**Clostridium difficile: a high-cost nosocomial pathogen**

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*C. difficile*-associated diarrhoea is increasing patients' suffering and bruising hospital budgets.

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**Bacterial Diseases of Cultivated Fish**

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Review of the bacterial causes of farmed fish diseases and the measures being taken to control them.
Clostridium difficile: a high-cost nosocomial pathogen

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Introduction
Over the last 15 years Clostridium difficile has risen from relative obscurity to be one of the most important hospital pathogens of the 1990s. Data from Sir Charles Gardiner Hospital (SCGH), in Perth, Western Australia, is typical of many, but not all, similar hospitals in developed countries. SCGH is a 650-bed adult university teaching hospital. During the period 1983 to 1992, C. difficile was detected in 917 patients who were being investigated for diarrheal illness. Most of these patients were elderly, with 63% aged 60 years or more, and 59% were female. The number of patients infected each year ranged from a low of 49 patients in 1983 to a high of 120 patients in 1990. When rates were calculated, using occupied bed days as the denominator, a similar trend was observed with the incidence increasing from 23/100,000 occupied bed days in 1983 to 56/100,000 occupied bed days in 1990.

As many pathogens that have emerged or re-emerged in the last two decades there are, after careful consideration, logical explanations for these apparent changes. Two factors may be particularly important in the rapid rise in infection with C. difficile. First, increased and inappropriate use of certain broad-spectrum antibiotics may be predisposing more patients to infection with C. difficile. Second, contamination of the hospital environment with C. difficile represents a significant problem. This paper will address these two aspects in some detail and then examine the implications for healthcare systems of the increasing incidence of C. difficile-associated diarrhoea (CDAD).

Furthermore, general information about C. difficile may be found in an excellent review, while other reviews deal with laborator intake and epidemiology of infection.

Agents capable of inciting C. difficile-associated diarrhoea
The ability of antimicrobial agents to induce diarrhoea was recognised long as a causative agent. Collitis is a common complication of ampicillin therapy, while clindamycin-associated colitis reached epidemic proportions in some parts of the USA in the early 1970s and was, in part, responsible for the research interest which eventually identified C. difficile as a causative agent.

The list of antimicrobial agents which have been shown to induce CDAD is endless though few studies have systematically assessed the risks of CDAD associated with exposure to a particular antimicrobial agent. In addition, much of the data pertaining to antimicrobial agents are old and do not take into account many newer agents, particularly third-generation cephalosporins (see below). Paradoxically, both vancomycin and metronidazole, agents which have been used successfully to treat CDAD, are also capable of inducing CDAD. Table 1 shows the spectrum of antimicrobial agents known to be related to the development of CDAD.

Several recent papers have indicated that exposure to clindamycin is still a significant risk factor predisposing to C. difficile infection. However, in terms of actual usage, third-generation or extended-spectrum cephalosporins are probably of greater importance. The increase in incidence of CDAD at SCGH between 1983 and 1990 correlated with an increase in the use of third-generation cephalosporins (Pearson's correlation coefficient, 0.80). Aronsson et al. investigated the relative risk of CDAD for different groups of antibiotics and found that cephalosporins were 40 times more likely to be implicated than ureidopenicillins, while Zimmermann, using a case-control methodology, determined an odds ratio for third-generation cephalosporins of 3.00 (p = 0.04), after controlling for horizontal transmission by matching on location. Third-generation cephalosporins are known to have a greater impact on colonisation resistance than many other antimicrobial agents.

In an apparently unique study, Yarinsky and Wheeler reviewed the appropriate-ness of prior antibiotic use and the develop-ment of CDAD. They found that only 26% of antibiotic administration in hospitalised patients was appropriate with positive culture results and organisms susceptible to the antibiotic(s). Another 26% of cases had positive cultures but the organisms were not susceptible to the antibiotic used while, in 50% of cases of CDAD, empiric antibiotic therapy in the absence of positive cultures led to the development of CDAD. While the costs to the healthcare system of CDAD are discussed more fully below, it may be worthwhile to highlight the cost of many of the antimicrobial agents known to be responsible for inciting CDAD. Apart from vancomycin, third-generation cephalosporins are among the most expensive antibiotics available today.

All the above studies have concentrated on antibiotics inciting CDAD in a hospital setting. Recently, Hirschhorn et al. examined CDAD in the community setting and calculated antibiotic-specific attack rates. These varied from 0 to 2040 cases per 100,000 exposures and were significantly higher for nitrofurantoin, cefuroxime, cephalaxin plus dicloxacillin, ampicillin/clavulanate plus cephaloridine, ampicillin plus dicloxacillin, and ampicillin plus cloxacillin.

Antibiotics are not the only agents capable of inciting CDAD. The role of antineoplastic agents in C. difficile infection was recently reviewed by Anand and Glatt. They reported on 23 cases of CDAD associated with antineoplastic therapy published in the literature. A variety of agents has been implicated, most commonly methotrexate. Laboratory evidence is available to support the case for these agents inciting CDAD, however, the mechanism of pathogenesis is less clear. Chemotherapeutic agents can alter the gut flora in a manner analogous to many antibiotics and this is probably the most important predisposing factor. The biggest problem in trying to ascertain the importance of antineoplastic agents in CDAD is that many patients who develop CDAD have been exposed to both antibiotics and antineoplastic agents.

Finally, it is worthwhile mentioning that any compound, process or illness which affects the gastrointestinal flora, either qualitatively or quantitatively, may reduce 'colonisation resistance' and thus predispose individuals to infection with C. difficile. At one stage it was thought that C. difficile may be responsible for inflammatory bowel disease and Crohn's disease when C. difficile could be isolated during exacerbations of these diseases. However, it is now apparent that the disease process itself alters the gut flora sufficiently to allow colonisation (at the very least) with C. difficile. In the same way, mechanical bowel preparations predispose patients who are
having gastrointestinal surgery. Also gastrointestinal infections caused by other bacteria, viruses or protozoa may alter the gut flora sufficiently to allow *C. difficile* to establish.

All the above, however, predates the requirement of exposure to viable cells of *C. difficile*. There is still great debate on what is the true reservoir of *C. difficile* and the importance of the various sources identified.

**Reservoirs and sources of *C. difficile***

Many sources of *C. difficile* have been recognised with the usual reservoir of *C. difficile* infecting humans is endogenous or exogenous, or if both are of epidemiological significance, has been debated for several years. As our knowledge increases, however, it seems less likely that carriage of *C. difficile* by healthy adults, reported as zero to 3%, is an important factor. In *in vitro* experiments it is suggested that the theory that *C. difficile* cannot survive in a healthy adult gastrointestinal tract; however, during infections the diseased gut disperses many organisms into the surrounding environment. The infected human gastrointestinal tract is probably the most important reservoir of infection.

Gastrointestinal carriage of *C. difficile* in humans is influenced by a number of factors including age, exposure to antibiotics and the environment to which the subject is exposed. As a consequence, reports on gastrointestinal carriage of *C. difficile* vary from country to country, hospital to hospital and, in some cases, ward to ward.

Between 15 and 70% of neonates and 30 to 65% of infants less than one year old are infected with *C. difficile*, both toxigenic and non-toxigenic, without any apparent ill-effect. The variation in levels of colonisation reflects environment contamination in nurseries, while colonisation occurs to the extent it does in some neonates and infants because of the lack of protective flora. The reasons why the vast majority of neonates and infants remain well are still being debated. Whether the absence of toxin receptors in the immature gastrointestinal tract or a protective effect of larger amounts of mucus as a more adult gastrointestinal flora becomes established towards the end of the second year of life, the proportion of infants colonised declines significantly.

Transient carriage in normal adults must occur frequently, particularly in hospital patients, as *C. difficile* spores will be ingested regularly. Whether true colonisation occurs, and for what length of time, is dependent on the extent of gut perturbation. As mentioned above, in the presence of a normal gastrointestinal flora *C. difficile* will not survive. Various *in vitro* experiments have confirmed that an abnormal gastrointestinal flora is required before *C. difficile* can flourish.

Diarrhoeal hospital patients provide a rich source of *C. difficile* to be spread either via person to person transmission (probably of less importance) or via the hospital environment. CDAD in other settings has not been investigated extensively although it is likely to be endemic in many nursing homes. Several recent studies, including that of Hirschhorn et al., have focused on the importance of the various sources identified.

### Table 2: Potential animal reservoirs of *C. difficile*.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Reservoir</th>
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<tbody>
<tr>
<td>Camels</td>
<td>Endogenous</td>
</tr>
<tr>
<td>Cattle</td>
<td>Endogenous</td>
</tr>
<tr>
<td>Donkeys</td>
<td>Endogenous</td>
</tr>
<tr>
<td>Horses</td>
<td>Endogenous</td>
</tr>
<tr>
<td>Antelopes</td>
<td>Endogenous</td>
</tr>
<tr>
<td>Kodiak bear</td>
<td>Endogenous</td>
</tr>
<tr>
<td>Dogs</td>
<td>Endogenous</td>
</tr>
<tr>
<td>Hamsters</td>
<td>Endogenous</td>
</tr>
<tr>
<td>Numbats</td>
<td>Endogenous</td>
</tr>
</tbody>
</table>

*Taken from Levett, 1981* and Riley et al., 1980

The extent and importance of this environmental contamination has become apparent following the recent study by McFarland et al., 1984 which clearly demonstrated an association between extensive contamination of the environment and acquisition of *C. difficile* among patients.

The state in which *C. difficile* is found in the environment is also likely to be of importance. Vegetative forms of *C. difficile* will not survive for a long time, however, *C. difficile* spores (see Figure 1) can remain viable for many months, resulting in a significantly increasing number of spores over time. Thus most infections will result from exposure to and ingestion of *C. difficile* spores.

![Figure 1: Electron micrographs of *C. difficile*:](a:termal s, b: discrete spores. (Courtesy of Professor S.P. Borriello.))
spores and more work is required to determine ways of reducing the spore load in the environment of susceptible patients. Bacterial spores are relatively resistant to many commonly used disinfectants and few studies have specifically examined inactivation of C. difficile spores by disinfectants. One such study showed that C. difficile spores were susceptible to glutaraldehyde-based disinfectants providing that a concentration of greater than 2% was used.

**Costs associated with CDAD**

What then are the costs attached to CDAD? Direct medical costs are relatively easy to measure although this has been done infrequently. We have recently estimated hospitalisation charges related to CDAD at SCGH with a matched retrospective cohort study using all SCGH discharges for the year 1990. Cases were matched with controls for age (±5 years), sex, date of admission (±1 month), diagnosis-related group and major diagnostic category. The median length of stay for cases was 17 days compared with 14 days for controls. Perhaps even more important, there was a significantly different mortality rate (21% for cases compared with 7% for controls, p = 0.0009) and morbidity rate (14% for cases compared with 4% for controls, p = 0.004). Clearly this is the most significant intangible cost of CDAD, relating to patient pain, suffering and quality of life.

Other categories of cost which need to be considered include the direct non-medical costs of accessing healthcare facilities; however, for CDAD these are likely to be negligible as, in the majority of cases, disease develops during a period of hospitalisation. Indirect costs associated with CDAD are likely to be similar to other situations which result in loss of productivity through work days missed. Unfortunately, few investigations of costs associated with infectious diseases take these into account; however, with an increased hospital stay on average of at least 18 days these costs are likely to be significant for those patients still in the workforce.

**Conclusions**

In summary, diarrhoea caused by C. difficile is certainly a costly problem, particularly if the figures presented here are representative of other hospitals in developed countries. Two factors, antibiotics and environmental contamination, make C. difficile such an important pathogen in hospitals today. Attempts to prevent nosocomial transmission in hospitals will only ever be partially successful due to the nature of the organism and environmental contamination. Until hospital administrators recognise that in order to control CDAD they need to control antibiotic policy, C. difficile will continue to be a costly problem.

**References**


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**New Oxoid High Resolution Medium for Antifungal Susceptibility Testing**

Unipath Ltd has introduced Oxoid High Resolution Medium to the standard range of culture media.

H R Medium is a chemically-defined medium used for the susceptibility testing of antifungal agents. It is the only commercially available culture medium which has been specifically developed for in vitro testing of fluconazole.

Opportunistic fungal infections have become increasingly important in recent years and are a major cause of morbidity and mortality in immunocompromised individuals. The increasing incidence and life threatening nature of these infections has resulted in more frequent use of systemic antifungal agents and the introduction of new agents.

Fluconazole is among the most notable of the new agents, having highly effective, broad-spectrum antifungal activity.

**References**


For further information contact: Valerie Kane, Unipath Limited, Wade Road, Basingstoke, Hants RG24 8PW, England. Tel: (01256) 841144. Fax: (01256) 463398.
Bacterial Diseases of Cultivated Fish

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Introduction
One textbook on bacterial fish pathogens lists 77 bacterial species associated with disease.1 Some of the more important bacterial diseases are listed in Table 1. Diseases of the salmon family will be highlighted in this article, reflecting the author’s main interests and the fact that these infections are of considerable economic importance and interest in many parts of the world.

Bacterial diseases of fish are most often considered in the context of fish cultivation. Large numbers of animals are reared in close proximity to one another and infection may readily be transmitted within a population. However, it should not be forgotten that most, if not all, bacterial diseases of fish originate in the wild and that some can have an important impact on wild fish stocks.

Furunculosis

Furunculosis, caused by Aeromonas salmonicida, is of great importance. The name is a misnomer as the necrotic swellings found occasionally in the musculature of chronically infected fish (Figure 1) are quite different from the pus-filled boils or abscesses which occur in mammals. Acute cases of disease in juvenile fish are characterised by a rapidly-fatal septicemia with few clinical signs.

A. salmonicida was first isolated in 1894 and has been recorded throughout Europe as well as in Canada, USA, Japan and Australia. Typical strains mainly affect salmonid fish, but atypical strains can cause disease in many different fresh water and marine fish species. Serious furunculosis epizootics have been recorded in wild fish populations, but the major impact of the disease in recent years has been on salmon farming. In the late 1980s, annual losses of an estimated 15-20% of stock due to BKD threatened the commercial viability of Atlantic salmon farming in Scotland.2

A. salmonicida is readily isolated from the major internal organs — especially kidney — on tryptone soya agar (TSA) or other routine media. The organism does not grow at 37°C, but at 22°C colonies are usually apparent within 48 hours (Figure 2). Isolation of the bacterium from asymptomatic carriers — which act as reservoirs of infection in wild or farmed fish populations — can be difficult due to the small numbers of bacteria present in such fish.

Bacterial Kidney Disease (BKD)
Bacterial kidney disease (BKD), caused by Renibacterium salmoninarum,3 was first described in the 1930s in wild Atlantic salmon in Scotland. It is a serious infection of farmed and wild salmonid fish and has been recorded in Canada, USA, Chile, Japan and several European countries. In British Columbia, annual losses due to BKD have been estimated at $30-40 million.

The disease is systemic, slowly progressive and often fatal. Severely affected fish may show no obvious external signs apart from abdominal distension. Internal signs may include fluid in the abdominal cavity, a membranous layer on one or more organs and, most characteristically, cream-coloured turbidity in the kidneys. Renibacterium salmoninarum, the causative agent, is a rod-shaped gram-negative bacterium that grows optimally at 22°C. It can be isolated from kidney, heart and other internal organs. The bacterium is not normally isolated from asymptomatic carriers, and has a low prevalence in wild fish populations. The main control strategy for BKD is the use of live vaccines, which work by stimulating the production of antibodies against the bacterium.

Table 1: Some important bacterial diseases of fish.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Pathogen</th>
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<tbody>
<tr>
<td>Furunculosis</td>
<td>Aeromonas salmonicida</td>
</tr>
<tr>
<td>Bacterial kidney disease (BKD)</td>
<td>Renibacterium salmoninarum</td>
</tr>
<tr>
<td>Enteric redmouth (ERM)</td>
<td>Yersinia ruckeri</td>
</tr>
<tr>
<td>Vibriosis</td>
<td>Vibrio anguillarum</td>
</tr>
<tr>
<td>Coldwater vibriosis</td>
<td>Vibrio salmonicida</td>
</tr>
<tr>
<td>Rainbow trout fry syndrome (RTFS)</td>
<td>Flexibacter psychrophilus</td>
</tr>
<tr>
<td>Columnaris disease</td>
<td>Flexibacter columnaris</td>
</tr>
<tr>
<td>Salmonid rickettsiosis</td>
<td>Piscirickettsia salmonis</td>
</tr>
<tr>
<td>Pasteurellosis</td>
<td>Pasteurella piscicida</td>
</tr>
<tr>
<td>Streplococcus</td>
<td>Straploccocus iniae</td>
</tr>
<tr>
<td>Edwardsiella septicaemia</td>
<td>Edwardsiella tarda</td>
</tr>
<tr>
<td>Edwardsiella ictaluri</td>
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</table>

*now Photobacterium damselae subsp. piscicida.16

Figure 1: Deep furuncle (arrowed) in the dorsal musculature of an adult wild Atlantic salmon.

Figure 2: Colonies of Aeromonas salmonicida on Tryptone Soy Agar (TSA) after 48 hours' incubation at 22°C.

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Rapid presumptive diagnosis of disease can be achieved by ELISA of kidney tissue.

3-5 weeks though in some cases can be alleviated by growing contaminants, and this problem must be alleviated by using selective media. Rapid presumptive diagnosis of BKD can be achieved by ELISA of kidney tissue.

Rapid presumptive diagnosis of BKD can be achieved by ELISA of kidney tissue.

Y. ruckeri is a small Gram-negative, peritrichous-flagellated, non-sporing rod. The organism is readily isolated from internal organs on routine media such as TSA. (At least six serotypes exist. Although some strains can grow at 37°C, incubation temperatures of 18-25°C are routinely used for isolation. Transmission of ERM occurs from fish to fish through water, though isolation of Y. ruckeri from sea gulls suggests a possible role for birds in the spread of infection.

Vibrios
disease is fastidious, having an absolute require­
ment for L-cysteine. and slow-growing is fastidious, having an absolute require­
ment for L-cysteine. and slow-growing (Figure 3).

Vibrio anguillarum infections are usually associated with high sea water temper­
atures and high population densities whether in farmed or wild fish. There are at least 10 serotypes of V. anguillarum. Acute disease can result in mortality of farmed salmon with few if any gross lesions. In contrast, we often observe deep-seated muscle lesions in chronically-infected wild salmon.

V. salmonicida is responsible for cold­
water vibriosis or Hitra disease in Atlantic salmon. This disease, which is most severe at low sea water temperatures, has caused heavy losses in Norway since the 1970s and has also been recorded in Scotland, Canada and the Faroe Islands. Clinical signs may include haemorrhage on the fin base and the abdominal wall. Internally, haemorrhage in the abdominal cavity, on the swimbladder and within the abdominal fat is typical (Figure 4). The organism can usually be isolated in large numbers from internal organs on NaCl-supplemented media at 15°C. In contrast to V. anguillarum, isolates of V. salmonicida from different geographical origins appear to be biochemically and serologically homogeneous.

Other significant diseases
Rainbow trout-fry syndrome (RTFS) is a disease of juvenile rainbow trout caused by systemic infection with the filamentous Gram-negative rod Flexibacter psychrophilus syn. Cytophaga psychrophila. The disease 'emerged' in Europe in the late 1980s and is now widely distributed. In fact the causative organism had been isolated in the USA more than 30 years earlier in association with a condition known as bacterial cold-water disease (Figure 5). In Scotland losses due to RTFS may exceed 50% on affected farms and it is becoming apparent that F. psychrophilus infections now pose a threat to the commercial success of trout farming in Europe. While

![Figure 2: Typical strains of A. salmonicida produce brown pigment on tryptone soya agar.](image2)

F. psychrophilus causes disease at relatively low water temperatures, the closely related organism F. columnaris — causative agent of columnaris disease — is more pathogenic at high water temperatures.

In southern Chile, a rickettsial disease has caused mortalities in salmonid fish since the late 1980s. Recurring epizootics have caused devastating losses in the region with mortalities approaching 90% in some coho salmon farms. The causative organism, *Piscirickettsia salmonis*, is a member of the Rickettsiaceae but 16S rRNA analysis has shown that it is not specifically related to any previously described intracellular pathogen. Similar organisms have also been reported recently in British Columbia, Norway and Ireland.

Other important bacterial fish diseases include pasteurellosis, a disease of sea bass and sea bream caused by *Pasteurella piscicida*, streptococcosis, a disease caused by *Streptococcus iniae* and other *Streptococcus* spp., which has been responsible for serious losses of yellowtail in Japan; and *Edwardsiella* septicaemia, a major disease of catfish in the USA caused by *E. tarda* and *E. ictaluri*.

**Disease control**

At the farm level the first approach to disease control is through husbandry practices aimed at minimising stress, overcrowding and physical trauma. Proper hygiene and management techniques also play an important role. For example, in Scotland it is common practice in salmon farming to grow just one year-class of fish at any one site. When the fish are harvested, all nets and equipment are cleaned and disinfected and the site remains empty or fallow for a period of weeks or months before the next year-class of fish is introduced and the cycle repeated.

Most bacterial diseases are potentially amenable to antibiotic treatment. In the UK, just four antibacterials are licensed for use in fish — oxytetracycline, oxolinic acid, trimethoprim-sulphadiazine and amoxycillin. All are prescription-only medicines and as such may only be administered under...
supervision of a veterinary surgeon. Antibiotics are usually given orally, by incorporation in the feed, though in certain circumstances they may be injected. To assure public safety, fish may be slaughtered for human consumption only after they have undergone a statutory withdrawal period following treatment to ensure that unacceptable antibiotic-residue levels are not present. None of the UK-licensed antibacterials are effective against BKD.

The development or acquisition of resistance to antibacterial agents imposes serious constraints on antibiotic therapy. Both plasmid-mediated and mutational resistance have been reported widely in fish pathogens. This makes susceptibility testing of clinical isolates essential and the choice of therapy at times problematical. Some workers have expressed concern that the potential exists for transfer of resistance genes from fish pathogens to human pathogens, though others have argued equally strongly that the risk to public health is very low.  

Highly effective vaccines have been developed against certain bacterial diseases of fish. In Europe, vaccines are now commercially available for the control of ERM, vibriosis, cold-water vibriosis and most recently furunculosis. The development of effective furunculosis vaccines followed a period of extensive investigation of virulence mechanisms of the causative agent. With the exception of some ERM vaccines, which may be administered by immersion, most vaccines are administered to fish by injection. In Scotland, our annual survey showed that some 20.7 of the 23.1 million farmed salmon smolts produced in 1994 were injection-vaccinated against furunculosis.

Legislation exists in some countries to prevent the introduction and spread of fish diseases. In the UK, two bacterial diseases—furunculosis and BKD—are notifiable under the Public Health Act, 1937 and 1983. Under this legislation live fish and eggs may be prevented from moving into or out of infected fish farm sites. In the case of BKD, for which there are no effective antibiotic treatments or vaccines, statutory movement restrictions have proven highly effective in restricting the spread of the disease.

Future research

Current methods of pathogen detection (e.g. direct culture, ELISA) are adequate for the diagnosis of clinical disease. However, they are not sufficiently sensitive for the detection of the very small numbers of pathogenic bacteria which may exist in asymptomatic carriers. Molecular biology techniques can provide sensitive tools for pathogen detection, as shown by the finding that a DNA probe coupled with PCR can be used to detect as few as two *R. salmoninarum* cells in a salmon egg. DNA probes are also available for *A. salmonicida* and a number of other pathogens. However there appear to be practical difficulties in the application of PCR to fish tissues and these need to be overcome before really sensitive techniques become available for pathogen detection in fish.

Vaccination of fish by injection is labour-intensive, and a major goal in fish vaccinology is the development of effective oral vaccines. The development of vaccines, oral or otherwise, against a number of economically important diseases, not least BKD, RTFS and salmonid rickettsiosis, are other important goals.

References