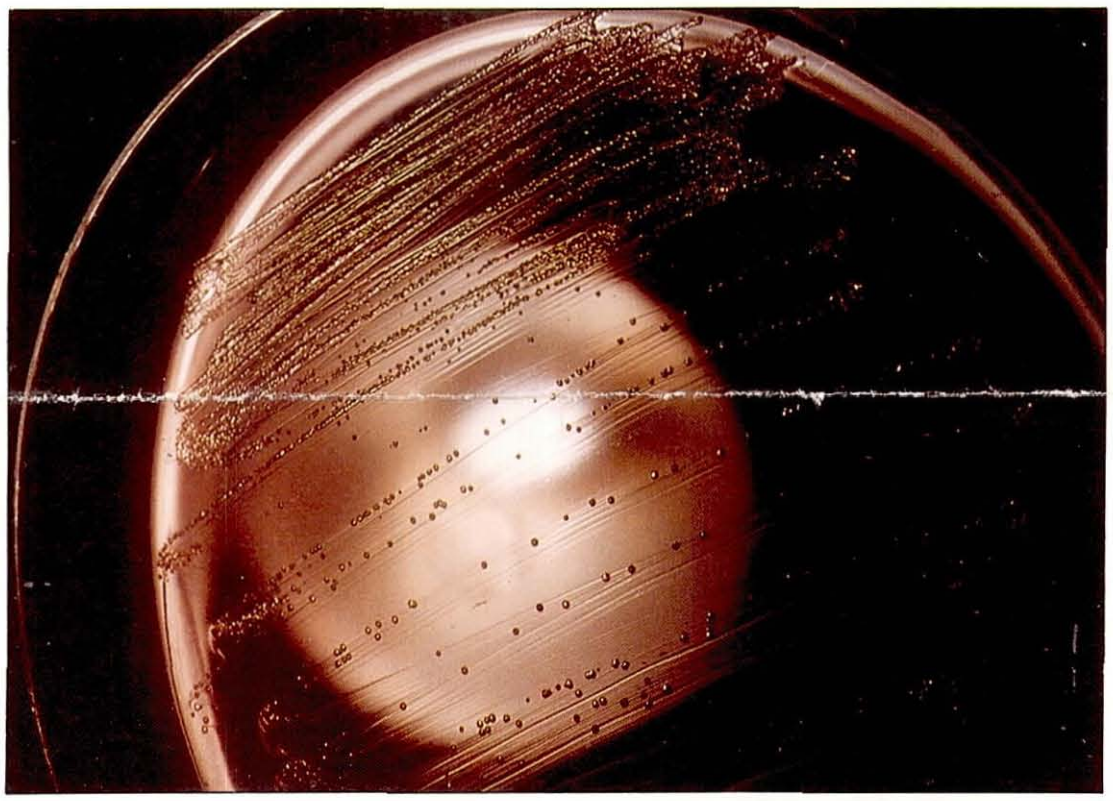

Figure 1a: *C.pylori* colonies growing on Brucella agar plates containing 10% sheep blood. Figure courtesy of R Sherburne.

Isolation of *C.pylori*

Biopsies were taken with a fibre optic endoscope, one set used for histology and the other for bacteriological culture. The latter was immersed in 0.2ml Brucella broth and processed within two hours of collection by aseptically macerating the tissue. Samples were then plated on Brucella agar containing 10 per cent sheep's blood. Plates were incubated under microaerophilic conditions in anaerobic jars without catalyst for four days at 37°C. The gas mixture (5 per cent O_2, 10 per cent CO_2 and 85 per cent H_2) was replaced every 24 hours. Alternatively, gas generation envelopes (Oxoid BR56) may be used or a CO_2 incubator containing 10 per cent CO_2. Other media appear to be equally effective for the isolation of *C.pylori*. Brain heart infusion agar containing 7 per cent horse blood can also be used.[7] *C.pylori* will also grow on Mueller-Hinton agar (Oxoid) with 0.1 per cent starch but colonies are smaller than on blood agar (Taylor and Chang, unpublished).

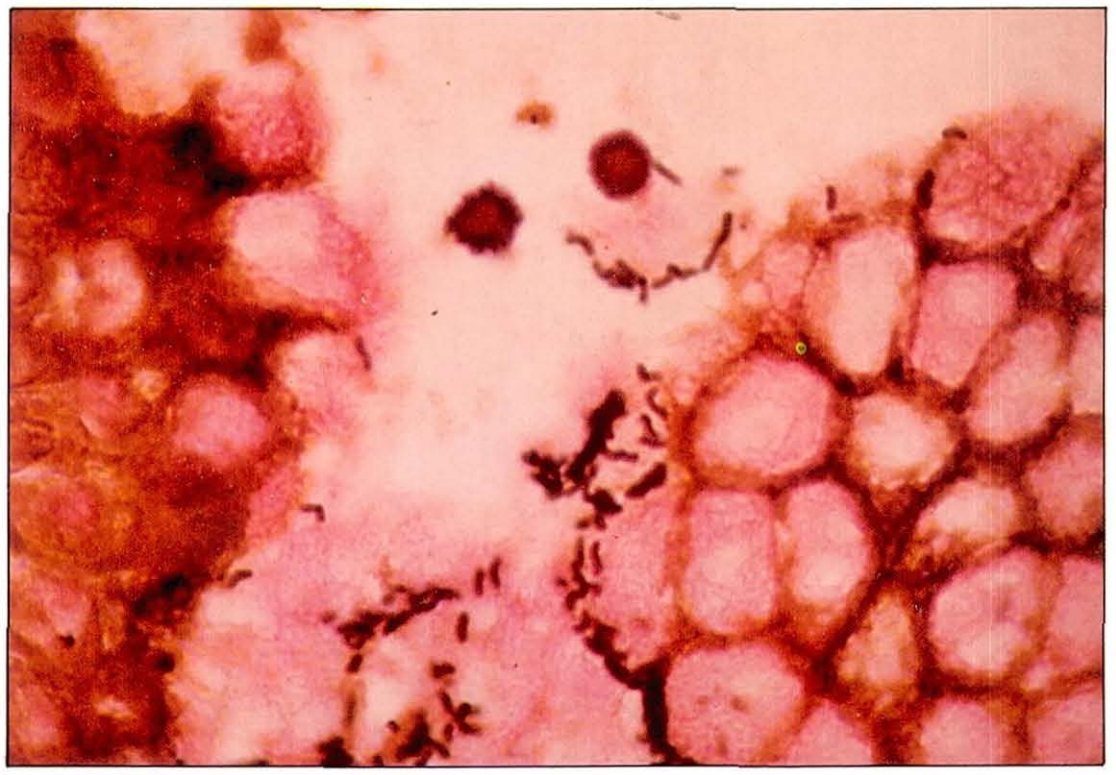

Figure 1b: Histological section stained by Warthin-Starry procedure showing *C.pylori* present in acutely inflamed and eroded gastric antrum. The spiral organisms are seen to advantage in this silver stained preparation. They lie in the gastric mucin, some are attached to the surface epithelium (x400). Figure courtesy of LD Jewell.

Characterisation of *C.pylori*

Growth — *C.pylori* form small transparent colonies best viewed by reflected light (**Figures 1a** and **1b**). They grow best at 37°C, but not at 25°C, although some strains grow at 42°C.

Morphology — *C.pylori* strains isolated from biopsy material are gram negative, spiral bacteria. Under the electron microscope they usually exhibit four flagella (range 3 – 5) at one pole (**Figure 2a**), although dividing cells may have flagella at both poles. At higher magnification, four flagellar discs may be seen associated with the flagella (**Figure 2b**). Terminal knobs are sometimes distinguishable.

Biochemical tests — **Table 1** shows the biochemical characteristics of *C.pylori*. Oxidase,[8] catalase[8] and alkaline phosphatase[8] tests were performed as described previously. The urease tests were performed using Christiansen's urea broth[10] and the hippurate tests as described by Harvey.[11] The DNase test used toluidine blue agar.[12] The positive urease test is the most important diagnostic test for *C.pylori*. The large amount of urease produced by the organism is thought to play a role in the pathogenic process. Moreover, a rapid diagnostic test based on urease production can be used directly on biopsy material.[13]

Classification of *C.pylori*

C.pylori has a DNA base pair ratio (guanine + cytosine) of 36 mole%[3] which is within the range of other Campylobacter species. However, a comparison of partial

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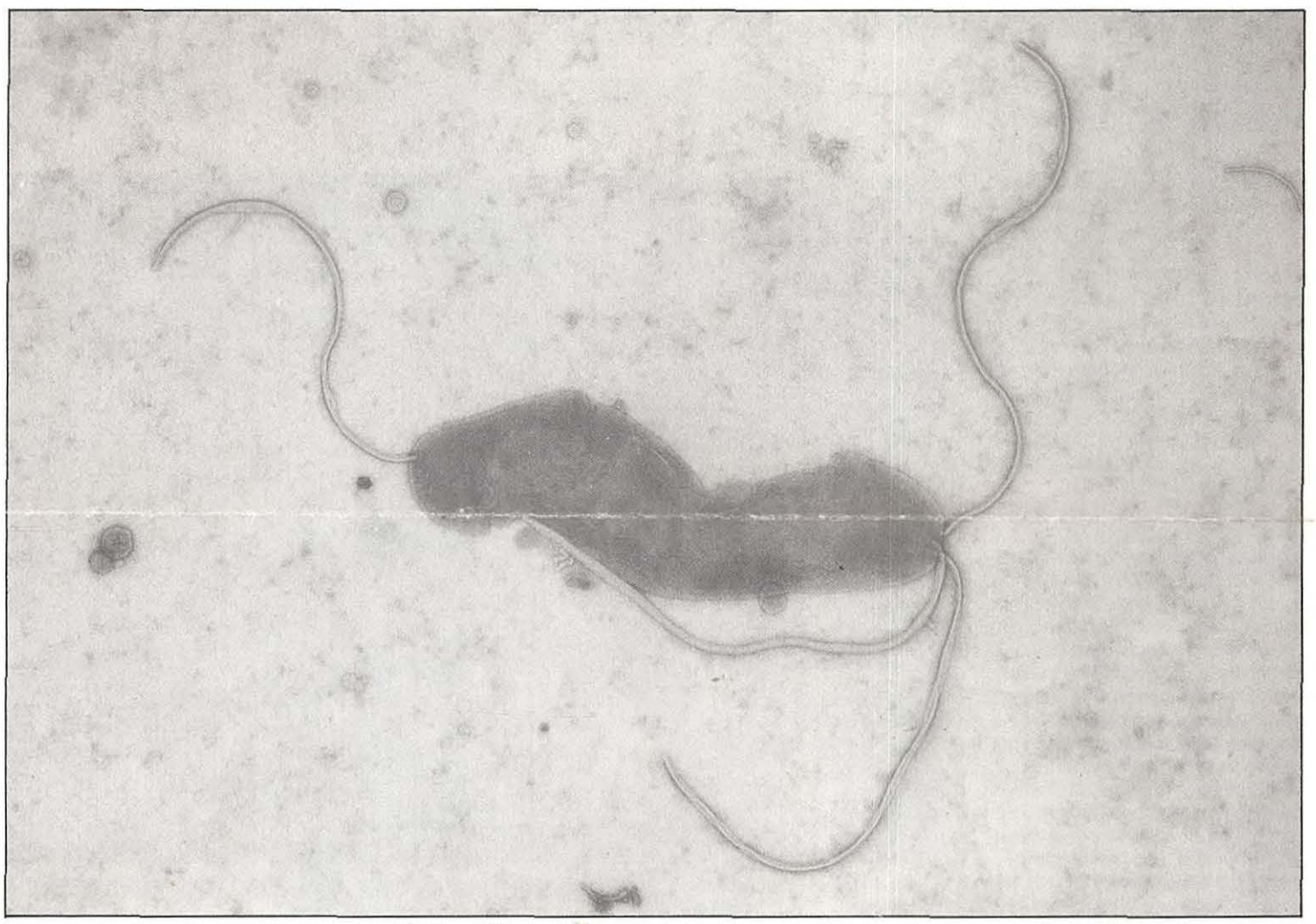

Figure 2a: Electron micrograph of *C.pylori* cell. The cells usually have four flagella at one pole. Only three are seen here. The flagellum at the other end is probably not attached to the cell but lies underneath it.

Table 2: Effect of antibiotics on growth of *C.pylori*.

Antibiotic	Concentration tested[a]	Comment
Ampicillin	8μg/ml	susceptible
amoxacillin	8μg/ml	susceptible
gentamicin	8μg/ml	susceptible
kanamycin	8μg/ml	susceptible
tetracycline	8μg/ml	susceptible
ciprofloxacin	1μg/ml	susceptible
novobiocin	1μg/ml	susceptible
erythromycin	1μg/ml	susceptible
nitrofurantoin	2μg/ml	susceptible
trimethoprim	256μg/ml	resistant
sulfamethoxazol	256μg/ml	resistant
nalidixic acid	40μg/ml	moderately resistant[b]

[a] Determined by testing 20 strains of *C.pylori* for growth on Mueller-Hinton agar (Oxoid) containing 10 per cent sheep's blood for the majority of antibiotics and 10 per cent lysed horse blood for trimethoprim and sulfamethoxazole at the antibiotic concentrations shown. Organisms ($10^6 - 10^7$ colony forming units) were applied to the agar surface using a Steer's replicator.

[b] *C.pylori* strain grew on 40μg/ml but were inhibited by 48μg/ml nalidixic acid.

Antibiotic susceptibilities of *C.pylori*

All strains of *C.pylori* were susceptible to the majority of antibiotics tested (**Table 2**). Several *Campylobacter* species were resistant to high levels of nalidixic acid [15] and this antibiotic is used to differentiate *Campylobacter* species. The moderate level of nalidixic acid resistance observed by us in these *C.pylori* strains was quite different from the response to nalidixic acid seen in other *Campylobacter* species.[15] In *C.jejuni* resistance to both tetracycline [16] and kanamycin [17] have been reported, both of which are plasmid mediated. Plasmid-mediated antibiotic resistance has not yet been encountered in *C.pylori*. Like most *Campylobacter* spp., *C.pylori* is resistant to trimethoprim and sulfamethoxazole. In our study all strains were susceptible to nitrofurantoin (**Table 2**). Variable resistance to metronidazole [18] has been reported with minimal inhibitory concentrations (MICs) ranging from 0.5 to 8μg/ml. Likewise, bismuth salts (tripotassium dicitratobismuthate and bismuth sodium tartrate) have moderate activity with MICs ranging from 2 to 32μg/ml.[18]

16S ribosomal RNA sequences from *C.pylori* and other *Campylobacter* species indicated that the evolutionary distance between *C.pylori* and other *Campylobacter* species is sufficient to exclude the pyloric organism from the *Campylobacter* genus. *C.pylori* appears to be more closely related to *Wolinella succinogenes* than to other *Campylobacter* species.[14] It has been proposed that *C.pylori* should no longer be considered a member of the *Campylobacter* species.[14]

Pathogenesis of *C.pylori*

Is *C.pylori* responsible for gastritis and ultimately for ulcers or is it a secondary invader? Work by Marshall and co-workers in which they attempted to fulfill Koch's postulates suggest that *C.pylori* plays a distinct role in gastrointestinal disease.[5] Moreover, evidence from the treatment of patients with gastrointestinal disorders also supports this view. PeptoBismol, which contains a bismuth salt, has been used for many years as an effective over-the-counter remedy for gastritis and ulcer sufferers. The antibiotic susceptibility tests described above indicate that *C.pylori* is moderately susceptible to bismuth compounds. The bismuth salts act locally in the stomach and since they are not absorbed into the blood may reach a fairly high concentration in the stomach. PeptoBismol can erradicate the organism in about 70 per cent of patients. Some individuals remain free of *C.pylori* for up to 12 months after a course of PeptoBismol treatment but about half experience a reoccurrence of the organisms on subsequent biopsy, either by recolonisation of a small number of organisms which remain in the stomach or possibly by reinfection from an external source.

Erythromycin is the treatment of choice for severe gastroenteritis caused by *C.jejuni*. However, erythromycin is likely to be less useful in the elimination of *C.pylori* because of its reduced activity in the stomach. Similarly, the activity of ß-lactam antibiotics could be altered considerably by the low gastric pH. Most antibiotics are not designed to be effective in the acid conditions encountered in the human stomach and consequently many antibiotics may not be useful for the erradication of *C.pylori*.

In China, furazolidone (a nitrofuran derivative) has been used to treat peptic ulcers since 1978.[19] A trial of two weeks' treatment with furazolidone or cimetidine yielded healing rates of 71 per cent and 55 per cent respectively. Relapse rates after four years were 10 per cent for the group treated with furazolidone and 33 per cent for the group treated with cimetidine.[19]

How does *C.pylori* cause gastritis and subsequently ulcers? It seems likely that these organisms which can live in the acid environment of the stomach, either digest or in some way damage the mucous

Table 1: Characteristic features of *C.pylori*.

Test	Reaction
Catalase	+
Oxidase	+
Urease	+
DNase	+
alkaline phosphate	+
hippurate hydrolysis	—
Growth at 25°C	–
Growth at 37°C	+
Growth at 42°C	variable
G + C content	36%

lining of the stomach, perhaps through the action of urease. Without the protective lining, acid irritates and causes holes in the stomach resulting in an ulcer. The epidemiology of the *C.pylori* infection is more controversial. Marshall has suggested that the organism may be spread through kissing because husbands with duodenal ulcers often (in about 50 per cent of couples) have wives with gastritis. Clearly, additional studies are needed to define the precise role *C.pylori* plays in the formation of gastritis and ulcers and to understand how the organism is acquired in the first place.

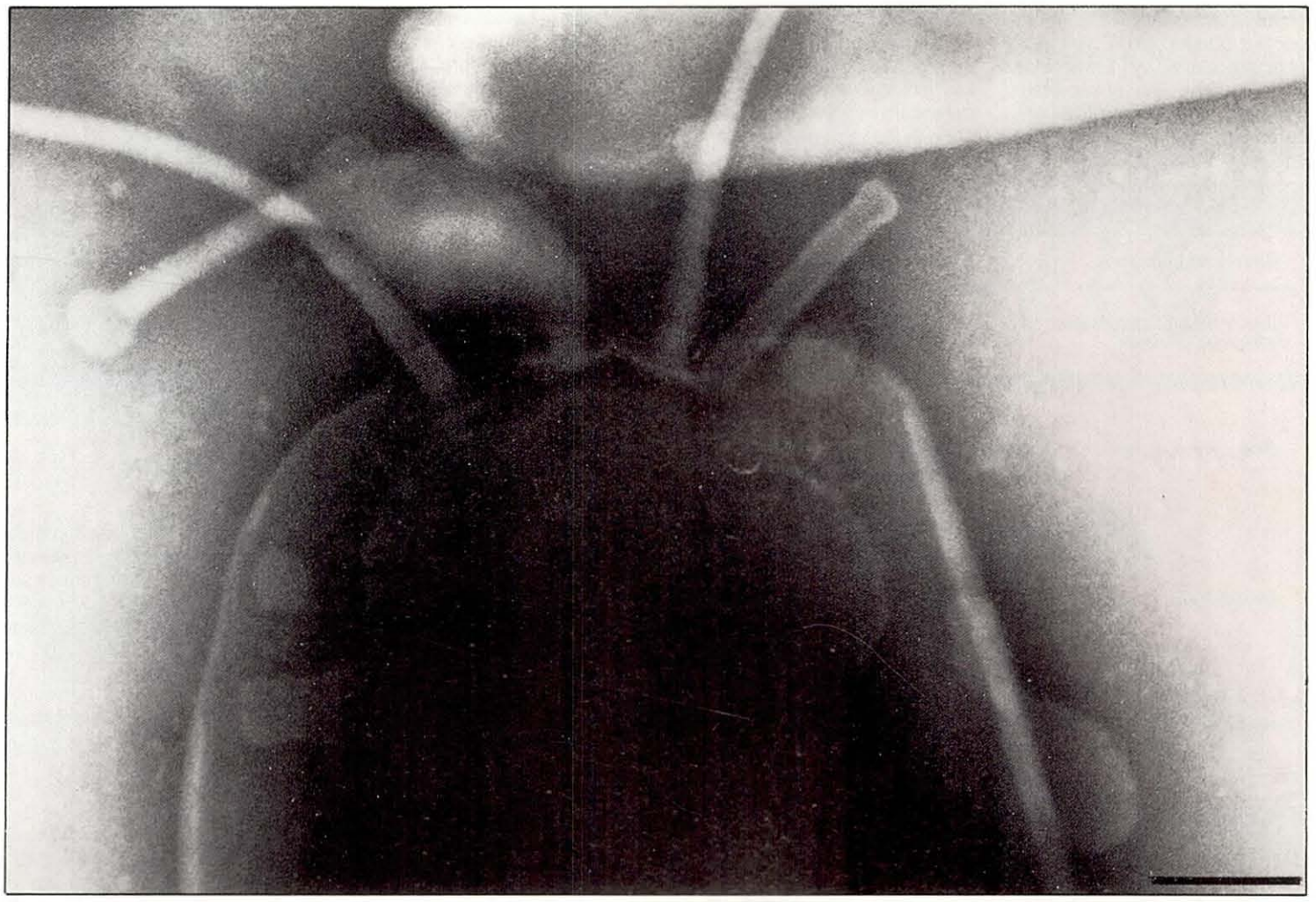

Figure 2b: Electron micrograph of a portion of *C.pylori* showing four flagella and associated discs. The bar represents 0.1μm. Reproduced with permission from the *American Journal of Clinical Pathology*, Volume 87, pages 49-54, 1987.

References

1. Krienitz, W. (1906). *Dtsch. Med. Wochenschr.*, **28:** 872-890.
2. Warren, J.R. and Marshall, B. (1983). *Lancet*, **i:** 1273-1275.
3. Marshall, B.J. and Warren, J.R. (1984). *Lancet*, **i:** 1311-1315.
4. Marshall, B.J. and Goodwin, C.S. (1987). *Int. J. Syst. Bacteriol.*, **37:** 68.
5. Marshall, B.J. *et al.* (1985). *Med. J. Aust.*, **142:** 439-444.
6. Taylor, D.E. *et al.* (1987). *Am. J. Clin. Path.*, **87:** 49-54.
7. Marshall, B.J. *et al.* (1985). *Med. J. Aust.*, **142:** 439-444.
8. Cowan, S.T. and Steele, J.K. (1974). *Manual for the identification of medical bacteria*, 2nd edition. London, Cambridge University Press.
9. Pennock, C.A. and Huddy, R.B. (1967). *J. Pathol. Bacteriol.*, **93:** 685-688.
10. Christensen, W.B. (1946). *J. Bacteriol.*, **52:** 461-466.
11. Harvey, S.M. (1980). *J. Clin. Microbiol.*, **11:** 435-437.
12. Lachica, R.V.F., Genigeorgis, C. and Hoeprich, P.D. (1971). *Appl. Microbiol.*, **21:** 585-587.
13. McNulty, C.A.M. and Wise, R. (1985). *Lancet.*, **ii:** 1443-1444.
14. Romaniuk, P.J. *et al.* (1987). *J. Bacteriol.*, **169:** 2137.
15. Taylor, D.E., Ng, L.-K. and Lior, H. (1985). *Antimicrob. Agents Chemother.* **28:** 708-710.
16. Taylor, D.E., *et al.* (1981). *Antimicrob. Agents Chemother.*, **19:** 831-835.
17. Lambert, T. *et al.* (1985). *Ann. Inst. Pasteur (Paris).*, **136:** 135-150.
18. McNulty, C.A.M., Dent, J. and Wise, R. (1985). *Antimicrob. Agents Chemother.*, **28:** 837-838.
19. Zheng, Z.-T. *et al.* (1985). *Lancet*, **i:** 1048-1050.

Bacteraemia and the immunocompromised host

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Infection in the immunocompetent patient is usually caused by a pathogen of high virulence with which the individual's defences cannot cope. In contrast, the immunocompromised patient can be infected by organisms of low virulence, which would normally be regarded as commensals. With the severely immunodepressed the dividing line between pathogen and non-pathogen disappears.

The different types of immunosuppression predispose the patient to different patterns of bacterial infection. There is a close relationship between a given range of host defences and the type of bacterium they control. The source and mode of entry of bacteria is another important determinant of bacteraemia in the immunocompromised. Many bacteraemias result from tissue infection. Those related to breaks in the skin will tend to have a different aetiology from those related to the gastrointestinal tract or the upper respiratory tract. Prolonged stay in hospital may have led to colonisation of these sites with typical antibiotic-resistant hospital species, giving rise to even greater therapeutic problems.

Figure 1: Protective Isolation Unit. *Photograph taken with permission and acknowledgement to the: Bone Marrow Transplant Unit, Westminster Children's Hospital, Vincents Square, SW1.*

Aetiology of bacteraemia and relationship to type of compromise and source of infection

Table 1 shows the relationship between bacterial species and the type of immune defect. There is a striking difference between the species involved in defects of cell-mediated immunity and those where humoral immunity is concerned. Species such as *Staphylococcus aureus* and *Staphylococcus epidermidis* feature where phagocytic defects are involved, whilst the Gram negative organisms, *Enterobactericae* and *Pseudomonas* are seen with both antibody and neutrophil defects. Bacterial species such as *Listeria monocytogenes* and *Myobacterium avium intracellulare* are

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Table 1: Infecting species associated with specific immune defects.

Immune defect	Bacterial species
Immunoglobulin	*Streptococcus pneumoniae* *Haemophilus influenzae* *Neisseria meningitidis* Enterobacteriacae *Pseudomonas aeruginosa* Group B streptococci
Cell-mediated immunity	*Listeria monocytogenes* *Salmonella spp.* *Mycobacterium spp.* Nocardia
Neutrophil	*Staphylococcus aureus* *Staphylococcus epidermidis* *Streptococcus pyogenes* *Pseudomonas aeruginosa* Enterobacteriacae
Splenectomy	*Streptococcus pneumoniae* *Haemophilus influenzae* *Neisseria meningitidis*

capable of survival within mononuclear phagocytes. Only macrophages activated by T lymphocytes can kill them. The encapsulated pyogenic cocci require anticapsular opsonic antibody to enhance phagocytosis and intracellular killing. Antibody plays a lesser role in *S.aureus* infection, neutrophil phagocytosis being the key factor. These patterns of host defence/infection are most clearly seen in the relatively rare primary immune deficiencies. Secondary immunodepression due to disease, drugs, irradiation, burns or major surgery is far more common. Patterns of infection may, therefore, be more complex, but often a particular defect predominates.

Table 2: Relationship between bacteria and source of infection.

Source of infection	Bacterial species
Skin (including intravenous catheters)	Staphylococci Streptococci Corynebacteria Acinetobacter Klebsiella
Gastrointestinal tract	*E.coli* *Ps.aeruginosa* *Klebsiella spp.* Other Gram negative bacilli *Clostridium spp.* Non-sporing anaerobes
Urinary tract	*E.coli* Proteus Pseudomonas Enterococci Staphylococci
Respiratory tract	*S.pneumoniae* *H.influenzae* *S.pyogenes* Staphylococci *E.coli* Pseudomonas spp. Other Gram negative bacilli Group B streptococci (in neonate)

Table 2 shows the bacterial species associated with different sites of origin. This list represents normal flora, with modifications resulting from time spent in hospital. Staphylococci, streptococci and corynebacteria are normally found on the skin. However, epidemic *Klebsiella* and *Acinetobacter* can colonise the skin of hospital patients. The presence of a foreign body such as an intravenous catheter can potentiate infection with low grade pathogens such as *S.epidermidis* as well as providing a direct route for infection.

Cytotoxic drugs can damage rapidly dividing cells such as those of the gastric mucosa. This allows invasion by gastrointestinal flora such as *Escherichia coli* or *Pseudomonas aeruginosa*. Despite their relatively low numbers in the gut, aerobic or facultative organisms are much more common causes of bacteraemia in this situation than are the anaerobes such as *Bacteroides fragilis*.

Gram negative bacilli have until recently been the most common cause of bacteraemia in the immunocompromised. *E.coli* is the most common species isolated. *Ps.aeruginosa* gives the highest mortality. Other genera, *Klebsiella* and *Proteus* also cause problems. Increasingly more resistant organisms such as *Acinetobacter, Enterobacter, Providencia* and *Serratia* are being encountered.

Recently there has been a resurgence of Gram positive organisms. *S.aureus* has always been a problem, but *S.epidermidis* and other coagulase negative species are increasingly common, usually associated with intravenous lines and other prostheses. The so called 'JK' coryneforms also cause bacteraemias and in some centres enterococci cause problems.

Neonates are a special group of immunocompromised patients. Group B streptococci, *E.coli* and *S.epidermidis* are the main causes of bacteraemia in this group with occasional infections caused by *Listeria monocytogenes* and *S.aureus*.

Table 3: Antibacterial agents used in decontamination and prophylaxis.

Topical	Oral
Chlorhexidine	Vancomycin
Hexachloraphene	Gentamicin
Iodophors	Colistin
	Framycetin
	Cotrimoxazole
	Amphotericin
	Nystatin

Prevention of infection

Prevention is nearly always better than cure. In the severely immunocompromised patient this is even more important, given the difficulty that may be experienced making an early diagnosis and the limitations of antibacterial chemotherapy in the absence of host defences. Preventive strategies can be divided into decontamination of potential sources of endogenous infection and prevention of cross infection by varying degrees of protective isolation. These measures are costly and time consuming and should be instituted as part of a carefully thought out strategy only for certain categories of severely compromised patients (e.g. acute leukaemias, bone marrow transplantation). In these cases half measures may be worse than useless. Strict preventive measures are not suitable for use on sporadic cases. To be effective they require expert team work.

Site decontamination and antibacterial chemoprophylaxis

The major sources of endogenous infection in the compromised host are skin, upper respiratory tract, gastrointestinal tract, female genital tract. Regular microbiological monitoring of these sites should be undertaken. Antimicrobial agents used for this purpose are listed in **Table 3**. Most interest centred originally on the oral regimens such as vancomycin/gentamicin or framycetin/colistin combined with nystatin or amphotericin. More recently, cotrimoxazole has been found effective in preventing bacteraemias and other serious infections originating from the gastrointestinal tract. The concept of colonisation, resistance and the use of agents that do not affect this adversely is now widely accepted.

Protected environment

Decontamination or prophylaxis should only be used in conjunction with a protected environment where air contamination is prevented by a plastic isolator or Laminar air flow. Arrangements also need to be made for sterile food and water. The environment needs regular monitoring as does the patient.

Diagnosis

The blood culture is the standard method of diagnosing bacteraemia. With the immunocompromised patient rapid sensitive diagnosis is important. Conventional blood culture systems using media designed to cope with the range of expected organisms work well, but organisms do need time to grow. The use of Gram staining, of antigen detection techniques applied either to blood or concentrated urine may speed up the process. However, these are limited by the availability of suitable reagents and are currently of little value for staphylococci and most Gram negative bacilli. Of course, with many organisms antigen detection will not give reliable guidance for antimicrobial chemotherapy. New methods, such as BACTEC and SIGNAL, may give a more rapid indication of a positive culture, but their full potential needs a system of 24h observation.

Bacteraemias often originate in the tissues and early diagnosis may necessitate aggressive invasive and non-invasive diagnostic techniques, e.g. biopsy, CT scan, Indium scan.

Therapy

The management of bacteraemia in the immunocompromised patient requires a clear policy of aggressive empirical chemotherapy with alternatives to be used if there is no initial response. Empirical therapy needs to be modified on the basis of the likely site of origin of the infection.

Combinations of aminoglycosides with broad spectrum antipseudomonal penicillins have been proved effective. Third generation cephalosporins are also used extensively. Both approaches require additional agents if staphylococcal or anaerobic infection is suspected (vancomycin, flucloxacillin and metronidazole respectively) while a cephalosporin regimen alone will not cover enterococci. The role of newer agents such as the monobactams 4-quinolones and imipenem/alastatin is currently being evaluated.

Immunotherapy with vaccines and antisera directed against pneumococci, pseudomonas and Gram negative core glycolipid is a potentially attractive addition to chemotherapy. However, outside the experimental sphere it is not yet practical.

Further reading

Proceedings of the Second International Symposium on Infections in the Immunocompromised Host. Easmon, C.S.F. and Gaya, H. (Eds). (1983). Academic Press, London.

Young, L.S. *et al.* (1986). *Rev. Infect. Dis.*, **8:** 1024-1033.

McGowan, J.E. (1985). *Rev. Infect. Dis.*, **7:** Suppl S 357.370.

Young, L.S. (1985). *Rev. Infect. Dis.*, **7:** S380-388.

Clasener, H.A.L. *et al.* (1987). *Rev. Infect. Dis.*, **9:** 295-328.

Cohen, J. (1986). *J. Antimicrob. Chemother.*, **18:** 436-439.

Trombley, C., and Anderson, J.D. (1987). *J. Clin. Microbiol.*, **25:** 2098-2101.

Printed by Williamson Printing Ltd, Chessington, Surrey, England.