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Sepsis, the Liver and the Gut

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/49962>

1. Introduction

The gastrointestinal tract has various functions including digestion, the production of hormones with local and systemic effects, a major role in immunological function, and acting as a barrier against antigens within its lumen. The intestinal microflora is an ecosystem which harbours over 400 bacterial species, predominantly anaerobes which outnumber facultative anaerobes. Most flora is present in the large bowel, mainly in the lumen and attached to the mucosa, but they do not normally penetrate the bowel wall. Intestinal bacteria form an important part of the enterohepatic circulation. Metabolites conjugated in the liver (including drugs and endogenous compounds) are excreted in bile to be deconjugated by bacterial enzymes in the intestine, so that they can then be absorbed across the intestine into the portal circulation and returned to the liver. Antibiotics that alter the intestinal flora can change the fecal excretion and the serum levels of these metabolites. Bacterial flora also increase fiber digestion and are believed to decrease the risk of gastrointestinal infections by interfering with gut pathogens. Our intestine harbours low concentrations of potentially pathogenic organisms (such as *Clostridium difficile*). Antibiotics that alter the normal intestinal flora can increase the risk of infection by exogenous pathogens or through the overgrowth of endogenous pathogens, like *Clostridium difficile*. If the bowel wall is damaged by trauma, burns or inflammation, intestinal bacteria may escape into the peritoneum to cause peritonitis and / or abscesses.[1]

Gastrointestinal dysfunction or gut failure frequently occurs in seriously ill patients and is responsible for bacterial translocation. This may in turn cause sepsis, with the initiation of a systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), and / or death.[2] Gut dysfunction is also present in other conditions, including inflammatory bowel disease, *Clostridium difficile* infection, and liver cirrhosis. In this chapter, we investigate common conditions affecting the liver and the gut and their relation to sepsis, as well as investigating the role of gut decontamination and probiotics in stabilising the gut flora.

2. Sepsis in liver cirrhosis

Liver cirrhosis occurs in response to chronic liver injury and involves the development of regenerative nodules surrounded by fibrous bands in the liver parenchyma. This in turn causes distortion of the hepatic vasculature, leading to portal hypertension and end stage liver disease. Cirrhosis leads to shunting of portal and arterial blood into the hepatic central veins, thus compromising the exchange between hepatic sinusoids and hepatocytes. Cirrhosis causes an impaired hepatocyte activity, portal hypertension and an increased risk of hepatocellular carcinoma. Hepatic vascular alterations and portal hypertension will in turn cause splanchnic vasodilatation, vasoconstriction and decreased renal perfusion, water and salt retention and an increased cardiac output.[3]

The estimated prevalence of cirrhosis in the United States is 0.15% [4], though this may be an underestimate due to the high prevalence of undiagnosed cirrhosis in hepatitis C and Non-Alcoholic Steatohepatitis (NASH). Similar numbers have been reported from Europe, and numbers are even higher in most Asian and African countries where chronic viral hepatitis B or C are frequent. Since compensated cirrhosis is frequently not detected until routine investigations are performed, a reasonable estimate is that up to 1% of the world population may have histological cirrhosis. Alcoholic liver disease and hepatitis C are the commonest causes of cirrhosis in the Western world, while hepatitis B is the most common cause in most parts of Asia and sub-Saharan Africa. Cryptogenic cirrhosis (cirrhosis without a recognised cause) is nowadays rarely diagnosed, particularly after the identification of the hepatitis C virus in the late 1980s and with the identification of nonalcoholic steatohepatitis in obese and diabetic subjects.[3]

Bacteraemic infections are more frequent in patients with hepatic cirrhosis. 9% of the overall number of bacteraemic episodes in newly-admitted patients occur in cirrhotic patients [5] and 46% of cirrhotic patients have bacterial infections on admission.[6] Advanced cirrhotics are more likely to have the systemic inflammatory response syndrome. This syndrome correlates with bacterial infection at admission and has been shown to be associated with a poor outcome.[7] Animal studies have identified the gut as the principal source of infection in liver cirrhosis, mainly through bacterial overgrowth and translocation in the small bowel. However, cultures of small intestinal mucosal bacteria in cirrhotic patients have shown that these microbiota are qualitatively and quantitatively normal. This has shifted attention towards factors that decrease gut integrity, or alter the removal of translocating bacteria as causative factors of bacteraemia in cirrhosis.[8] It is hypothesized that in cirrhosis the intestine is more permeable, allowing bacteria easy access into the circulation through the gut mucosa with consequent macrophage activation. This permeability is further increased in patients with portal hypertension. Serum levels of interleukin-6 and soluble receptors of tumor necrosis factor were shown to be significantly higher in HIV-HCV co-infected and HCV mono-infected patients with decompensated cirrhosis when compared with those with compensated liver disease.[9] This susceptibility was also demonstrated in non-alcoholic steatohepatitis.[10] In patients with cirrhosis and severe sepsis, high production of pro-inflammatory cytokines seems to cause a deterioration in liver function and predisposes to the development of shock, renal failure, acute lung injury or acute respiratory distress

syndrome, coagulopathy, or hepatic encephalopathy. Variants of the NOD2 gene (100fs and G908R) appear to increase bacterial translocation in cirrhotics and have been associated with spontaneous bacterial peritonitis in a recent study.[11] There is an increased risk for culture-positive spontaneous bacterial peritonitis and infected ascites in cirrhotic patients with these variants.[11]

The second theory is that patients with chronic liver disease tend to have impaired bacterial clearance. This was demonstrated when quantitative real-time polymerase chain reaction (PCR) using primers that amplify all known bacteria was used to measure bacteraemia following tooth-brushing. The investigators showed greater than 75% bacteraemia following tooth-brushing, but while control subjects were able to clear this bacteraemia, subjects with cirrhosis had prolonged bacteraemia, suggesting that cirrhotic patients may be more susceptible to sepsis because of ineffective bacterial clearance.[12]

The mortality rate of patients with liver cirrhosis is significantly higher than that of patients with other diseases when they develop bacteraemia, and underlying cirrhosis is an independent risk factor for mortality in bacteraemic patients. In-hospital mortality rate in patients with liver cirrhosis and sepsis was shown to be up to 30% [13-16], with another 30% dying by 1 year.[16] Factors which are significantly associated with in-hospital mortality are the presence of more than 1 site of infection, pneumonia, Child's C status and a model for end-stage liver disease (MELD) score of 17 or more. In-hospital mortality rate increases as the number of factors increases (7% with one factor, 21% with two factors, 87% with three factors and 100% with four factors).[13] The initial CRP level does not predict mortality secondary to sepsis in liver cirrhosis patients. However, serial CRP measurements during the first week of antimicrobial therapy may be a useful prognostic factor for mortality in cirrhotic patients.[14] In a nationwide Korean surveillance study comparing bacteraemia in patients with liver cirrhosis with bacteraemia in patients with other liver diseases, patients with cirrhosis were shown to be more likely to have *Klebsiella pneumoniae* bacteraemia (20.1% vs 14.3%, $P=0.018$) but less likely to have coagulase-negative staphylococcal bacteraemia (5.1% vs 10.4%, $P=0.028$).[14]

One of the sequelae of cirrhosis is the development of ascites. Patients with ascites have an increased risk of developing spontaneous bacterial peritonitis (SBP) with a prevalence of 10-30%. Even with early diagnosis and management of spontaneous bacterial peritonitis, mortality is still 31% at 1 month and 66% at 12 months.[16] SBP is a very common bacterial infection in patients with cirrhosis and ascites.[17] Bacterial translocation is believed to be responsible for the first step in the pathogenesis of spontaneous bacterial peritonitis. Translocation is only possible because of the concurrent failure of the defensive mechanisms in cirrhosis. Research has confirmed an increased bacterial translocation in cirrhotic rats. There is also pronounced impairment of gastrointestinal tract motility in cirrhosis. A disturbance of the gut microflora thus occurs and this, in association with changes in the permeability of the gastrointestinal tract, causes the passage of microorganisms and endotoxins to the mesenteric lymph nodes.[18] The diagnosis of SBP is based on diagnostic paracentesis. Half the episodes of SBP are present on hospital admission while the rest are acquired during hospitalization.[19] SBP may present with peritonitic signs (abdominal

pain, tenderness, vomiting, ileus), fever, elevated white cell counts, tachycardia, hypotension, worsening of liver function, hepatic encephalopathy, renal failure and gastrointestinal bleeding. However, cirrhotic patients with SBP may be completely asymptomatic. Empirical antibiotics should be started immediately following the diagnosis of SBP. The first line antibiotic treatment in SBP are the third generation cephalosporins, as the commonest causative organisms are Gram-negative aerobic bacteria.[20] Other options include co-amoxiclav, ciprofloxacin and ofloxacin (though quinolones should not be used in patients who are using these antibiotics for SBP prophylaxis, in areas where there is a high prevalence of quinolone resistance or in nosocomial SBP). Antibiotics are effective in the management of SBP in approximately 90% of patients. Failure of antibiotic therapy usually occurs due to bacterial resistance or because of missed secondary bacterial peritonitis. If secondary bacterial peritonitis has been excluded, the antibiotic needs to be changed according to the culture and sensitivity results of the isolated organisms, or else modified to an alternative empiric broad spectrum agent.[21]

Hepato-renal syndrome (HRS) refers to the rapid deterioration of renal function in patients with liver cirrhosis. It occurs in approximately 30% of patients with SBP treated with antibiotics alone and is associated with a very poor survival. Albumin administration (1.5 g/kg at diagnosis and 1 g/kg on day 3) decreases the frequency and mortality of HRS in cirrhotic patients with SBP. For this reason, the European Association for the Study of the Liver (EASL) guidelines recommend that all cirrhotic patients who develop SBP should be treated with intravenous albumin and empirical antibiotics.[21]

In patients at high risk of developing SBP, antibiotic prophylaxis is recommended.[21] Since it is hypothesised that SBP occurs following the translocation of enteric Gram negative bacteria from the gut to the circulation, the ideal prophylactic antibiotic needs to be effective at decreasing the amounts of these organisms in the gut without altering the protective anaerobic flora. The use of prophylactic antibiotics should be strictly restricted to patients at high risk of SBP to decrease the risk of developing resistance. These high-risk patient populations include cirrhotics with acute gastrointestinal hemorrhage, those with low total protein content in ascitic fluid and no prior history of SBP (primary prophylaxis) and patients with a previous history of SBP (secondary prophylaxis). In such high-risk patients, antibiotics should be started immediately (i.e. following upper gastrointestinal bleed, after a first episode of SBP or upon finding low total protein) and are recommended life-long, or until liver transplant is performed.

Bacterial infection is a major problem in cirrhotic patients with acute gastrointestinal hemorrhage, occurring in 25 - 65% of these patients.[22] Bacteraemia in patients with variceal hemorrhage is associated with a decreased ability to control bleeding [23], an increased rebleeding rate, and increased hospital mortality.[24] Antibiotic prophylaxis has been shown to prevent infection in patients with gastrointestinal bleeding and decrease the rate of rebleeding. A meta-analysis of five studies performed in patients with gastrointestinal bleeding [25-29] has shown that antibiotic prophylaxis significantly decreased both the incidence of severe infections (SBP and/or sepsis) and mortality. The preferred antibiotic for SBP prophylaxis is norfloxacin (400 mg/12 h orally for 7 days) which

provides selective intestinal decontamination. Norfloxacin is a quinolone antibiotic with antibacterial activity against Gram-negative bacteria but not against Gram-positive cocci or anaerobic bacteria. However, in view of the increasing incidence of quinolone-resistant bacteraemia [30-32], and because a substantial number of infections in patients with gastrointestinal hemorrhage are caused by Gram-positive bacteria, ceftriaxone has been studied as a prophylactic agent in cirrhotics with gastrointestinal bleeding. A study comparing oral norfloxacin with intravenous ceftriaxone for the prophylaxis of bacterial infection in cirrhotic patients with gastrointestinal bleeding showed that ceftriaxone was more effective than norfloxacin in the prevention of infections.[33] The main disadvantage with ceftriaxone is that it must be given intravenously and is therefore limited to hospital use. Cirrhotic patients with low protein concentrations (<10 g/L) in their ascitic fluid and/or high serum bilirubin levels are at an increased risk of developing SBP.[34] Studies have shown that norfloxacin (400 mg/day) is effective as a prophylactic agent against SBP and improves survival in patients with low total protein in their ascitic fluid.[35-37] Following an episode of SBP, the cumulative recurrence rate at 1 year is approximately 70% [38], with a 1-year survival probability of 30–50% and a 2-year survival probability of 25–30%. Prophylactic norfloxacin (400 mg/day, orally) reduces the risk of recurrent SBP. Other antibiotics which may be used in SBP prophylaxis after the first episode of SBP include ciprofloxacin (750 mg once weekly, orally) or co-trimoxazole (800 mg sulfamethoxazole and 160 mg trimethoprim daily orally), but the evidence with these antibiotics is not as strong as with norfloxacin. The EASL guidelines also recommend that patients recovering from SBP should be considered for liver transplantation.[21] The American Association for the Study of the Liver and the British Society of Gastroenterology guidelines [39,40] have similar recommendations for the management of spontaneous bacterial peritonitis and its prophylaxis.

Terlipressin is a vasoactive agent used in patients with septic shock and which has a selective affinity to vascular V1 receptors. It is an effective pressor agent in patients with catecholamine-unresponsive septic shock. Additional studies are needed to identify the best time to start terlipressin, the efficacy and dosages of continuous infusion versus bolus administration as well as the safety and efficacy of this compound in comparison with other vasoactive drugs.[41,42]

3. Acute cholangitis

Acute cholangitis and biliary sepsis are severe infectious diseases, frequently observed in patients with obstructive jaundice. The presence of bacteria in the biliary tract increases in the presence of biliary obstruction, particularly in the presence of foreign bodies like stones, but also in the presence of malignant obstruction secondary to pancreatic head carcinoma or cholangiocarcinoma. Reflux of bacteria from the biliary tract to the systemic circulation is believed to be the primary etiologic factor in bacteraemia and the development of sepsis in cholangitis. Biliary tract obstruction is the initiating factor in the pathogenesis of acute cholangitis causing elevated intraluminal pressures, and subsequent infection of the normally sterile bile. Bacteria may infect bile retrogradely from the gut (through the

ascending route), through the haematogenous route or via lymphatics. The presence of bacteria in the biliary tract (bactibilia) increases rapidly with the development of biliary obstruction, particularly in the presence of foreign bodies like stones. Biliary obstruction causes local and systemic changes in the host defenses. There is decreased bile passage into the small bowel and decreased secretory IgA from the gastrointestinal tract. This promotes changes in the gut bacterial flora which in turn cause loss of mucosal integrity, decreased endotoxin inactivation and bacterial overgrowth. These changes cause portal bacteremia, endotoxemia and increased translocation of endotoxins to the liver, resulting in sepsis and also decreasing the hepatic Kupffer cell function in these patients. In view of these pathophysiological changes, early biliary decompression is necessary to restore normal function of the Kupffer cells in the liver and thus prevent functional alterations in the liver because of chronic, long-standing obstruction and cholestasis. Early biliary decompression also decreases postoperative morbidity and mortality.[43] The increased expression of triggering receptor expressed on myeloid cells (TREM-1) in the peripheral blood mononuclear cells of sepsis patients with acute cholangitis suggests an important role of TREM-1 in the development of acute cholangitis.[44, 45]

The predominant pathogens cultured from bile specimens in patients with obstructive jaundice (samples obtained at endoscopic retrograde cholangiopancreatography (ERCP) or percutaneous transhepatic biliary drainage) were gram-negative bacteria (68%) followed by gram-positive bacteria (26%), anaerobes (3%) and *Candida* (3%).[46] The predominant gram-negative pathogens were *Escherichia coli*, *Acinetobacter baumani* complex, *Klebsiella pneumonia* and *Enterobacter cloacae*. The most effective antibiotics against the gram-negative bacteria were shown to be imipenem (susceptibility: 97.9%), cefoperazone/sulbactam (89.4%), piperacillin/tazobactam (85.1%) and cefepime (85.1%).[46] Another study on patients with acute cholangitis [47] confirmed that gram-negative organisms are responsible for most bacteraemias (95%), with the commonest ones being *Escherichia coli* (62%), and *Klebsiella pneumonia* (26%). This study found that bacteraemias caused by biliary tract infection represented 5.5% of all causes of bacteraemias. Thirty-day mortality among these patients was 14% with 57% of these patients dying secondary to septic shock.[47] The management of ascending cholangitis involves the use of appropriate antibiotics and drainage of the biliary tract. Treatment should target Enterobacteriaceae with a cephalosporin, and if the patient becomes hypotensive, an aminoglycoside effective against ESBL-producing *E. coli* or *Klebsiella pneumonia* should also be administered. Biliary drainage, by ERCP or percutaneous transhepatic cholangiography, is frequently needed for adequate biliary decompression.[47]

Patients undergoing ERCP tend to be at high risk of sepsis because of the underlying biliary obstruction which predisposes to cholangitis and because of the invasive nature of the procedure. The use of prophylactic antibiotics before ERCP is therefore recommended by all major international gastroenterological societies, especially in the presence of an obstructed biliary system.[48-50] The use of prophylactic antibiotics attempts to decrease or eliminate the incidence of cholangitis, sepsis and pancreatitis after the procedure.[48] During ERCP, bacteraemia is believed to occur because of the injection of contrast and the iatrogenic introduction of foreign substances in the bile of patients who already have underlying

pathologies such as biliary obstruction or pancreatic pseudocysts. Bacteraemia during ERCP is relatively uncommon in patients who do not have evidence of biliary or pancreatic ductal obstruction.[49] Bacteraemia is however well recognised during ERCP for biliary obstruction with pancreatic or biliary infection occurring following 0.4–0.8% of endoscopic biliary procedures. These episodes must always be taken seriously because of the associated 8–20% mortality risk.[50] Biliary dilatation, the insertion of biliary stents, prolonged procedure time and hilar cholangiocarcinoma have been shown to give an increased risk of post-ERCP cholangitis.[51] The British Society of Gastroenterology and the American Society of Gastrointestinal Endoscopy have similar recommendations on the prophylactic use of antibiotics for ERCP.[52,53] Patients with ongoing cholangitis who will be needing therapeutic endoscopic intervention should always be on appropriate antimicrobial therapy upon admission to hospital. Additional pre-ERCP antimicrobial prophylaxis is not normally recommended for those who are already taking antibiotics therapeutically for cholangitis. Routine prophylaxis for ERCP is not usually necessary, unless it is not possible to adequately decompress the biliary system during the procedure, in which case a full antibiotic course is indicated until adequate drainage can be achieved. Indications for routine antibiotic prophylaxis during ERCP include specific biliary disorders, such as primary sclerosing cholangitis or hilar cholangiocarcinoma (where complete biliary drainage will be difficult or impossible to achieve during one procedure), patients with a history of liver transplantation, patients with pancreatic pseudocysts, patients with severe neutropenia and / or advanced haematological malignancy. When antibiotic prophylaxis for ERCP is given, oral ciprofloxacin or intravenous gentamicin is usually recommended.

4. Inflammatory bowel disease and sepsis

Bacteria play an important role in the pathogenesis of inflammatory bowel disease (IBD), its complications and its symptoms. In IBD, antibiotics can decrease tissue invasion and eliminate aggressive bacterial species. Antibiotics are also used in IBD to treat infective complications and for altering bacterial flora, which may result in specific anti-inflammatory effects. The antibiotics which are used most frequently in IBD are metronidazole and ciprofloxacin, which may be effective in Crohn's colitis and ileocolitis, perianal disease and pouchitis.[54]

The pathophysiology of both Crohn's disease (CD) and ulcerative colitis (UC) involves dysfunction of the intestinal barrier, which then causes leak flux diarrhoea and the facilitated uptake of noxious antigens into the systemic circulation. Barrier dysfunction in IBD involves a reduction in epithelial horizontal tight junctions (TJ) and an abnormal TJ protein expression. An increased incidence and frequency of apoptosis as well as erosions and ulcerations in the gastrointestinal mucosa can add to the leakiness of the gut. The dysfunction of the intestinal barrier occurs because of the increased expression of pro-inflammatory cytokines like Tumor Necrosis Factor alpha, Interferon gamma, Interleukin 1 β , and Interleukin 13 in the chronically inflamed intestine. Chronic inflammation in IBD is believed to result from genetic polymorphisms which cause an inadequate immune response as well as changes in the intestinal microbiota. Probiotics may offer some benefit in

IBD by stabilising the barrier function through TJ protein expression and distribution.[55] In CD, an increased presence of *Campylobacter concisus* and *Escherichia coli* as well as a substantial decrease in the amount of the anti-inflammatory commensal *Faecalibacterium prausnitzii* has been reported, while it has been suggested that *Fusobacterium varium* can promote the development of UC.[56-60] Cultures of *Mycobacterium avium* subspecies paratuberculosis (MAP) in the peripheral blood of CD patients and controls have revealed that MAP is commoner in CD patients, thus suggesting that MAP may have a role in the aetiology of CD.[61] Smokers with CD have also been shown to have luminal microbiota that consist of significantly higher bacteroides (38.4%) than non-smokers (28.1%).[62] While these microbiota frequently do not cause sepsis, sepsis is significantly commoner in IBD, both in immunosuppressed patients and in patients who are newly diagnosed and not on immunosuppressive therapy.[63-64] An increased incidence of bacterial endocarditis in both UC and CD has also been reported.[65] Rifaximin appears to be a promising antibiotic in inducing remission of CD (69% in open studies and significantly better than placebo in double blind trials) and UC (76% in open studies and significantly better than placebo in controlled studies). It may also have a role in remission of UC and pouchitis.[56]

Genetic polymorphisms play a major role in the aetiology of inflammatory bowel disease (IBD). Major advances in the aetiology of CD came from the discovery of polymorphisms in the NOD2 (nucleotide-binding oligomerization domain containing 2), autophagy-related susceptibility genes ATG16L1 (Autophagy-related 16-like gene) and IRGM (Immunity-Related Guanosine Triphosphate) in patients. The identification of the presence of adherent-invasive *E. coli* (AIEC) which are able to resist killing by macrophages on the ileal mucosa was another step forward in understanding the aetiology of Crohn's disease.[66] Mutations in NOD2 gene which cause loss of function of NOD proteins are strongly associated with ileal Crohn's disease. NOD2 is one of the genes controlling microbiota in the intestine, with studies showing loss of regulation of microflora in the terminal ileum of NOD2-deficient mice. Paneth cells, which regulate ileal microbiota by the production of anti-microbial compounds, show an elevated expression of the NOD2 gene, and therefore ileal intestinal epithelial cells which lack NOD2 are unable to destroy bacteria effectively. NOD2 mutations in CD therefore appear to increase disease susceptibility by disrupting the interaction between mucosal immunity and the ileal microflora.[67] NOD2 appears to activate pro-inflammatory signalling cascades once bacterial muramyl dipeptide has been sensed by the epithelial cells. It also seems to be involved in antiviral and anti-parasitic defence programs.

On the other hand, ATG16L1 is a protein necessary for autophagosome formation once bacterial or parasitic components are introduced into cells. Gene polymorphisms resulting in dysregulated immune responses to invasive micro-organisms, including those in the NOD2 and ATG16L1 genes, facilitate microbial replication and loss of the functional integrity of the epithelial barrier with an increase in permeability. The access to sub-epithelial tissues by the invasive micro-organisms may cause local chronic inflammation and microbial dissemination which may result in systemic inflammatory responses. The associated impaired response of myeloid cells to this microbial insult also increases the risk of chronic, low grade infection and inflammation.

5. Pouchitis

Restorative proctocolectomy with ileal-pouch anal anastomosis is the operation of choice for UC patients requiring surgery. It is also used for patients with familial adenomatous polyposis (FAP). Chronic pouchitis is an important long-term complication following ileal-pouch anal anastomosis, accounting for 10% of pouch failures and occurring in 50% of patients after pouch formation for UC. It is however rarely seen in FAP, suggesting that pouchitis tends to occur because of the inflammatory process occurring in UC. Antibiotics are effective in reducing the symptoms of pouchitis, implicating bacteria in its development.[68] Studies have revealed that patients with pouchitis have different bacterial families (Peptostreptococcaceae, Clostridiaceae) from patients with normal pouches (Ruminococcaceae, Bifidobacteriaceae).[69] Bacterial species in pouchitis are important because of the benefit that some probiotics have been shown to offer to these patients, as indicated in the next section.

6. Immunosuppressants in IBD

The increased risk of sepsis and bacteraemia in IBD patients has already been established. The treatment of IBD frequently involves the use of potent immunosuppressing agents including steroids, azathioprine, 6-mercaptopurine, methotrexate and biological drugs including infliximab and adalimumab. Potential complications with the use of these agents in IBD patients include sepsis. A recent meta-analysis which reviewed early post-operative infectious complications in UC patients undergoing colectomy showed no significant difference in the rate of infectious complications between patients who were treated with infliximab and those who were not.[70] In an analysis of serious infections (defined as infections requiring hospital admission) among 489 IBD patients receiving anti-TNF α therapy across Australia and New Zealand, only 14 (2.2%) serious infections were reported. These infections included 3 cases of Varicella Zoster, 2 cases of *Pneumocystis jiroveci* pneumonia, 2 flu-like illnesses, two cases of *Staphylococcus aureus* bacteraemia and five other bacterial infections.[71] Another single-centre analysis on the safety of infliximab in CD, revealed that in 297 patients on infliximab there was a 2.7% rate of serious infection, with 0.33% resulting in fatal sepsis.[72] Case reports of sepsis in patients treated with biological therapy are also numerous.[73-77] Active sepsis is an absolute contraindication for anti-TNF therapy use, as this risks overwhelming sepsis. Reactivation or development of tuberculosis has been reported in 24/100,000 patients with rheumatoid arthritis on anti-TNF therapy, compared with 6/100,000 not receiving such treatment.[78,79]

Reports of severe sepsis in patients with IBD while taking Azathioprine have also been described.[80,81] Azathioprine and 6-mercaptopurine are used in patients with moderate to severe CD or UC. Azathioprine has a complex, heterogeneous thiopurine methyltransferase (TPMT) metabolism which may affect required dosages and may increase the risk for adverse events. Routine TPMT activity testing before starting Azathioprine may decrease the risk of early leukopenia and avoid potentially life-threatening myelotoxicity.[82] The risk of severe sepsis increases further if combination immunosuppressants (such as

combinations of azathioprine and anti-TNF α agents) are used.[83] The TREAT registry showed that while unadjusted analysis indicated that Infliximab is associated with an increased risk of infection, multivariate logistic regression analysis suggested that Infliximab was not an independent predictor of serious infections and the increased risk was associated with disease severity and concomitant prednisone use.[84] The REACH study, evaluating the efficacy of Infliximab in children with moderate to severe CD refractory to immunomodulatory treatment, reported serious infections as the major adverse events with their frequency being higher with shorter treatment intervals. The combination of immunosuppressive medications appears to increase the risk of opportunistic infections.[85]

7. *Streptococcus gallolyticus* and colorectal tumours

Streptococcus gallolyticus, previously called *S.bovis* biotype I (Table 1) is a gram positive bacterium found in the colon of 10% of healthy individuals. It is an opportunistic pathogen as it can cause bacteraemia and endocarditis, especially in the presence of colorectal cancer (CRC). In the International Collaboration on Endocarditis Prospective Cohort Study, *S. gallolyticus* accounted for a very significant 12.5% of the cases of infective endocarditis in patients over 65 and 5.4% in those 18-65 years of age.[86] The association between *S.bovis* bacteraemia and colonic neoplasia was first reported in the literature in 1951 by McCoy and Mason.[87] In a recent meta-analysis [88], among the *S.bovis*-infected patients who underwent colonoscopy, 60% of patients had underlying adenomas or carcinomas. One hypothesis on the association between CRC and *S. gallolyticus* suggests that colorectal malignancy specifically allows for colonisation and translocation of the bacterium through the altered mucosa. An alternative theory proposes that the organism itself promotes carcinogenesis by interacting with the colonic mucosa. Several studies comparing faecal carriage of *S. gallolyticus* in patients with colorectal cancer or adenomatous polyps with normal controls failed to show a significant difference.[87,89,90] However, studies on patients with proven *S. gallolyticus* bacteraemia consistently showed that 25 to 80% of patients with the infection had colorectal tumours. Similarly, 18 to 62% of patients with *S. gallolyticus* endocarditis have been diagnosed with colonic neoplasia.[91]

Old nomenclature	Later nomenclature	Recent nomenclature
<i>S. bovis</i> biotype I	<i>S. gallolyticus</i>	<i>S. gallolyticus</i> subsp. <i>gallolyticus</i>
<i>S. bovis</i> biotype II/1	<i>S. infantarius</i> <i>S. infantarius</i> subsp. <i>coli</i>	<i>S. infantarius</i> subsp. <i>infantarius</i> <i>S. lutetiensis</i>
<i>S. bovis</i> biotype II/2	<i>S. pasteurianus</i> <i>S. macedonicus</i>	<i>S. gallolyticus</i> subsp. <i>pasteurianus</i> <i>S. gallolyticus</i> subsp. <i>macedonicus</i>

Table 1. Nomenclature of *Streptococcus gallolyticus*

7.1. Virulence factors and possible carcinogenic effect of *S. gallolyticus*

Boleij et al [92] reconstructed the route of infection in vitro on a continuous cell line of heterogenous human epithelial colorectal adenocarcinoma cells that can be synthesized into

a monolayer and which simulate the intestinal epithelium. Cellular immune responses upon infection and bacterial biofilm formation were analysed. The *S. gallolyticus* strains have a relatively low adhesiveness and are unable to internalise epithelial cells. However, they are able to cross a differentiated epithelium without inducing an interleukin 8 or 1β response within the epithelium. The organism has a particular ability to form biofilms on collagen-rich surfaces (representing heart valves in vivo). The authors concluded that *S. gallolyticus* has the ability to evade the innate immune system of the intestinal epithelium and the potential to form vegetations over collagen-rich surfaces as is observed in vivo.

7.2. Association with liver disease and extracolonic malignancy

S. gallolyticus has also been associated with chronic liver disease. Tripodi et al prospectively studied 199 patients with infective endocarditis and found that 30 of these were attributable to *S. bovis* biotype I (*S. gallolyticus*). [93] 56.7% of these patients had advanced liver disease, compared with only 15.3% of patients with non-*S. bovis* endocarditis, while colonic adenomas were present in 46.7% of cirrhotics. Alazmi and his team [94] retrospectively analysed microbiology data from 46 patients (38 adult and 8 paediatric) with proven *S. gallolyticus* bacteraemia and found that 19% had end-stage liver disease while colonic neoplasia was found in 6 of 10 adult patients in whom colonoscopy was performed. 7 of the adult patients had AIDS while no significant association with gastrointestinal disease was found in the paediatric population. An association between *S. gallolyticus* and extracolonic malignancy is less well established. Gold et al [95] report a series of 45 patients with documented *S. gallolyticus* bacteraemia. Eight of these patients had malignant lesions arising within the gastrointestinal tract, and 5 patients had extraintestinal malignancies. Vergara-López et al looked at 93 patients with *S. gallolyticus* bacteraemia [96] and found that 25% of individuals had a colonic neoplasm while 14 patients (15%) were diagnosed with non-colonic neoplasms including biliary and pancreatic (6.5%) and esophagogastric (3.2%) neoplasms. In view of these observations, the authors recommend that in the absence of colonic neoplasms clinicians should do a thorough investigation of the gut with gastroscopy and appropriate imaging of the hepatobiliary system.

8. Conclusion

A considerable body of evidence links colonic neoplasms with *S. gallolyticus* bacteraemia but many unanswered questions remain about this association. Evaluation of the colon by colonoscopy is essential in all cases of *S. gallolyticus* bacteraemia. In view of the high incidence of chronic liver disease and extracolonic neoplasms in some studies, formal evaluation of the liver may be warranted with or without cross sectional imaging of the abdomen. In the future, biomarkers for this organism may allow early diagnosis of colonic neoplasia.

8.1. Selective decontamination of the digestive tract

In critical illness, sepsis plays a major role in morbidity and mortality. Bacterial translocation from the gut is believed to occur following loss of the barrier function of the intestinal

mucosa. Selective decontamination of the digestive tract (SDD) involves the use of local and systemic antimicrobial agents to clear potentially pathogenic organisms from the gastrointestinal tract, especially Gram negative organisms, *Staphylococcus aureus* and yeasts, while avoiding agents that inhibit the anaerobic flora. Reduction of the Gram negative bacterial load would be followed by a decrease in sepsis and bacteraemia. However, in spite of the evidence in favour of SDD, it is still not in widespread use in intensive-care units (ICU).

SDD involves the combination of orally administered non-absorbed antibiotic and antifungal agents with an intravenous broad spectrum antibiotic. A regimen that has been used in several major studies consists of orally administered amphotericin-B, tobramycin and colistin.[97,98] Along with the topical agents, intravenous cefotaxime is also given for the first four days of ICU stay. The systemic antibiotics should cover both community-acquired organisms and hospital-acquired organisms while having minimal influence on the normal bowel flora and good penetration to bronchial secretions, making cefotaxime an ideal candidate.[99] The enteral non-absorbable antibiotics are intended to prevent secondary endogenous infections but they fail to cover resistant organisms such as MRSA. Silvestri et al [100] have added oral vancomycin to Polymyxin E, tobramycin and amphotericin B in an attempt to decrease the incidence of MRSA ventilator-associated pneumonias (VAP). This combination was effective in reducing the incidence of VAP and secondary carriage of MRSA with no reported cases of vancomycin-resistant Enterococci or vancomycin-intermediate *Staphylococcus aureus*. In most randomised controlled trials, SDD has been compared to Selective Oropharyngeal Decontamination (SOD) and standard care. SOD involves local application of non-absorbable antibiotics restricted to the oropharynx, usually applied in the form of a gel. The topical antimicrobial combination for SOD is usually similar to the combination used in SDD. Studies have shown that both SDD and SOD are useful in preventing sepsis in critically ill patients but few studies have analysed the effect of their use on the prevalence of resistant organisms within ICUs. This remains an area that needs further study and is a major issue that precludes the widespread use of SDD.[101]

8.2. The evidence on SDD

Many randomised controlled trials (RCT) have been performed over the last decade studying the benefits and risks of SDD. An important recent RCT studied the effect of SDD and SOD on 28-day mortality in ICU patients.[97] 5939 patients in 13 different ICUs in the Netherlands were enrolled to receive either standard care, SDD or SOD. SDD included the application of topical tobramycin, colistin and amphotericin B to the oropharynx and stomach along with the intravenous administration of cefotaxime for the first four days of ICU stay. 28-day mortality was marginally reduced from 27.5% in patients treated with standard care to 26.6% and 26.9% in the SDD and SOD groups respectively. Another RCT looked at the role of oropharyngeal and intestinal colonisation with gram-negative bacteria as a source of ICU-acquired bacteraemia.[102] This trial randomised a total of 6778 ICU patients to receive SDD, SOD or standard care. The outcomes measured included the incidence densities (episodes per 1000 ICU patient days) of ICU-acquired gram-negative

bacteraemia and rectal colonisation with gram-negative bacteria. SOD gave a 33% reduction while SDD gave a 45% reduction in the incidence of Gram-ve bacteraemia.

In another study [103], 107 patients with more than 20% burns and/or suspected inhalation injury were randomised to receive SDD or placebo and mortality rates and incidence of pneumonias were measured. A similar antibiotic regimen to the one used in [97] was used but topical polymixin E substituted colistin. Results showed an ICU mortality of 27.8% in the placebo arm compared to 9.4% in the SDD arm. Rates of pneumonia were 30.8 and 17.0 per 1000 ventilator-days in the placebo and the SDD arms respectively. The authors also noted that MRSA infection was commoner in the SDD group amounting to 26.4% versus 20% in the placebo group. Various other trials have been summed up by three major meta-analyses (Table 2).[104-106]

Name and year of meta-analysis	Number of trials/number of patients	Clinical end points studied	Results	Conclusions
Silvestri et al 2007 [106]	51 trials 8065 patients	BSI Causative organisms Total mortality	Significantly reduced in SDD group OR 0.73 Significantly reduced G-BSI without increasing G+BSI Reduced in SDD group	NNT to prevent 1 G-BSI is 20 NNT to prevent one death is 22
Silvestri et al 2008 [105]	54 trials 9473 patients	Carriage of G- bacteria Carriage of G+ bacteria G- RTI G- BSI	Significantly reduced Not significantly changed Significantly reduced Significantly reduced	SDD mainly targets G- bacteria and does not show a significant increase in G+ bacterial infections. SDD was better than SOD at reducing carriage of and severe infections due to G- bacteria
Liberati et al 2009 [104]	36 trials 6914 patients	Rate of RTI Mortality	Significantly reduced in both SOD and SDD groups Significantly reduced in SDD but not in SOD	SOD alone reduces RTI but not mortality while SDD reduces both

Table 2. Meta-analysis on benefits of SDD in preventing sepsis. (BSI blood stream infection, RTI respiratory tract infection, SDD selective decontamination of the digestive tract, SOD selective oropharyngeal decontamination, G+ gram positive, G- gram negative, NNT numbers needed to treat, OR odds ratio)

8.3. Prevention of Ventilator-Associated Pneumonia (VAP)

Pneumonia is a major cause of mortality in critically ill and ventilated patients. The incidence of VAP in different studies ranges between 7 and 40% while mortality ranges from 25 to 50%.[107] In an important meta-analysis carried out by Liberati et al [104], 36 RCTs studying the effects of different combinations of SDD and SOD in ICU patients on the incidence of VAP were analysed. This showed that in trials comparing combined topical and systemic antibiotics to controls, there was a significant reduction in both VAP and mortality in the treated group. In trials comparing topical antibiotics to controls, a significant reduction in VAP (but not in total mortality) was shown.[108] Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a common cause of VAP. In a study by Silvestri et al [106], oropharyngeal vancomycin was applied along with standard SDD using only enteral non-absorbable antibiotics in a group of ventilated ICU patients. The rate of pneumonia due to MRSA was reduced in the vancomycin group when compared to controls who received only the topical SDD. Patients in this study were also investigated for the emergence of vancomycin-resistant Enterococci and vancomycin-intermediate *S. aureus* but these bacteria were not isolated. This suggests that the addition of topical glycopeptides to the SDD regimen may help reduce the rate of MRSA though further studies are needed before this approach can be recommended.

8.4. Evidence supporting use in surgical patients

Roos et al [109] studied the incidence of infections and anastomotic leakage 30 days following surgery in 289 patients receiving either topical SDD or placebo. Results show that 19.6% of the SDD group had infectious complications when compared to 30.8% in the placebo group. Anastomotic leakage was also reduced in the SDD group (6.3% vs 15.1%). In spite of this, there was no significant difference in mortality or hospital stay between the two groups. Melsen et al [110] compared the benefits of SOD and SDD in surgical and medical ICU patients. 2762 surgical and 3165 non surgical patients were randomised to receive SDD, SOD or standard care. Compared with standard care, mortality was comparable in SDD treated surgical and non surgical patients though the duration of ventilation, ICU and hospital stay were significantly reduced in the surgical patients. SOD failed to reduce mortality when compared to standard treatment in the surgical cohort while providing a reduced mortality by 16.6% in non-surgical patients. Patients undergoing liver transplant are very vulnerable to infection during the early post-operative period, particularly with gram-negative organisms. SDD has been studied in these patients in several RCTs [111-113] and meta-analyses [114]. The results have been conflicting and several small RCTs failed to show any benefit of SDD over standard care following liver transplant.

9. Conclusion and recommendations

The evidence so far shows a decrease in 28 day mortality and reduction in bacteraemia in high risk patients and suggests that SDD should be regularly used in ICU settings. However SDD is still not common practice in most ICUs as many intensivists still question its safety

and efficacy. In a UK based survey of ICUs to document the use of SDD [106], 95% of British centres did not use SDD, mainly because of concerns regarding resistance. In addition there is a reluctance to use intravenous antibiotics in many of those who used SDD in intubated patients. Convincing the medical world of the effectiveness and safety of SDD will require more robust data about antibiotic resistance with SDD and SOD.

9.1. *Clostridium difficile* infection

Clostridium difficile is an anaerobic, spore-forming, gram-positive rod found in the intestines of 2-5% of the healthy human population [115] but responsible for 16-25% of hospital-acquired antibiotic-associated diarrhoea.[116] It has been recognised as an important cause of antibiotic-associated colitis since the introduction of clindamycin in 1977 when it was understood that the disturbance of bowel flora by antimicrobial agents allowed overgrowth and subsequent infection by *Clostridium difficile*. [117] Transmission occurs through the feco-oral route via contact with contaminated surfaces, with the hands of healthcare workers being potential routes of contamination. Vegetative bacterial cells produce spores in conditions of stress, making them resistant to commonly used techniques of surface disinfection such as alcohol handrubs, most disinfectants and antibiotics. It is however susceptible to chlorine-based antiseptics such as diluted bleach.[118] The spectrum of disease is wide and ranges from asymptomatic carriage to fulminant pseudomembranous colitis which may be fatal.

Toxin synthesis by *C. difficile* mediates disease progression and the severity of illness. The potent exotoxins produced by *C. difficile* have been labelled A and B. They are both large monoglycosyltransferases that catalyse the glucosylation and inactivation of Rho-GTPases, the small regulatory proteins of the actin cell cytoskeleton, leading to disruption of the cell cytoskeleton and subsequent cell death. Some strains of *C. difficile* produce an unrelated binary toxin which consists of two separate components: CDTa and CDTb. CDTb mediates translocation of CDTa into cells which allows the disruption of cytoskeleton proteins through phosphorylation, ultimately causing cell death.[118] The virulence of different strains of *C. difficile* is related to the rate of toxin production. Hypervirulent strains such as the molecular type NAP1/027/BI, have been found to have more robust toxin production and show an earlier spore-formation than other strains thus causing more severe infections.[119] Excessive toxin production in this strain has been traced to a mutation in the Toxin B encoding gene sequence.[120] Another emerging strain is the PCR ribotype 078, which is associated with community-associated *C. difficile* infection and has been isolated in animal and food products.[121]

9.2. Epidemiology

The emergence of *C. difficile*-associated disease (CDAD) can be traced back to the start of the antibiotic era. Antibiotic-associated diarrhoea and colitis became well established and *C. difficile* was identified as the cause of most of these cases in 1978. The earliest cases were

attributed to clindamycin but later, as the use of cephalosporins and wide spectrum penicillins increased, these antibiotics were increasingly implicated as causes of CDAD. An important outbreak in the US between 1989 and 1992 was traced to a strain of *C. difficile* with resistance to clindamycin.[122] Since 2003, an increase in the incidence of CDAD was observed, along with a decrease in their response to the standard antibiotic regimens. The hypervirulent strain NAP1/027/B1 was identified as a cause of several outbreaks in North America and Europe and is believed to be related to the increase in use of fluoroquinolones, to which this strain is particularly resistant.[123,124]

9.3. Risk factors for CDAD

Antibiotic use is the strongest factor associated with CDAD. The most important mechanism involves the disruption of normal colonic commensal bacterial populations providing a niche for *C. difficile* to multiply and produce toxins. Resistance to antibiotics plays an important role in infections due to strains with increased virulence such as the NAP1/027/B1 strain.[125] Antibiotics commonly implicated include fluoroquinolones, clindamycin, broad-spectrum penicillins and cephalosporins. However, all antibiotics (including metronidazole and vancomycin) can predispose to *C.difficile* infection by disrupting the anaerobic gut flora. In fact it is hypothesised that the same antibiotics used for treating CDAD might be responsible for the recurrence of CDAD after treatment.[126] It has been shown that both the use of broad-spectrum antibiotics and prolonged courses of antimicrobials increase the risk of CDAD.[127,128] Advanced age is also an important risk factor associated with CDAD prevalence and severity. The increased frequency of comorbidities places elderly patients at higher risk of mortality and serious infections though compromised immune function also plays an important role.[126] The role of gastric acid suppression with proton pump inhibitors has also been implicated in the pathogenesis of CDAD though the evidence is equivocal.

9.4. Diagnosis and investigations

In most cases of suspected CDAD, the clinical presentation and microbiological evidence of toxin-producing *C. difficile* in stools is sufficient for diagnosis. The clinical picture may include bloody diarrhoea with abdominal pain and tenderness and ileus with abdominal pain, vomiting and reduced bowel motility. Pseudomembranous colitis can be diagnosed by the visualisation of pseudomembranes at endoscopy while toxic megacolon presents with characteristic radiologic findings.[129] The markers of disease severity are outlined in Table 3. Microbiological evidence of infection is obtained from stool culture or assay for stool *C. difficile* toxin (CDT). Different tests can be used to detect the toxins. The most widely used test is the enzyme immunoassay (EIA) for toxin A, toxin B or both. The EIA CDT assay has sensitivities and specificities of 50-90% and 70-95%, respectively. Diagnostically, *C.difficile* cell culture cytotoxin assay remains the gold standard with sensitivity and specificity of 93% and 89%.[130]

Physical findings	Blood investigations	Imaging studies
Fever, rigors, haemodynamic instability (including vasodilatory or septic shock), signs of peritonitis, (including decreased bowel sounds, abdominal tenderness, rebound tenderness and guarding), signs of ileus (including vomiting and absent passage of stool). Admixture of blood with stools is rare in CDI and the correlation with severity of disease is uncertain	marked leukocytosis (leukocyte count $> 15 \times 10^9/L$) marked left shift (band neutrophils $>20\%$ of leukocytes) rise in serum creatinine ($>50\%$ above the baseline) elevated serum lactate distension of large intestine	colonic wall thickening including low-attenuation mural thickening pericolonic fat stranding ascites not explained by other causes The correlation of haustral or mucosal thickening, including thumbprinting, pseudopolyps and plaques with severity of disease is unclear.

Table 3. Markers of severe disease [127]

9.5. Treatment

The management of CDAD is tailored to the severity of the condition. The treatment recommended by the ESCMID guidelines (2009) [129] is summarised in Table 4.

Degree of severity	Mild (stool frequency <4 times daily, no signs of colitis)	Moderate (no markers of severe disease)	Severe (any marker of severe disease)
Oral treatment possible	Stop antibiotics and observe closely	Metronidazole 500 mg tds orally for 10 days	Vancomycin 125 mg qds orally for 10 days (A-I)
Oral treatment not possible		Metronidazole 500 mg tds intravenously for 10 days (A-III)	Metronidazole 500 mg tds intravenously for 10 days (A-III) + intracolonic vancomycin 500 mg in 100 mL of normal saline every 4–12 h (C-III) and/or vancomycin 500 mg qds by nasogastric tube (C-III)

Table 4. Treatment of *C.difficile* infection [129]

Oral vancomycin may be replaced by teicoplanin 100mg twice daily. Other antibiotics have been shown to be effective in CDAD but are not as yet recommended for routine use. In a phase 3 clinical trial [131], fidaxomicin had a better response rate and a lower recurrence rate than standard dose vancomycin. Oral rifaximin was studied on comparatively smaller

numbers. Neff et al [132] report three liver transplant patients with moderately severe CDAD who had relapsed after treatment with metronidazole and did not tolerate vancomycin. All three showed a good response after 28 days of rifaximin 400mg three times daily. In another small study [133], there was only one recurrence after treatment of 8 patients with rifaximin for ten days. If severe disease does not respond to medical therapy, surgical intervention may be necessary. Indications for colectomy include perforation of the colon and systemic inflammation with deteriorating clinical condition not responding to antibiotic therapy. This includes the clinical diagnoses of toxic megacolon and severe ileus. Colectomy should preferably be performed before colitis is very severe. Serum lactate may serve as a marker of severity with surgery ideally performed before lactate exceeds 5.0mmol/L.[127]

9.6. Recurrence and the role of fecal transplant

Recurrence of infection is defined as the recurrence of symptoms due to incomplete clearance of the initial infection. 15-30% of patients with CDAD experience recurrent infections in spite of seemingly adequate treatment.[134] Various combinations of antibiotics (Table 5) have been suggested for the management of recurrent infections as well as measures to normalise the intestinal flora using probiotics or fecal transplantation. Healthy donor fecal installation has been proposed as a way to restore normal bowel flora in patients with CDAD recurrence not responding to antibiotics. Several studies have been performed to date with most showing favourable results [135] but the lack of well designed RCTs makes the evidence weak and more studies are needed before it can be formally recommended in the guidelines.

First recurrence	Second recurrence	Third recurrence
<i>Mild to moderate infection -</i> Metronidazole at a dose of 500 mg orally three times daily for 10 to 14 days <i>Severe infection or unresponsive to or intolerant of metronidazole -</i> Vancomycin at a dose of 125 mg orally four times daily for 10 to 14 days	Prolonged vancomycin orally in tapered and pulsed doses, for example: 125 mg four times daily for 14 days 125 mg twice daily for seven days 125 mg once daily for seven days 125 mg once every two days for eight days (four doses) 125 mg once every three days for 15 days (five doses)	Vancomycin at a dose of 125 mg orally four times daily for 14 days, combined with any of the other options for recurrent infection (not evidence based): Intravenous immunoglobulin at a dose of 400 mg per kg body weight once every three weeks, for a total of two or three doses depending on effect. Vancomycin, followed by rifampicin at a dose of 400 mg twice daily for 14 days Healthy donor fecal implantation

Table 5. Management of CDAD recurrence [134]

10. Conclusion

International guidelines [136] have issued a list of evidence-based infection control measures intended to contain outbreaks of CDI within hospitals. Measures include the strict use of hand hygiene using soap and water, the use of gloves and gowns when approaching an infected patient, isolation of infected patients in single rooms and maintaining contact precautions for the duration of diarrhoea. Routine identification and treatment of carriers is not recommended. Identification of potential sources of infection, such as rectal thermometers, can help reduce the incidence of CDAD. Frequent use of chlorine-containing cleaning agents to disinfect the clinical area along with routine environmental screening for *C.difficile* are also recommended. Restricting the use of cephalosporins and clindamycin may also be useful. The frequency, duration of antibiotic courses and number of agents used should be as recommended by international guidelines. Implementing these recommendations was shown to be of benefit by the Centre for Disease Control and Prevention [137] in several hospitals in the USA with a decline in *C. difficile* infection (CDI) rate of 20% among 71 hospitals participating in the CDI prevention program, thus confirming that with *C. difficile* infections, prevention is better than cure.

10.1. Probiotics

The term *probiotic*, first introduced in 1965 by Lilly and Stillwell, describes bacterially-derived factors that stimulate the growth of other organisms. This definition was updated by Fuller in 1989 who defined probiotics as viable organisms with a beneficial effect on the host. Fermented ingredients containing no viable organisms but that still cause beneficial changes in the intestinal flora are termed *prebiotics*, while *symbiotics* are mixtures of pre- and probiotics. *Lactobacillus*, *Bifidobacterium*, *Eschericia coli*, *Saccharomyces cerevisiae* and *Bacillus* species are the microflora most commonly used in probiotics.[138] The healthy human gut hosts a large community of microorganisms that interact with the host in a positive manner. Disruption of the normal gut flora by antibiotics or infections causes a change in bowel function, most frequently resulting in diarrhoea. It has been proposed that the normal commensal flora occupies most binding sites on the intestinal mucosa and out-competes potentially pathogenic organisms, thus providing a protective effect on the host. Probiotics are believed to function in a similar way to the normal commensal flora by colonising the intestinal contents so as to prevent the proliferation of potentially pathogenic organisms by competing for resources and intestinal binding sites. Probiotics may also lead to an improvement in intestinal barrier function, modulation of the immune system by induction of protective cytokines and modulation of pain perception.

Probiotics have been around for decades and are available in different formulations including capsules, powders and fermented milk products. However, evidence of their benefit has been relatively scarce until recently as most studies were hampered by poor standardisation in view of the different species and strains used. Species used vary widely as do the number of viable organisms and their resistance to gastric acid. Some examples of commercially available probiotics are: Erceflora (*Bacillus clausii*), Align® (*B. infantis*),

Bioflor® (*Saccharomyces boulardii*), Culturelle® (*L. rhamnosus* GG), DanActive® (*L. casei*), Mutaflor® (*E. coli* Nissle 1917), Florastor® (*Saccharomyces boulardii*), and VSL#3® (*Bifidobacterium breve*, *B. longum*, *B. infantis*, *Lactobacillus acidophilus*, *L. plantarum*, *L. paracasei*, *L. bulgaricus*, *Streptococcus thermophilus*). The evidence on the use of probiotics in inflammatory bowel disease and pouchitis has been described earlier.

10.2. Infectious diarrhoea

Infectious diarrhoea is a major cause of morbidity and mortality especially in third world countries. Most studies with probiotics analysing the effect on diarrhoea duration have been in paediatric patients and they show a significant benefit. In a meta-analysis [139] of 63 studies of which 56 involved infants and young children, there was a significant decrease in the mean duration of diarrhoea (mean difference 24.76 hours; $n=4555$, trials=35), diarrhoea lasting ≥ 4 days (risk ratio 0.41; $n=2853$, trials=29) and stool frequency on day 2 (mean difference 0.80; $n=2751$, trials=20). However, there was a wide variation in the probiotics used, patient characteristics and clinical settings. When probiotics are used in conjunction with rehydration therapy they appear to be safe and have clear benefits in shortening the duration of diarrhoea and reducing stool frequency in acute infectious diarrhoea.

4 randomised controlled trials ($n=464$) comparing specified probiotic agents with placebo or no treatment in children with persistent diarrhoea (diarrhoea lasting more than 14 days) [140] showed that probiotics reduced the duration of persistent diarrhoea by a mean of 4.2 days and significantly reduced stool frequency at day 5. In a randomised controlled trial that randomised 88 children younger than two years old with acute diarrhoea to receive *S.boulardii* or placebo there was an average reduction in the duration of diarrhoea of 1.44 days in the treatment arm along with a significant reduction in stool frequency at day 4.[141] The dose-dependent effect of administering *L. rhamnosus* on fecal shedding of rotavirus was analysed in another study. 23 children with acute rotavirus infection were randomised to placebo, low-dose or high-dose *L. rhamnosus*. [142] This trial showed no significant reduction in viral shedding in the low dose group but a significant reduction in the high dose group suggesting that a minimum of 6×10^8 CFU (colony forming units) for 3 days has to be given to paediatric patients to achieve a good effect. Other studies [143] also suggest a definite but modest benefit in probiotic use in acute infectious diarrhoea, especially in rotavirus-induced diarrhoea.

10.3. Antibiotic-associated diarrhoea and *Clostridium difficile*-associated disease

Antibiotic-associated diarrhoea (AAD) occurs in about 25% of patients receiving antibiotics, with rates varying between different populations and according to the type of antibiotic used.[144] *Clostridium difficile* accounts for only 10-20% of cases and a causative agent is frequently not found. Diarrhoea may begin following a single dose of antibiotic or up to 6 weeks after treatment [145] and can range in severity from mild symptoms to the life-threatening colitis usually associated with *C. difficile*. Risk factors for AAD include oral broad-spectrum antibiotics, advanced age and prolonged hospital stay. Probiotics have been

advocated to reduce the incidence of AAD since they help re-establish beneficial intestinal flora after disturbance by antibiotics. Probiotic organisms that have been studied for preventing AAD include *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus rhamnosus* GG and *Saccharomyces boulardii*.

Several meta-analysis have highlighted the positive effects of probiotics on AAD. In [146], 8 RCTs (n=1220) evaluating the effectiveness of probiotics in preventing AAD and CDAD were analysed. Probiotics used included *S. boulardii* in 3 studies and various strains of *Lactobacillus*, *Bifidobacterium bifidum* and *Streptococcus thermophilus* in different combinations in the other 5 studies. Results were found to be protective for AAD (Risk Ratio [RR]: 0.56; 95% CI, 0.44–0.71) as well as for CDAD (RR: 0.29; 95% CI 0.18–0.46). In [147], different strains of *Lactobacillus* as single agents in the prevention of AAD were analysed in 10 RCTs (n=1862). The total daily dose of *Lactobacillus* ranged from 2×10^9 to 4×10^{10} CFUs and was administered throughout the entire antibiotic treatment (5-14 days) for all patients. The combined RR of developing AAD was significantly lower with *Lactobacillus* when compared with placebo (RR 0.35, 95% CI 0.19-0.67). In a subgroup analysis, this benefit was seen among adult but not among pediatric patients (RR 0.24, 95% CI 0.08-0.75 and RR 0.44, 95% CI 0.18-1.08, respectively). Considerable evidence backs the use of probiotic agents (especially *Lactobacillus* species and *S. boulardii*) as an extra measure to prevent AAD and CDAD.

10.4. Probiotics in IBD

Probiotics alter the microbial concentrations of the intestines and may also be used to deliver microbial metabolic products which affect intestinal mucosal inflammation in IBD. There is little evidence of benefit with currently available probiotics in CD though newer probiotics composed of other micro-organisms may prove beneficial in the future. On the other hand, studies have shown a benefit of probiotics in recurrent and relapsing antibiotic sensitive pouchitis and in mild UC. In fact, recent practice guidelines [148] on the management of pouchitis suggest that in patients with prompt recurrence of pouchitis following antibiotic cessation, and in those with multiple recurrences of pouchitis despite antibiotics, either VSL#3™ or chronic use of antibiotics may be helpful. These guidelines however do not recommend probiotics in the acute treatment of pouchitis.[148]

Probiotics may prevent relapse in chronic pouchitis and ulcerative colitis, and may also prevent the development of pouchitis postoperatively. However, further studies are needed to identify optimal dosing, duration of therapy, delivery methods and whether blends of different strains of probiotics are superior to single strains.[149] Following a systematic review of studies using VSL#3™, *E.coli* Nissle 1917 and Yakult™, Mallon et al concluded that the addition of probiotics to conventional medical therapy had no effect in overall remission rate in mild to moderate UC.[150-152] Other randomized controlled trials have also shown conflicting results with some showing a higher rate of remission (with VSL#3 or a combination of a prebiotic and *B.longum*) [153-155] and others showing little or no benefit (with *E.coli* Nissle 1917).[156] There are no recommendations regarding the use of probiotics

as maintenance therapy in UC. Few studies have been carried out using single or combined strain probiotics as maintenance therapy in UC with 3 of 4 single probiotic trials using *E.coli* strain Nissle 1917. The results from these reports showed that probiotics had similar efficacy to 5-aminosalicylates.[153,157-159] In children with active distal ulcerative colitis, decreased mucosal inflammation was noticed following rectal infusion of *Lactobacillus reuteri*. [160] Even non-living probiotic bacteria may prevent the onset of severe intestinal inflammation by strengthening the integrity of the intestinal barrier and stabilising the environment for gut microbiota.[161,162]

The risks of probiotic use are generally low, but cases of fungaemia in ICU patients on *S. boulardii* and a case of sepsis from a *Lactobacillus* strain in a UC patient have been reported.[163,164] An important consideration before starting probiotics is whether the patient is on immunosuppressing agents. There is no evidence for the use of probiotics in severe IBD and little clinical evidence on the safety of probiotics in severely immunocompromised IBD patients.

10.5. Mortality of preterm infants with necrotising enterocolitis

Necrotising Enterocolitis (NEC) is an important cause of morbidity and mortality in preterm and very low birth weight (<1500g) infants. There is strong evidence [165-167] that the administration of enteral probiotics plays an important role in establishing benign commensal flora and preventing NEC and its complications. In these studies, the most commonly used species were *Bifidobacterium* and *Lactobacillus* species.[168] Very few adverse events from probiotics have been reported and they are thus being recommended as evidence-based treatment.[169]

10.6. Irritable Bowel Syndrome

Irritable Bowel Syndrome (IBS) is a heterogeneous group of disorders characterised by functional bowel symptoms such as abdominal pain, bloating and changes in bowel habit in the absence of other pathologies which might explain these symptoms. IBS is typically difficult to treat as its aetiology is still poorly understood. Targeting the intestinal flora with probiotics has been an attractive potential treatment and has shown some promise in several meta-analyses.[170-174] These studies showed a modest improvement in the patients' symptoms when using strains like *S. boulardii* and *Lactobacillus rhamnosus* GG. Longer term studies with specific strains are warranted to clarify the most appropriate species and long-term effects with probiotics.

11. Conclusion

The emergence of probiotics as a popular type of alternative medicine has preceded by several decades their promotion as an evidence-based treatment. Their role in treatment or prevention for several important conditions namely NEC, UC, pouchitis and AAD is expected to fuel further research as many unanswered questions still remain. In spite of

many large trials the data is still relatively weak to allow specific recommendations on which probiotics to prescribe in specific conditions. Optimum dose recommendations also remain to be clarified.

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