

*Clostridium Difficile*  
Infection

Prevention and Management

A Report by a Department of Health/  
Public Health Laboratory Service  
Joint Working Group

## Foreword

There has been increasing concern in recent years about hospital outbreaks of infection due to *Clostridium difficile* which have been prolonged and difficult to control. The infection causes illness ranging from brief diarrhoea to life threatening pseudomembranous colitis and in some outbreaks has led to a number of deaths. Patients who have received antibiotic therapy, particularly the elderly, those with serious underlying disease and surgical patients are at the greatest risk. It is likely that the infection will be a continuing problem as demographic changes create an increasingly elderly in-patient population.

There has been uncertainty about the most effective methods of preventing and controlling the infection and in particular about the role of asymptomatic colonised patients and the hospital environment as possible sources of infection. This has led to increasing numbers of requests to the Public Health Laboratory Service and the Department of Health and for advice on the management of outbreaks. In response, a joint DH/PHLS Working Group was established under the Chairmanship of Dr. E. Mary Cooke, Deputy Director of the PHLS. This is the report of the Working Group.

The report is based on information derived from an extensive review of the literature and also to a large extent on the practical experience of the members of the group. It became apparent during the course of the review that published research is lacking in the areas of pathogenesis and modes of spread of the infection.

We hope that you will find the guidance in the report useful in managing any outbreak, in developing policies for the management of individual cases of *C. difficile* infection and in contributing to the development of effective antibiotic prescribing policies.

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The Prevention and Management of  
***Clostridium Difficile***  
Infection

A Report by the Joint DH/ PHLS  
Working Group

Department of Health  
Public Health Laboratory Service

1994

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## Summary

- ◆ ***C. difficile* infection causes serious illness and outbreaks among hospital in-patients. It affects especially the elderly, the debilitated and patients who have had antibiotic treatment.**  
Paragraphs 2.9-2.18
- ◆ **Appropriate antibiotic usage in hospitals is essential in the prevention and control of *C. difficile* infection. Adoption of an antibiotic policy is recommended.**  
Paragraphs 2.10-2.13 and 5.2 – 5.3
- ◆ **The infection may be acquired directly from other infected patients, from health care staff or from the environment.**  
Paragraphs 2.22-2.28
- ◆ **Routine infection control procedures, including thorough hand-washing by all staff are essential for prevention of *C. difficile* infection.**  
Paragraphs 5.4-5.7 and Appendix V
- ◆ **The diagnosis of *C. difficile* infection is based on the detection of toxin in the stools.**  
Paragraphs 1.5 and 6.1-6.4
- ◆ **Patients who are colonised with or transient carriers of the organism are not an important source of infection. Screening and treatment of asymptomatic patients is unnecessary.**  
Paragraphs 2.33 and 5.21
- ◆ **Patients with diarrhoea and suspected of infection should be isolated until they have formed stools.**  
Paragraphs 5.13-5.18 and Appendix V
- ◆ ***C. difficile* spores persist in the hospital environment. Thorough cleaning is important. The use of environmental disinfectants is unnecessary.**  
Paragraphs 2.26-2.28, 5.27-5.29, Appendix V

## Chapter 1– Introduction

### The need for guidance

- 1.1 There has been a steady rise in the number of *Clostridium difficile* infections, particularly over the last decade. Laboratory reports to the Communicable Disease Surveillance Centre (CDSC) have shown an eight-fold and fourteen-fold increase for isolates and for *C. difficile* toxin respectively, both rising sharply for 1991-1992 due to one large outbreak<sup>1</sup>. There is concern that outbreaks in hospitals are increasingly prolonged and difficult to control. The rise in the number of infections has been accompanied by an increase in the number of requests to the Department of Health and Public Health Laboratory Service (PHLS) for advice.
- 1.2 In response a joint Department of Health/Public Health Laboratory Service working group chaired by the PHLS Deputy Director, Dr. E. Mary Cooke has produced this guidance for infection control teams, consultants in communicable disease control and clinicians.

### The nature of the organism

- 1.3 *C. difficile* is a gram-positive anaerobic bacillus that forms subterminal spores. These are resistant to exposure to air, drying and heat, survive in the environment and are considered to be the main transmissible form of the organism. *C. difficile* is distinguished from other clostridia on the basis of biochemical tests and the toxins it produces. These include two major toxins that are linked to its pathogenicity – toxin A, which causes loss of fluid from the gut mucosa, and toxin B, a potent cytopathic toxin.

### Clinical features

- 1.4 *C. difficile* infection is nearly always associated with, and triggered by, the use of antibiotics, prescribed to treat another condition of given prophylactically. It affects predominantly the colon and may result in a wide spectrum of disease ranging in severity from trivial diarrhoea, through moderately severe disease with abdominal pain, diarrhoea and systemic upset to life-threatening pseudo-membranous colitis (PMC) with toxic megacolon, electrolyte imbalance and even perforation of the bowel. Most patients experience abdominal pain with explosive watery foul-smelling diarrhoea<sup>2,3</sup>. Some also have a fever. The diarrhoea can be constant for about forty-eight hours and is therefore very distressing and debilitating, particularly for elderly patients. Recurrence of diarrhoea following apparently successful treatment is common, occurring in up to 20% of cases<sup>4,5</sup>. This is thought in most cases to be due to the germination of persistent spores rather than to new infection. These patients may continue excreting *C. difficile* for long periods.
- 1.5 Examination of the stools of patients with *C. difficile* diarrhoea reveals not only the presence of the organism but also, and more importantly for diagnostic purposes, the toxins it produces. Throughout this document, the term “*C. difficile* infection” refers to patients who exhibit symptoms due to *C. difficile* infection and who are toxin positive.

- 1.6 Systemic infection with *C. difficile* is rare and usually occurs in association with infection with other organisms. Previously suggested links with a number of other conditions have not been substantiated. However, fourteen cases of gut infection associated with reactive arthritis have been reported in the literature; six of ten cases tested were HLA B27 positive, and toxin A may be the trigger factor<sup>6</sup>.
- 1.7 Investigations during hospital outbreaks have revealed that many patients in the vicinity of affected cases become colonised with the organism and may remain asymptomatic carriers, probably for a considerable period. No toxins can be demonstrated in stools from these patients. The management of carriers and the extent of their role in the transmission of infection are issues about which there has been much uncertainty and to which the Working Group has given particular attention.
- 1.8 *C. difficile* infection appears to be a disease largely confined to hospital in-patients, reflecting the fact that this group is more vulnerable, more likely to be given antibiotic treatment and more likely to be exposed to the infection. No outbreaks have been identified in the general community, although sporadic cases occur as they do in hospitals<sup>7</sup>. Many of these infections may well be of endogenous origin. In the community these patients are unlikely to create any public health hazard and may often pass unrecognised.

### **Epidemiological trends**

- 1.9 Surveillance of *C. difficile* infection in England and Wales is maintained by the CDSC of the PHLS through the national voluntary laboratory reporting system. Isolates of the organism and detection of *C. difficile* toxins are both reported.
- 1.10 Annual totals of reports to CDSC of infections caused by *C. difficile* increased markedly between 1982 and 1992 (Table 1). These data should, however, be treated with caution because they may, at least in part, reflect increased investigation and reporting rather than a true increase in incidence of *C. difficile* disease. In 1981 reports were received from 48 laboratories compared with 141 in 1992; some reported only isolates, some only toxin and some both and the figures suggest difference in practice between laboratories. These data do not distinguish between colonised and infected patients.
- 1.11 The increase in *C. difficile* infections has been most marked amongst older patients especially those over age 65 (see paragraph 2.14). Outbreaks are likely to become increasingly common in the future, at least in the developed world, as the average age of hospital patients rises.

### **Costs to the health care system**

- 1.12 There are few data concerning the cost of *C. difficile* infections in the UK. However, one outbreak involving 175 cases with 17 deaths in three hospitals over six months was estimated to cost in excess of £100,000. This included the following components, for which precise costing was possible. The list does not

include the costs incurred when service was disrupted due to closure of wards and cancellation of routine admissions, which are much more difficult to quantify.

Antibiotic therapy:	Vancomycin	£12,797
	Metronidazole	£580
Major identifiable costs:	Nursing Time	£50,000
	Laboratory costs	£5,000
	Ward cleaning	£6,581
	Consumables	£10,600
	Posters and written publicity <sup>8</sup>	£2,600

*C. difficile* outbreaks are clearly expensive and disruptive to hospital activity. In-patient care of affected patients is prolonged. Wards are likely to have to be closed temporarily with consequent difficulties in continuing to admit both urgent and waiting list patients. Prompt and vigorous control measures taken as soon a problem of *C. difficile* infection is recognised are in the interest of vulnerable patients, and extremely cost effective.

### **Future research and work**

1.13 There is still much that is not known about the pathogenesis of *C. difficile* disease. A clearer understanding of the infecting dose and routes of transmission of the organism as well as of the factors mediating susceptibility to it is crucial to improve prevention of *C. difficile* infection. The role of the environment in cross-infection and how best to decontaminate it require further investigation, as does the possibility that severe cases may be due to particularly virulent clones. The likelihood of cross-infection occurring would be reduced by therapeutic advances to reduce the recurrence rate. The best treatment approach for recurrences of infection is not yet certain and future research into this area is also needed. Infection control doctors (ICDs), Consultants in Communicable Disease Control (CCDCs), clinicians and academics could all contribute and the Working Group would urge them to undertake studies to investigate *C. difficile* infectivity, pathogenicity, epidemiology and control.

**TABLE 1**

*Clostridium difficile*  
**Laboratory Reports to CDSC 1982 – 1991**  
**England and Wales**

<b>Year</b>	<b>Faecal isolates</b>	<b>Toxin in stool</b>
1982	165	121
1983	263	216
1984	240	300
1985	426	308
1986	480	594
1987	492	412
1988	682	430
1989	638	483
1990	673	555
1991	821	761
*1992	1263	1681
*1993	2074	3163

\* provisional data

## Chapter 2 – Current Knowledge of *C. difficile* infection

### Recognition of the Pathogenic Role of *C. difficile*

- 2.1 Although PMC has been known as a clinical entity since the late 19<sup>th</sup> century, interest in its aetiology was stimulated by reports of cases associated with tetracycline or chloramphenicol usage in the 1950s and 60s<sup>9,10</sup> and clindamycin usage in the 1970s<sup>11</sup>. The cases in the 1950s were attributed to *Staphylococcus aureus*<sup>10</sup> though retrospective analysis of the evidence, particularly in view of the present knowledge of the role of *C. difficile*, casts doubt on the general conclusions reached then<sup>12</sup>. However, this does not exclude the possibility that some past and present cases are due to *S. aureus*<sup>13</sup>.
- 2.2 In 1975 (published 1977<sup>14</sup>) a cytopathic effect was observed when a faecal filtrate from a patient with PMC was applied to a tissue culture. Use of a rodent model of antibiotic-associated diarrhoea<sup>15,16,17</sup> revealed a similar effect from the faecal contents of hamsters<sup>18</sup> and it was found that these effects could be neutralised by antitoxin to *C. Sordellii* toxin<sup>17</sup>. These observations led to the suggestion that *C. Sordellii* was implicated in the pathogenesis of PMC, despite the fact that it was not a component of the faecal flora of either disease hamsters or patients with PMC. Shortly afterwards, *C. difficile* (which had first been described in 1935<sup>19</sup>) was isolated from diseased hamsters and was shown to induce disease in healthy animals and to produce a cytotoxin which could be neutralised by *C. Sordellii* antitoxin<sup>20,21</sup>. The demonstration that *C. difficile* was also present in the faeces of patients with PMC soon followed<sup>22-25</sup>.
- 2.3 It is now well established that infection with *C. difficile* is most frequently nosocomial<sup>1</sup> and that outbreaks of antibiotic-associated diarrhoea and/or PMC occur. In 1979 retrospective analysis of stool samples collected from a cluster of clindamycin-associated cases reported in 1974 showed that they had been due to *C. difficile*<sup>12</sup>, and this represented the earliest proven outbreak.
- 2.4 Appendix II lists the critical observations in the history of our understanding of *C. difficile* infection. Those interested in this subject are directed for more details information to publications by Bartlett<sup>26,27</sup>, Willis<sup>13</sup> and Borriello<sup>28</sup>.

### Published Information

- 2.5 There is abundant evidence in the literature that *C. difficile* may be acquired nosocomially and cause outbreaks of disease. However, interpretation of the reports is difficult. Some authors fail to distinguish clearly between patients with symptomatic infection and those who were only colonised, and different definitions or diarrhoea and methods of diagnosis are used. The difficulty in developing typing methods that are sufficiently unambiguous and sensitive and that are readily available for routine use has hampered research into this infection.
- 2.6 Similarly, there is confusion over what constitutes an outbreak of *C. difficile* disease. In addition to the problems already mentioned, it can be difficult to

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<sup>1</sup> Hospital acquired

determine whether what is being reported is a “true! Single strain outbreak or results from better ascertainment of unrelated cases because of increased awareness of the problem. This would lead to increase investigation of in-patients with diarrhoea in an environment in which antibiotics are used widely. It can have expensive consequences, as shown by Hall *et al*<sup>7</sup> (See Chapter 4). Evidence to support the occurrence of cross-infection has generally been indirect, often with no typing of the strains involved, and the mode of transmission remains uncertain.

- 2.7 The Working Group has assessed the published evidence on nosocomial transmission, risk factors for infection and methods of prevention and control of outbreaks. Appendix III lists a number of outbreak reports and epidemiological studies. They reflect the diversity in the literature in terms of number, age and types of patients, diagnostic methods and the period over which the study was conducted. The Appendix also summarises the identified risk factors and the control measures instituted or recommended by the authors.
- 2.8 The information presented in the literature can be sub-divided into factors relating to patients’ susceptibility to infection and factors relating to the mode of transmission and these are considered in turn. Each section sets out the views of the Working Group, based on the information available and the members’ experience, on the main factors that are of practical importance in preventing both sporadic cases and outbreaks of *C. difficile* disease. We are conscious that there are areas where lack of scientific information makes it very difficult to give definitive advice; in these instances the advice offered is based on the combined experience of the Group.

### **Factors Affecting Susceptibility to Infection**

- 2.9 Many risk factors have been proposed for acquiring *C. difficile* infection<sup>29-39</sup> but few studies have quantified their magnitude in a clinically useful way. Proposed risk factors have included antibiotic treatment, age, cytotoxic agents, intensive case, naso-gastric intubation, concurrent illness and alteration in gut motility.

#### *Antibiotic treatment*

- 2.10 The administration of antibiotics is the most significant and most frequently reported predisposing factor for *C. difficile* infection and has been implicated in most nosocomial outbreaks, for instance in those reported by Worsley<sup>1</sup> and by Thibault *et al*<sup>38</sup>. It appears that the main defence against *C. difficile* infection is the possession of normal bowel flora. Disruption of the normal flora as a result of antibiotic treatment allows *C. difficile* to establish itself. However, other factors are also involved in the pathogenesis of the infection as the disease was recognised in the pre-antibiotic era and may occur in patients who have not received antibiotics. These cases may follow disruption of the bowel flora through other mechanisms.
- 2.11 Although most antibiotics have been associated with predisposition to *C. difficile* infection, the most commonly implicated have been clindamycin, the cephalosporins and penicillins whether used alone or in combination<sup>40, 41</sup>. MacFarland and colleagues<sup>29</sup>, for instance, showed that the short term use (<1

week) of high dose cephalosporins (>10g cumulative) or the use of a broad-spectrum penicillin for > 7 days were associated with the occurrence of *C. difficile* infection. However, it is difficult to extrapolate from frequency of association to relative degree of risk of infection, as the former will also be dependent on the extent of usage of the antibiotic in question. By way example, clindamycin is no longer the most commonly implicated antibiotic, but this is probably a reflection of its decreasing use rather than any change in its tendency to predispose to *C. difficile* infection. Studies of oral antibiotics using the hamster model of disease demonstrated that the major difference between them was not the degree of susceptibility induced but rather the duration of that susceptibility<sup>42</sup>. Whether this is true in man remains to be established.

2.12 The use of multiple antimicrobial agents, whether given concurrently or sequentially, is particularly likely to increase the risk of infection<sup>38, 43</sup>. In the report of Gerding and co-workers<sup>43</sup>, patients with *C. difficile* infection, when compared with a suitable matched control group, were more likely to have received courses of multiple antibiotics used therapeutically. By contrast, controls had received antibiotics prophylactically more frequently than cases.

2.13 The route of administration is also important, as routes which minimise the effect on the gut flora are less likely to predispose to the infection. However, disease has been associated with oral, intramuscular, intravenous and even topical antibiotics. Parenterally administered aminoglycosides given alone have not been associated with the infection, and quinolones, trimethoprim and co-trimoxazole, also given alone, seem to be relatively low-risk<sup>2, 44-46</sup>. In studies with animal models chloramphenicol, tetracycline and sulphonamides have also failed to cause the condition or produced inconsistent results<sup>2</sup>. However the relevance of this work to human infection is uncertain, particularly since gentamicin, which seems not to cause disease in man, does do so in animals<sup>2</sup>.

### Age

2.14 *C. difficile* infection is more common in the elderly. The reasons for this have not been fully explained although there is some evidence that these patients have a less effective natural barrier to infection<sup>47</sup>. The importance of age as a risk factor is reinforced by the age distribution in laboratory reports received by CDSC during 1990-92. These are summarised in Table 2 which illustrates the bias towards older age groups, particularly those over 65 years, who in 1992 made up nearly two thirds of the reports. It is notable that whereas numbers of reports showed little change in this period among young children and a modest increase among older children and adults under 45, there was a more marked increase in those over 45, and a particularly steep rise in reports of patients aged over 65. Although denominator data are not available, these descriptive epidemiological findings are compatible with those of case control and other studies reported in the literature that age over 65 years is an independent risk factor for *C. difficile* disease.

2.15 Published reports of outbreaks<sup>31, 48</sup> indicate that most cases occur in the over 50s. Some case control and prospective cohort studies have included only the older age groups<sup>49</sup> or have controlled for age so that the variable has been lost

from the analysis. However, Borriello and Larson<sup>40</sup> reported that of 310 stool samples from patients suspected of suffering from PMC which they examined for *C. difficile* and its toxins, a disproportionate number of positive results were in patients over 50. Subsequently, a prospective cohort study of 399 consecutive adult patients of all ages revealed age as an important risk factor for both asymptomatic carriage and infection. Indeed age over 75 was found to be associated with the greatest relative risk, even when compared with antibiotic treatment, laxative abuse, the use to neo-gastric tubes, length of stay and severity of underlying illness<sup>29</sup>.

- 2.16 It has been suggested that *C. difficile* is endemic in many long-stay facilities for the elderly<sup>50</sup>. This has not been substantiated by others<sup>49</sup>, who suggest that the differences may be due to case mix, in particular whether many of the patients were admitted from other affected hospitals. Elderly patients also tend to stay longer than other patients in acute wards. Their high risk of infection with the organism may therefore to some extent simply reflect increased exposure to nosocomial pathogens and to antibiotics<sup>29, 43, 51-53</sup>.
- 2.17 The changing demography of the population in Western countries is likely to mean that the number of cases of *C. difficile* infection among hospital in-patients will increase steadily over the next decade. Figure 1 illustrates the projected increase in the “very elderly” population into the next century.
- 2.18 However, it must be remembered that the elderly are not the only patients who are vulnerable to this infection. The preponderance of reported cases in this group may to some extent reflect a disproportionate effort to isolate the organism from them when *C. difficile* is recognised as a problem in a hospital.

#### *Underlying Disease*

- 2.19 Published reports suggest that general surgical<sup>31</sup> and oncology patients<sup>30, 35-37, 54, 55</sup> and those with chronic renal disease<sup>32, 33</sup> are at a particular risk of infection. Of the surgical specialties, abdominal surgery has been associated with the greatest risk. Gerding *et al* showed a *C. difficile*-associated diarrhoea rate of 6-9% for general and vascular surgical patients compared with 0.2-0.6% for orthopaedic, ENT and thoracic surgical patients<sup>43</sup>. Another study confirmed localisation of *C. difficile* infection on the surgical unit, whereas carriers were spread evenly on all three study wards (surgical, medical and orthopaedic)<sup>51</sup>.
- 2.20 The higher risk of oncology patients acquiring *C. difficile* is presumably as a result of frequent hospital admissions, chemotherapy<sup>36</sup> and numerous courses of antibiotics, both as treatment and prophylaxis<sup>34</sup>. Carriage rates of 35-38% have been reported in neutropenic patients<sup>30, 35</sup> while general medical patients on the same ward had rates of only 12%. In both studies a significant proportion of those from whom *C. difficile* was isolated were colonised, not infected. Paediatric oncology units have been similarly affected<sup>34, 37</sup>.
- 2.21 *C. difficile* infection has also been reported from renal units<sup>32, 33</sup>, usually as outbreaks. Due to the chronic nature of their illness these patients too have attended hospital frequently, have had more admissions and more courses of

antibiotics for intercurrent infections than most other patients<sup>32</sup>. An outbreak over a 10 month period was described in which five patients, all sharing one of two cubicles, four within one month, were shown to have the same strain. Four of the patients died<sup>33</sup>. This not only suggests the possibility of cross-infection, it also highlights the severity of the disease in a vulnerable group.

### **Transmission from Infected Patients**

2.22 The extent to which exogenously infected patients acquire the organism directly from other patients with diarrhoea or from colonised patients is a matter for debate. The route of transmission may be direct, via healthcare workers or via the environment.

#### *Patient to patient spread*

2.23 Patients with *C. difficile* infection may excrete up to  $10^9$  organisms per gram of faeces<sup>56</sup> and the explosive nature of the diarrhoea, as well as causing contamination of the environment, may lead to considerable soiling of the patients skin, giving rise to the possibility of direct spread from patient to patient by the faecal-oral route, although the evident that this occurs is only circumstantial<sup>57</sup>. It is also conceivable though unproven, that the organism and its spores may be transmitted by aerosol.

2.24 Transmission of *C. difficile* leading to illness in healthcare workers has been documented<sup>58</sup>, though this is extremely uncommon.

#### *Spread via health care workers*

2.25 The organism can be isolated from the hands of health care workers treating patients infected with *C. difficile*<sup>57</sup> and this is a likely route of spread, as it is for many other nosocomial infections. Johnson *et al*<sup>59</sup> demonstrated a significant reduction in infection and carriage rates on two high risk hospital wards following the use of gloves when handling body substances.

#### *Spread via the environment*

2.26 The organism is widely distributed in the environment and has been isolated from soil, domestic pets and other animals<sup>60-62</sup>. However, the frequency with which it normally occurs in the hospital environment is difficult to ascertain.

2.27 Despite recent improvements in laboratory techniques, isolation of the organism from the environment is not easy and may be intermittent. Nevertheless, the spores of *C. difficile* have been found in abundance in the environment of infected patients, for example, from commodes, toilets, wheelchairs, floors, sinks and linen. They may persist on fomites and surfaces for long periods - five months or longer<sup>63</sup>. The spores may persist despite disinfection. In one report a bedpan and bedpan washer were repeatedly positive both before and after the washing cycle<sup>34</sup>. Areas of the hospital associated with more *C. difficile* infection have found to have higher rates of contamination when compared to the rest of the hospital<sup>54, 64</sup>, and in those environments contamination is highest in close

proximity to patients with diarrhoea<sup>65</sup>. In some studies, typing of the isolates has not been carried out and it has only been assumed that those patients and from the environment were the same<sup>34, 43, 64</sup>. However, in other studies, typing has confirmed the association. For instance, in one report of 18 cases<sup>57</sup>, isolates from the environment and from patients were of the same immunoblot type, and in another report<sup>66</sup> isolates were of the same bacteriophage-pacteriocin type. These findings suggest that transfer from symptomatic patients to the environment and from the environment to patients can occur.

2.28 Accounts of attempts to clear *C. difficile* from the hospital environment have not been encouraging. Kaatz *et al*<sup>66</sup>, using a hypochlorite spray containing 500 parts per million available chlorine achieved an 80% reduction in colony-forming units. Streulems *et al*<sup>67</sup> claimed a similar reduction using a disinfectant based on aldehydes, the concentration of which was sufficiently low as to make sporicidal action a remote possibility in the short time available before evaporation of a thin layer from a surface would have occurred<sup>68</sup>. Both these reduction are more consistent with the physical removal of bacteria during cleaning processes than of chemical disinfection<sup>69</sup>. Kaatz *et al*<sup>66</sup> also used 1600 parts per million available chlorine buffered to pH 7.6, a very rapidly sporicidal agent and found a reduction of 98% in numbers of *C. difficile*, a result more consistent with true disinfection. Unfortunately, this concentration and pH of hypochlorite is highly corrosive and unsuitable for long-term use on either hard or soft surfaces in the hospital environment. Decontamination of medical equipment is fortunately more straightforward; those items not amenable to steam sterilisation can be adequately decontaminated by a ten-minute expose to 2% alkaline buffered glutaraldehyde<sup>68</sup>.

### Asymptomatic Carriers

2.29 Various studies indicate that 2-3% of healthy adults and up to 36% of hospitalised patients harbour *C. difficile*<sup>70, 71</sup> in their faecal flora. Although *C. difficile* disease is not a problem in neonates, studies in new born infants have found higher rates of up to 65% in hospitalised infants<sup>72</sup>. These carriage rates may be explained by lack of colonisation resistance, associated with a developing and incomplete intestinal flora<sup>28</sup>. Following weaning and establishment of an adult pattern of gut flora, carriage rates fall dramatically.

2.30 All the evidence suggests that in patients with illness due to *C. difficile*, the stools always contain toxin and that in the great majority of asymptomatic carriers, no toxin is present. These patients are colonised by, rather than infected with, the organism. There has been much controversy about the part played in nosocomial transmission of infection by these colonised patients. Some authors consider that they may act as a reservoir<sup>50, 55, 71</sup>. However, some reports, which suggest that they were the source of cross-infection in particular instances, do not make clear how much prior environmental contamination had already occurred and whether there were other sources of infection. Anecdotal evidence indicates that patients colonised with *C. difficile* are not a significant factor in outbreaks. Experience with other pathogens which can be carried asymptotically for long periods, such as salmonella, is that patients without diarrhoea and with normal hygiene standards are very unlikely to give rise to spread of infection.

- 2.31 It seems reasonable to suppose that colonised patients are at risk of endogenous infection if they are treated with broad spectrum antibiotics, although this seems not to have been demonstrated. Whether patients colonised with *C. difficile* disseminate spores of the organism if they develop diarrhoea from some other cause is a question which merits further research.
- 2.32 There is little evidence to suggest that asymptomatic bowel carriage in medical and nursing staff is a risk either to the staff themselves or to patients.
- 2.33 Attempts to eradicate carriage by treatment with Vancomycin or Metronidazole have been reported<sup>50, 55, 71, 73</sup>. Vancomycin was effective at clearing the stools but was associated with a high recurrence rate; Metronidazole was no better than placebo<sup>73</sup>. The lack of evidence that colonised patients act as a source of infection, combined with the high cost and lack of proven efficacy of the treatment of colonised patients have led the Working Group to a firm view that they should not be treated with antibiotics. (See paragraph 5.21 on control of outbreaks).

**TABLE 2****Reports of C difficile Isolates and Toxin Detection to  
the Communicable Disease Surveillance Centre (CDSC)****Age Distribution 1990 – 1992**

	0-4y	5-44y	45-64y	>65y	Age not stated	Total
<b>1990</b>						
Isolates	97	93	93	325	65	673
Toxin	38	73	71	313	60	555
<b>1991</b>						
Isolates	85	106	96	462	72	821
Toxin	37	98	117	439	70	761
<b>*1992</b>						
Isolates	85	134	156	783	105	1263
Toxin	71	170	187	1141	112	1681
<b>Ratio 1992:1990</b>						
Isolates	0.9	1.4	1.7	2.4	1.6	1.9
Toxin	1.9	2.3	2.6	3.6	1.9	3.0

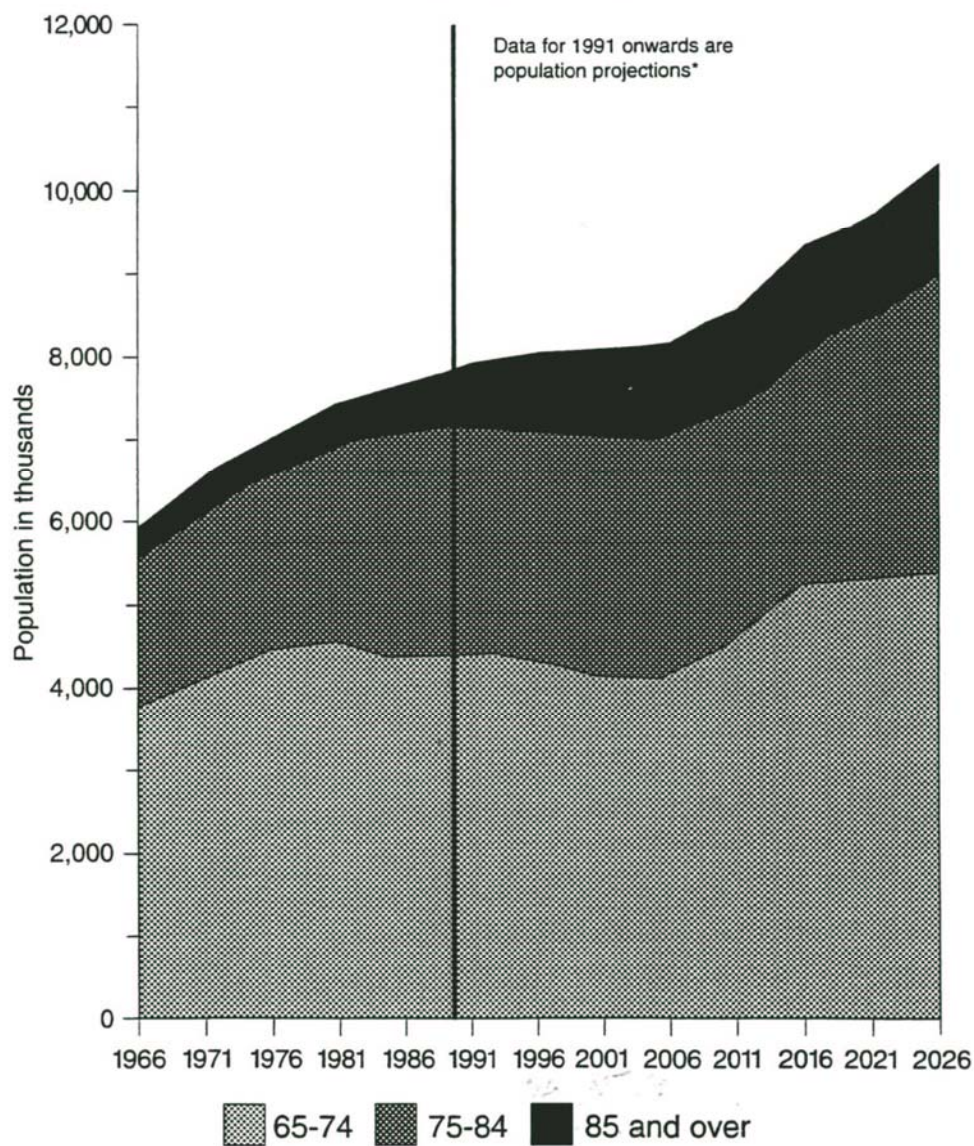
\* provisional data

Figure 1

# Elderly Person Population Trends

England and Wales 1966-2026

Taken from the central Health Monitoring Unit Epidemiological Overview Series  
The Health of Elderly People



\* based on Registrar General's mid 1989 estimates

Source: OPCS Population Trends 51 and Monitor PP2 91/1

## Chapter 3 – Antibiotic Policies

- 3.1 Because of the importance of antibiotic treatment in predisposing patients to *C. difficile* infection, the Working Group believes it may be helpful to give some general guidance on antibiotic policies. More specific advice on the features of the policy which are important in preventing *C. difficile* infection is given in Chapter 5.
- 3.2 Antimicrobial agents are widely prescribed, second only to cardiovascular agents in frequency of use. In hospitals they are given to 20-40% of patients and account for about 25% of the total drug expenditure. There has been an awareness of their overuse and misuse for several decades<sup>74-76</sup> and many surveys have defined the worldwide extent of the problem. Studies<sup>77-80</sup> have suggested that on almost 50% of occasions antimicrobial agents are used unnecessarily of the wrong drug, dose or duration of therapy is prescribed (whichever of a number of criteria for acceptable usage is adopted).
- 3.3 The factors leading to inappropriate use of antimicrobial agents have been extensively reviewed<sup>81, 82</sup>. Various methods have been suggested to improve the use of antibiotics<sup>82-85</sup> (Table 3), but whether they are effective in practice depends on compliance with them which in turn depends on whether they meet the needs to the clinicians<sup>86-90</sup>.
- 3.4 Many hospitals in the UK now attempt to exercise some control of prescribing<sup>91</sup>. The most widely used control measures are the adoption of an antibiotic formulary that limits the availability of specific antimicrobial agents and a local policy that advises appropriate therapy for specific indications. The Working Group endorses the view, now generally held, that all hospitals should have an antibiotic policy.
- 3.5 The purpose of the policy is to promote the rational, safe, effective and economic use of antibiotics. The type of policy must be tailored to the needs to the particular hospital, bearing in mind the type of patient treated and the organisms commonly encountered. If a policy is too restrictive then it will be counter-productive and a degree of flexibility is paramount. Certain broad guidelines can be suggested but in choosing specific agents to be included it is important to consider local factors.
- 3.6 A policy will inevitably impose restrictions and can be implemented effectively only after careful consultation with all the different professional groups involved, including physicians, surgeons, microbiologists and pharmacists. External expert advice may be useful. The chosen policy should contain a narrow range of familiar drugs. The decision as to which drugs to include will depend upon the antibiotic susceptibility of the common organisms prevalent in the hospital. Spectrum of activity is not, however, the only consideration. Different groups of antibiotics usually have overlapping rather than identical spectra. Furthermore, although certain drugs might have identical antibacterial activity *in vitro*, different pharmacokinetics – absorption, distribution and elimination – may produce very different results in the patient. Other non-microbiological factors to consider include differences in the frequency of administration, the availability of different formulations, the duration of therapy needed and whether oral therapy can replace expensive intravenous use of another agent. The benefits to the hospital, both in

cost and in reduction of adverse effects and bacterial resistance, are likely to be appreciable.

- 3.7 Once a hospital policy has been adopted and a formulary compiled the information should be widely disseminated in order not only to promote awareness and education among staff, but to put in context the advertising material produced by the pharmaceutical industry. There is a need for continuous intervention, audit and other review and feedback, otherwise any gains achieved may soon be lost and adverse events and drug costs will increase<sup>92</sup>. The use of computerised audit systems may facilitate this process<sup>93,94</sup>.

**TABLE 3**

**Components of a Policy to Improve the Usage of  
Antibiotics in Hospitals**

1. Hospital formulary and guidelines on antibiotic usage.
2. Education programmes.
3. Appropriate (restricted) reporting of antibiotic susceptibility by microbiology laboratory.
4. Written justification on prescriptions.
5. Automatic stop dates.
6. A requirement for consultation in specified circumstances with a member of the hospital staff with particular expertise in antimicrobial chemotherapy.
7. Limiting contact with pharmaceutical industry.

## Chapter 4 – Identification of Outbreaks

- 4.1 Many outbreak descriptions do not make clear whether a true clinical outbreak has occurred and there is a need for a note of caution about over-zealous investigation and management of clusters of *C. difficile* infection. The detection of *C. difficile* in an “index” case, perhaps a patient with PMC or severe antibiotic-associated diarrhoea, commonly leads to increased awareness of *C. difficile* as a pathogen to be sought in patients with diarrhoea, and then increased investigation of such patients.
- 4.2 In one such “outbreak” in 1982 when the numbers of positive reports were expressed as rates per number of stools investigated for *C. difficile*, it was shown that the increase in identified cases could have been due to increased investigation<sup>7</sup>. In that outbreak it was not possible to ascertain whether there had been any change in the incidence of diarrhoea in the affected wards because of inadequate recording and description in medical and nursing case notes of patients’ bowel movements. The costs were considerable, for example, 685 vials of Vancomycin (cost £14, 645 at 1993 prices) were used during the “outbreak” period, compared to 20 in the same period in the previous year. In addition, ward closures led to major problems with hospital admission in the district.
- 4.3 The same difficulties occurred during the investigation of a cluster of cases of *C. difficile* diarrhoea in a tertiary care hospital in Belgium; apparent failure of control measure to reduce the incidence of *C. difficile* infection was related to increased “case” ascertainment following contact screening and a resultant threefold increase in the number of stools examined for *C. difficile*<sup>67</sup>.
- 4.4 Another reason for an increase in *C. difficile* infection which may appear to be a genuine outbreak could be an increase in the number of susceptible patients, in particularly the elderly, being prescribed broad spectrum antibiotics. There may be a change cluster of such individuals in a particular ward causing a pseudo-outbreak.
- 4.5 It is important to be aware of the “background rate” of diarrhoea in each ward, particularly those with elderly patients, since loose stools are common in this group – an annual rate of 30-35% among patients in nursing home has been observed<sup>50</sup>; furthermore approximately a third of elderly hospitalised patients may carry *C. difficile*<sup>70</sup>. Thus, the symptoms and the microbiology may be unrelated and clear case definitions (which much include toxin detection, not just presence of the organisms to define a case) are needed to make informed decisions about the recognition and management of an outbreak.

## Chapter 5 – Prevention and Control

### Prevention of *C. difficile* Infection

#### *Introduction*

5.1 Prevention of *C. difficile* infection relies on ensuring that patients do not become susceptible through disruption of their normal gut flora and on preventing as far as possible their exposure to the organism. These approaches are implemented through careful measures to control antibiotic usage and through routine infection control procedures.

#### *Control of Antibiotic Usage*

5.2 Chapter 4 provides a general review of the purposes, benefits and methods of seeking to improve the use of antibiotics in hospitals. For the purposes of preventing *C. difficile* infection, the Working Group believes that the main component of an antibiotic policy should be:

- ◆ The avoidance of unnecessary antibiotic use;
- ◆ The use of narrow spectrum antibiotics whenever the causative pathogen is known;
- ◆ Review of “blind” empirical antibiotic therapy as soon as the causative pathogen has been identified.
- ◆ Avoidance, wherever possible, of the use of antibiotic ‘cocktails’.
- ◆ Regular chart review to ensure that antibiotics are discontinued as soon as possible;
- ◆ Strict control of the use of antibiotics for surgical prophylaxis; they should be given for as short a period as possible, i.e. per-operatively.

5.3 In addition, particularly if *C. difficile* is known to be a problem in the hospital;

- ◆ It should be noted that parenteral aminoglycosides, when given alone, have never been associated with *C. difficile* infection, although their potential toxicity must be recognised.
- ◆ Consideration should be given to whether the intramuscular or intravenous route should be used, since parenteral antimicrobials can be less likely than oral preparations to predispose to *C. difficile* infection.

## *Infection control procedures*

- 5.4 The other important aspect of prevention is good routine infection control, especially;
- ◆ Hand-washing by staff and patients
  - ◆ Adherence to disinfection and sterilisation policies for environment
  - ◆ Environmental cleaning
  - ◆ Isolation of all patients with diarrhoea pending diagnosis (see paragraphs 5.13 – 5.17 below)
- 5.5 Poor hand washing has frequently played a key role in the spread of infection in hospitals<sup>95-98</sup>. It is essential that all staff understand the need for hand washing procedures to be stringently observed. There is no evidence that alcohol-based hand rubs are effective in killing *C. difficile* spores on hands<sup>99</sup>. It is therefore appropriate to concentrate on physical removal using an inexpensive, convenient soap or detergent which is acceptable to staff. Because of the frequency with which staff should be washing their hands the provision of an emollient product and of soft paper towels is advisable.
- 5.6 We recommend that detailed procedures are drawn up to cover the cleaning of the environment and other necessary measure following an episode of diarrhoea, including reporting such incidents to the infection control team.
- 5.7 Although effective infection control procedures are essential in preventing outbreaks of *C. difficile* infection, ample advice on these procedures is available elsewhere and we do not consider it appropriate to provide it in any detail here. However, a brief summary is in Appendix IV, together with further sources of information.

### **Control of Outbreaks**

- 5.8 Once an outbreak has been identified, routine infection control measures, including those designed to prevent other patients becoming vulnerable to *C. difficile* infection, become even more important. Reviewing antibiotic policies and monitoring compliance with them are essential if an outbreak is to be controlled.
- 5.9 It is also important to reiterate the need for thorough hand-washing and the use of enteric precautions, by all staff who are likely to come into contact with the patient – including medical, nursing, paramedical, domestic and portering staff and any students. They should be reminded of these procedures, if necessary by a programme of continuing education.
- 5.10 Attention has already been drawn in Chapter 4 to the importance of exercising care in investigating clusters of *C. difficile* infection and of considering the normal ‘background’ rate of sporadic infection and of diarrhoea in general in the affected ward before deciding that an outbreak is occurring.

5.11 Guidance on management arrangements and steps to be taken for the control of outbreaks in hospitals are given in “Hospital Infection Control”, a joint DH/PHLS guidance document issued in 1998<sup>100</sup> (currently under revision). Containment and control of a *C. difficile* outbreak should form part of the general plan for managing any outbreak of infection in the hospital. The plan should specify the circumstances in which a formal Outbreak Control Group will be convened. Criteria for declaring an outbreak over should also be included in the plan.

#### *Case and Outbreak Definitions*

5.12 A case definition of *C. difficile*-associated diarrhoea and a definition of a *C. difficile* outbreak should be drawn up by the Infection Control Team. The Working Group recommends the following definitions, which are modified from examples provided in the literature<sup>51, 57, 67</sup>, for use in identifying and managing an outbreak:

- ◆ *C. difficile* diarrhoea: diarrhoea not attributable to any other cause which occurs at the same time as a positive toxin assay (with or without a positive *C. difficile* culture) and/or endoscopic evidence of PMC;
- ◆ An outbreak of *C. difficile* diarrhoea: two or more related cases satisfying the above criteria over a defined period based on the date of onset of the first case.

The incidence of *C. difficile* infection differs markedly from one hospital to another. Some institutions have a background level of more than two cases per weeks; others do not and so the response threshold will be different. Thus changes from a background level of infection to an outbreak must be judged in each hospital, and the point at which an outbreak control group should be convened agreed. For these reasons, the Working Group has concluded that the most appropriate “defined period” in the definition given above will be best determined locally, in the light of knowledge of the background rate. The development of more accurate and rapid epidemiological typing methods will certainly be of assistance here, although some outbreaks may involve more than one strain.

#### *The management of affected patients*

5.13 The Working Group reiterates the view that, as part of the institution of enteric precautions, patients with antibiotic-associated diarrhoea should be isolated until formed stools have been obtained, whether toxin or culture positive or negative.

5.14 The Working Group recommends that, wherever possible, affected patients (including patients with diarrhoea who have not yet been confirmed as *C. difficile*-positive) are transferred to an isolation ward. If an isolation ward is not available, the patients should be managed in side rooms. Each room to be used should have its own handbasin for the use of the patient, staff and visitors and, if at all possible, it should also have its own lavatory. Negative pressure ventilation is not required. In a large outbreak it may not be possible to isolate all affected patients in side-rooms and it may then be necessary to cohort nurse them in a dedicated area of a ward.

- 5.15 It is sometimes argued that side-room isolation is not necessary and that affected patients can be nursed in the main ward with other patients when, because of a rigorous antibiotic policy, the latter are not susceptible to *C. difficile* infection. However, patients may become vulnerable to the infection through mechanisms other than antibiotic treatment, as was noted in paragraph 2.10, and the Working Group recommends that patients with *C. difficile* infections should not be managed on an open ward unless part of cohort nursing.
- 5.16 The psychological state of the patients and their ability to be self-caring should be assessed before they are transferred to isolation facilities, and the decision as to their best management should take this into account. It may be better to cohort nurse elderly patients who may become confused rather than to move them into unfamiliar surroundings or to isolate them in single rooms.
- 5.17 As *C. difficile* infection is most frequent and severe in the elderly or debilitated – particularly patients in intensive care, oncology, burns and surgical units – such patients should not be admitted to or visit wards affected by an outbreak.
- 5.18 Recommended procedures for the movement of infected patients are given in Appendix V.
- 5.19 It is important to communicate with General Practitioners (GPs) and the Primary Health Care Team about infected patients who are being discharged, particularly in view of the significant proportion who may relapse and the fact that further antibiotic treatment may increase this risk. The recent guidance produced by the committee chaired by Dr. Michael Adrums, Public Health: Responsibilities of the NHS and the Roles of Others, issues under cover of HSG (93)56 in 1993 includes relevant advice on communication with GPs in such circumstances<sup>101</sup>.

#### **Antibiotic treatment of *C. difficile* infection**

- 5.20 Prompt treatment of the condition should be instituted for its beneficial effect on the patient, and also to reduce further environmental contamination.
- 5.21 There has been a limited number of studies to date on the antibiotic treatment of *C. difficile* infection and these were reviewed by Wilcox and Spencer in 1992<sup>102</sup>. Although not all patients who were treated in these reviewed studies were proven to be suffering from *C. difficile* infection, no statistically significant difference in the efficacy of oral Vancomycin compared to oral Metronidazole was noted. Response rates for both agents are impressive – 88-100% for Vancomycin, 125-500mg 6-hourly and 77-100% for Metronidazole, 200-400mg 6-8 hourly, both given for 7-10 days. There is no evidence, particularly for Vancomycin, that the higher dosages are any more effective. The cost differential between the two antibiotics is considerable: about £120 for Vancomycin compared with about £2 for Metronidazole at 1994 prices, based on a 10-day course. No data exist to support the suggestion that the clinical response to Vancomycin is quicker than to Metronidazole.

5.22 The best treatment approach for relapse (or re-infected) cases is even less certain than for primary infection. More often than not, repeat courses of either Vancomycin or Metronidazole are successful<sup>5, 103</sup>. A regimen of tapering doses, followed by pulse treatment with Vancomycin has proved effective, the rationale being to allow resistant spores to germinate, and thus become susceptible to later antibiotic administration<sup>104</sup>. A variety of treatments has been tried in very resistant cases and these have been successful in individual situations<sup>105-109</sup>.

#### *Colonised patients*

5.23 The Working Group has considered very carefully the difficult question of the extent to which patients who are colonised with *C. difficile*, that is, those who are asymptomatic and whose stools are toxin-negative, are a significant factor in the spread of infection. In paras 2.30-2.33 we stated our conclusion that they were not a source of infection. Therefore we believe that screening the stools of patients without diarrhoea is unnecessary and recommend that it should not be undertaken. If a colonised patient with formed stools is identified because the stools are examined for some other reason, we recommend that the patient should not be managed with enteric precautions, isolated or given antibiotic treatment to eradicate *C. difficile* and that there is no need to examine further specimens for clearance of the organism. Nor is there any purpose in seeking clearance specimens from a patient who has had *C. difficile* infection, once he or she has formed stools. However, if a patient who has recovered from *C. difficile* infection or is found to be colonised has diarrhoea from any cause, even if the cause is known not to be infective, we would suggest isolation of the patient, because of the uncertainty referred to in paragraph 2.32 as to whether the patient might then disseminate the organism. A 'recovered' patient who develops diarrhoea is in any case likely to be among the c. 20% of cases who suffer recurrent infection.

#### *Acquisition of C. difficile by hospital staff*

5.24 The transmission of *C. difficile* infection to a very few members of hospital staff has been reported<sup>58</sup>. It must be stressed that in these instances the disease was both mild and short lived. There is no evidence that nursing staff who are asymptomatic carriers of *C. difficile* present a risk to patient, or are at risk themselves, and there is no reason why they should not continue their normal duties. Nor is there any evidence to suggest that there is a need to exclude health care staff who are receiving antibiotic treatment from work during an outbreak. We recommend that there is no necessity to screen any asymptomatic staff for carried of the organism.

#### *Submission of C. difficile isolates for typing*

5.25 Typing of micro-organisms responsible for outbreaks of infection is well established as a valuable epidemiological tool in investigating the source and routes of spread in both community and hospital outbreaks. Although no single typing system is yet generally accepted for *C. difficile*, methods are being evaluated and it seems reasonable to assume that such research will prove useful in managing *C. difficile* outbreaks. The systems currently available are described in Chapter 6; some have proved useful in confirming some single strain outbreaks

and showing that others were due to several strains, but as yet experience of their use is limited. Further development can be achieved only if isolates associated with outbreaks are submitted to reference laboratories and the Working Group recommends that this should be done.

5.26 The questions which we would expect typing to answer are:

- ◆ Are all the patients infected with the same strain? Strains may vary in different wards or at different times and typing may provide evidence that the epidemiology is changing or explain why control measures have apparently failed.
- ◆ Is relapse due to the same strain?
- ◆ Are patients infected with more than one strain at a time?

It will be particularly important to consider these questions if new, speculative or expensive control measures are under consideration in the future.

5.27 Reference services are available in a number of centres, including the PHLS Anaerobe Reference Unit in Cardiff. The reference unit should be consulted before isolates are sent to it. Batches rather than single isolates should be sent and selected to reflect the full spectrum of the known epidemiology (time, place and person). It is often helpful also to have isolates from patients with severe and with mild infections, from patients thought to have been admitted with *C. difficile* infection (so called “invaders”) and from the environment.

5.28 The epidemiological data should be available to the typing laboratory so that they can best decide which, if any, of the isolates should be typed using more than one method, and whether additional batches of isolates will be needed. In research projects<sup>110</sup>, if the possibility of multiple strains is being investigated, typing of up to 10 colonies of *C. difficile* from some of the patients may be required and the reference laboratory should be consulted in advance.

## **Control of the environment and equipment during an outbreak**

### *The environment*

5.29 Thorough cleaning of the environment is particularly important in an outbreak of *C. difficile*. Given its resistance to disinfectants<sup>66,67</sup> and its long persistence in the environment<sup>63</sup>, there has been much discussion about the usefulness of different agents in environmental decontamination. However, it is generally accepted that some attempt should be made to reduce the environmental spore load when several cases occur and we recommend that beds, surfaces, laboratories and commodes should be thoroughly cleaned daily with detergent. There is no evidence that any disinfectant compatible with routine environmental use is more effective than cleaning with soap or detergent in reducing the level of environmental contamination and the Working Group sees no role for the routine use of environmental disinfectants in a *C. difficile* outbreak. It may be necessary to close and empty the ward and, in these circumstances, it should be thoroughly

surface cleaned before reopening, including wiping of all mattress covers and horizontal surfaces and laundering of curtains.

### *Equipment*

5.30 It is also important to ensure that all equipment that comes into close contact with patients is properly disinfected or sterilised. The best form of equipment sterilisation is by moist heat (autoclave), but instruments which will not survive moist heat sterilisation should be disinfected by exposure to 2% alkaline-buffered glutaraldehyde for 10 min. If patients with *C. difficile* infection are not being cohort nursed, single use equipment may be preferable.

### *Laundry*

5.31 Patients' clothes and linen should be bagged according to the local policy for fouled/infected laundry. Normal hospital laundering processes are effective at removing *C. difficile* contamination. Department of Health guidance on arrangements for dealing with foul or infected linen is contained in Health circular 87/30<sup>111</sup>. This is currently in the process of revision, and will be re-issued as Health Service Guidance.

## Chapter 6 – The Laboratory Investigation of *C. difficile* Associated Diarrhoea

6.1 Laboratory investigations of suspected *C. difficile*-associated diarrhoea commonly employed in the UK are directed towards the detection of either the cytopathic effect, or specifically toxin A, in the stool and/or isolation of the organism. Detection of either toxin A or B, or both, in faecal samples provides the definitive confirmation of *C. difficile* infection. Isolation of *C. difficile* from faecal samples is not diagnostic but is necessary for typing as part of the epidemiological investigations of outbreaks.

### Detection of toxin in stools

6.2 *C. difficile* produces two potent exotoxins, A and B. Toxin B is mainly cytotoxic and can be demonstrated by exposing tissue culture cell monolayers to faecal extracts. African Green Monkey Kidney (Vero) cells are very sensitive to toxin, but other cell lines such as Hep2, Hela, or MRC5 fibroblasts, which are commonly used in diagnostic virology laboratories, may be utilised. The specificity of the cytopathic effect (CPE) produced may be confirmed by neutralisation of the CPE in a second monolayer of cells protected with antitoxin raised either to *C. Sordellii* or to *C. difficile* toxin. The test usually requires overnight incubation. Toxin A (the enterotoxin) can be detected with commercially available enzyme immuno-assay (EIA) kits. Various kits have been evaluated; most have adequate sensitivity and specificity<sup>112-114</sup>. Such kits enable laboratories without tissue culture facilities to offer a service for the diagnosis of *C. difficile* infection with results on the same day. The cost is relatively high if tests are done singly and it is more economical to batch tests on a daily basis. Stool samples that cannot be processed promptly should be refrigerated.

### Isolation of *C. difficile* from faecal samples

6.3 As with other enteric pathogens, isolation of *C. difficile* from stool samples is facilitated by the use of selective media. The original formulation of Cycloserine-Cefoxitin Fructose Agar (CCFA) described by Georgia *et al*<sup>115</sup> contained concentrations of selective agents that may be inhibitory to some strains of *C. difficile*<sup>116</sup>. The current recommended concentration of cycloserine is 250mg/L and of cefoxitin is 8mg/L. Selective agar may either be inoculated directly or the stool sample can be pre-treated with alcohol to select for spore forming clostridia<sup>117</sup> (See Appendix VII). The PHLS Anaerobe Reference Unit in Cardiff has developed a modification of CCFA (see Appendix V11) incorporating several ingredients that enhance typical colonial characteristics of *C. difficile*. This selective agar, Cefoxitin-Cycloserine Egg Yolk (CCEY) agar, stimulates spore germination and stimulates production of a metabolite almost unique to *C. difficile*, p-cresol.

6.4 *C. difficile* is distinguished from other clostridia on the basis of biochemical tests and production of its specific toxins. Confirmation of the identity of *C. difficile* isolates can be done by three simple tests. Initially recognised by their distinctive odour (likened variously to horse or elephant dung), their identity can be confirmed quickly by a latex slide agglutination test for somatic antigen (see

Appendix VII), and/or by viewing colonies under long wave UV light for their characteristic yellow/green fluorescence<sup>115</sup>. Mis-identifications of other clostridial species as *C. difficile* are possible however, due to shared properties of UV fluorescence with *C. innocuum*, and agglutination of the *C. difficile* latex particle reagent by *C. Sordellii*, *C. bifermentans* and *C. glycolicum*<sup>118</sup>. If further identification is needed, *C. difficile* produces neither lipase nor phospholipase activity on egg yolk medium, is not haemolytic, does not produce indole or reduce nitrate; it may liquefy gelatine and is weakly saccharolytic. It differs from other clostridia in producing both iso-caproic acid and p-cresol.

### **Strategies for routine diagnosis and investigation of outbreaks**

6.5 Toxin detection alone is an adequate laboratory investigation for sporadic cases. In outbreaks, when epidemiological studies are required, the dual approach of toxin detection and isolation of *C. difficile* is optimal. However, the workload may be such that a laboratory might find it difficult to cope with performing both examinations. In such situations the laboratory should perform toxin tests, but also store the stool specimens at 4C or -20C for later culture. In this way, the diagnosis of *C. difficile* infection can be confirmed and any immediate action to control the outbreak taken. Essential epidemiological data can then be obtained by culturing the stored sample and typing the isolates. Proof of cross-infection with *C. difficile* is possible only by comparing isolates. Without these investigations, it is impossible to tell whether there is simply an increase in sporadic unrelated cases, or if cross-infection is indeed occurring. Laboratories unused to culturing *C. difficile* should contact their local Public Health Laboratory or the PHLS Anaerobe Reference Unit, Cardiff, for further advice.

6.6 Screening of patients for carriage of *C. difficile* is necessary only for research purposes and is not part of the investigation of outbreaks.

### **Environmental sampling**

6.7 Environmental sampling as a routine monitoring procedure is not recommended because a negative result may give a false sense of security and a positive result, without further typing for comparison with isolates from patients, yields little useful information. Any sampling should be part of an organised epidemiological investigation including typing of isolates. Sampling methods are given in Appendix VII.

### **Typing of *C. difficile***

6.8 The typing of *C. difficile* isolates is not necessary as part of the routine investigation of sporadic cases of infection but is essential for providing laboratory confirmation of clinically suspected single strain outbreak and episodes of cross-infection and allows reliable epidemiological data to be gathered. The indications for typing are considered in more detail in sections 5.23-5.26.

6.9 The typing systems that have been used in epidemiological studies of *C. difficile* were reviewed by Tabaqchali and Wilks<sup>119</sup>. Studies of *C. difficile* infection have been hampered by the lack of a single generally accepted and widely available

typing system. The laboratory aspects of typing *C. difficile* isolates for epidemiological investigations were addressed recently by a PHLS working group<sup>120</sup>.

- 6.10 Typing methods that have been used for *C. difficile* include bacteriophage/bacteriocin typing and serotyping<sup>119</sup> but these are not generally available. Twenty serotypes have been described and some serotypes may represent more virulent strains of *C. difficile* than others<sup>121</sup>. However, the sera require extensive cross-adsorption and it is reported that there are still some cross-reactions. The cost of establishing the full range of either polyclonal antisera or monoclonal antibodies of sufficient quality is considered at present to be too great. Antibiotic susceptibility patterns have been useful markers in some outbreaks but cannot be relied upon to be sufficiently discriminatory.
- 6.11 An alternative approach is to use fingerprinting systems based on protein or DNA profiles or on whole cell analysis, e.g., by pyrolysis mass spectrometry (PyMS). Such methods are able to distinguish between isolates examined together in single batches. It has not been possible with fingerprinting methods to build up a robust and consistent database of types for monitoring trends in longitudinal studies or to compare strains between different laboratories, or even on different days in the same laboratory. Whole cell analysis by PyMS has been used successfully in the investigation of hospital outbreaks of *C. difficile* infection<sup>122, 123</sup>. It should continue to be available in one or more Reference Centres to provide a rapid typing response for batches of isolates that appear to be epidemiologically related on clinical grounds. Facilities are currently available in the PHLS Anaerobe Reference Unit in Cardiff, where most of the workload will be handled, and also through the PHLS in Newcastle.
- 6.12 The examination of cell-surface-protein profiles obtained by SDS-PAGE is perhaps the simplest approach to fingerprint typing. The analytical problems can be simplified and the discrimination improved by combining SDS-PAGE with immunoblotting<sup>124</sup>. Other SDS-PAGE approaches have included one based upon the profiles of <sup>35</sup>S-methionine labelled proteins<sup>125</sup>. The automated equipment needed for this analysis is no longer produced but it can be carried out manually.
- 6.13 The development of genotypic approaches (i.e. DNA fingerprinting) has had problems. Plasmid analysis cannot be used because up to 70% of *C. difficile* isolates do not harbour them. Chromosomal DNA from *C. difficile* has been difficult to handle but several new techniques are showing promise – e.g., Restriction Fragment Length Polymorphism (RFLP) with frequent cutting endonucleases or with rare cutting enzymes and pulsed field gel electrophoresis, ribotyping, Polymerase Chain Reaction (PCR) with various (predominantly random) primers – and it is likely that development of these methods will provide an appropriate typing system.

## **APPENDIX I**

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## APPENDIX II

### History of the recognition of the pathogenic role of *Clostridium difficile*

- 1893 First description of pseudomembranous colitis (PMC) (diphtheritic colitis)<sup>126</sup>.
- 1935 First description of *C. difficile* (*Bacillus difficilis*) and its toxigenicity<sup>19</sup>.
- 1943 Rodent model of antibiotic-associated diarrhoea (AAD) described<sup>15</sup>.
- 1969 First description of *C. difficile* causing intestinal disease (in mono-associated rats).<sup>127</sup>
- 1974 Cytopathic effect in gut contents of rodents (hamsters) with AAD first described<sup>128</sup>.
- 1977 First description of a cytopathic protein toxin in the stools of patients with PMC<sup>14</sup>.
- 1977 First description that a *Clostridium* sp. (BVA 17H F1-9) caused AAD in hamsters (subsequently identified as *C. difficile*)<sup>18</sup>.
- 1978 Identification of *C. difficile* as the cause of PMC in man<sup>22-25</sup> (references in chronological order).
- 1979 Development of a selective medium for *C. difficile*<sup>115</sup>.
- 1981 Demonstration that *C. difficile* produces two toxins<sup>129, 130</sup>.

## APPENDIX III

### Review of the Literature on Outbreaks and Epidemiological Studies

REFERENCE	STUDY	NUMBER OF PATIENTS (MEAN AGE)	SPECIALTY	METHOD OF DETECTION	RISK FACTORS	INFECTION CONTROL PROCEDURES: ADOPTED (RECOMMENDED)
1) Rogers TR <i>et al</i> , 1981 <sup>34</sup>	Outbreak over 8 months	10 (6)	Paediatric Oncology	Culture Toxin	Antibiotics	Isolation with enteric precautions Protective isolation for susceptible patients
2) Greenfield C <i>et al</i> , 1981 <sup>131</sup>	Outbreak over 21 days	8 (50)	Medical	Culture Toxin	Antibiotics	Isolation with enteric precautions
3) Bruce D <i>et al</i> , 1982 <sup>32</sup>	Outbreak	4 (52)	Renal	Toxin	Antibiotics Renal Disease	(Isolation)
4) Malamou-Ladas H <i>et al</i> , 1983 <sup>54</sup>	Survey of patients and environment	27 17 3 51	Oncology Urology ITU Newborn	Culture	Environment	Decontamination of faecal soiled items Handwashing Isolation
5) Delmee H, Michaux JL, 1986 <sup>55</sup>	Prospective over 29 months	376	Leukaemia	Culture Toxin	Environment Carriers	Vancomycin for all culture positives Cleaning
6) Gerding D <i>et al</i> , 1986 <sup>43</sup>	Prospective case control over 1 year	149 (64)	Medical Surgical	Culture Toxin	Surgery Antibiotics	Antibiotic review
7) Cumming A <i>et al</i> , 1986 <sup>33</sup>	Outbreak over 10 months	18	Renal	Culture	Antibiotics Renal disease	Antibiotic review Isolation and enteric precautions Vancomycin if symptoms
8) Bender B <i>et al</i> , 1986 <sup>50</sup>	Outbreak over 7 months		Long stay Elderly	Culture Toxin	Long-stay facility carriers	(None effective)
9) Lewis R, 1987 <sup>132</sup>	Retrospective over 1 month	29	All	Culture Toxin	None stated	(Use toxin not culture results as basis for control measures)

REFERENCE	STUDY	NUMBER OF PATIENTS (MEAN AGE)	SPECIALTY	METHOD OF DETECTION	RISK FACTORS	INFECTION CONTROL PROCEDURES: ADOPTED (RECOMMENDED)
10) Testore GP <i>et al</i> , 1988 <sup>31</sup>	Outbreak over 6 weeks	10 (52)	Surgical	Culture Toxin	Age Antibiotics Surgery Environment	Cleaning with glutaraldehyde Disposable equipment Handwashing No isolation
11) Cefai C <i>et al</i> , 1988 <sup>49</sup>	Prevalence at 6 monthly intervals x 2	65 54 (73)	Long-stay Elderly	Culture Toxin	None stated	(Awareness of local carriage rates)
12) Silva J, Jezzi C, 1988 <sup>64</sup>	Prospective over 6 months	521	Medical (high and low risk)	Culture Toxin (diarrhoea stools only)	None stated	(Isolation Cleaning)
13) Gerard M <i>et al</i> , 1988 <sup>35</sup>	Prospective over 7 months	156	Oncology Leukaemia	Culture Toxin	Antibiotics Chemotherapy	Non stated
14) Heard SR <i>et al</i> , 1988 <sup>30</sup>	Prospective over 6 months	248	Oncology Medicine	Culture Toxin	Acute Leukaemia	None stated
15) Brunetto AL <i>et al</i> , 1988 <sup>37</sup>	Outbreak over 1 year	21	Paediatric Oncology	Culture Toxin	Antibiotics Neutropenia	Antibiotic review Isolation of cases Vancomycin to all culture positive patients
16) Kaatz GW <i>et al</i> , 1988 <sup>66</sup>	Outbreak over 19 weeks	7 (62)	Medical	Culture Toxin	Environment	Cleaning with hypochlorite
17) D'Innocenti R <i>et al</i> , 1989 <sup>48</sup>	Outbreak over 3 weeks	5 (70)	Orthopaedic	Culture Toxin	Age Antibiotics	None stated
18) Strimling MO <i>et al</i> , 1989 <sup>58</sup>	Case report Staff	1 (19) 3 (30)	Long-stay/ Medical Nurses	Toxin	None stated	(Enteric precautions required)

REFERENCE	STUDY	NUMBER OF PATIENTS (MEAN AGE)	SPECIALTY	METHOD OF DETECTION	RISK FACTORS	INFECTION CONTROL PROCEDURES: ADOPTED (RECOMMENDED)
19) McFarland L <i>et al</i> , 1989 <sup>57</sup>	Prospective over 11 months	428	Medical	Culture Toxin Clinical features	Environment Staff hands	Handwashing Gloves Body substance precautions for all patients Frequent cleaning
20) McFarland <i>et al</i> , 1990 <sup>29</sup>	Prospective over 11 months	399	Medicine	Culture Toxin	Age Length of stay Severity of illness Antibiotics	(Handwashing Gloves Cleaning)
21) Johnson S <i>et al</i> , 1990 <sup>51</sup>	Prospective over 9 weeks	282 (63)	Surgical Medical Orthopaedic	Culture Toxin Clinical features	Antibiotics Abdominal surgery Staff	None stated
22) Issak MI, Elliott TSJ, 1990 <sup>39</sup>	Outbreak over 1 month	13 (53) 3 died	Not stated	Culture Toxin	Antibiotics	Isolation till culture negative Antibiotic review
23) Johnson S <i>et al</i> , 1990 <sup>59</sup>	Prospective Two 6 month studies before and after gloves	N/A	Medical Surgical	Culture Toxin	Staff hands	Gloves for handling body fluids
24) Kerr R <i>et al</i> , 1990 <sup>71</sup>	Outbreak and control	56 (80)	Long-stay Elderly	Culture Toxin	Carriers	(Metronidazole for carriers)
25) Thibault A <i>et al</i> , 1991 <sup>38</sup>	Outbreak case control	26	Medical Surgical	Culture Toxin	Surgery Antibiotics	Antibiotic review

<b>REFERENCE</b>	<b>STUDY</b>	<b>NUMBER OF PATIENTS (MEAN AGE)</b>	<b>SPECIALTY</b>	<b>METHOD OF DETECTION</b>	<b>RISK FACTORS</b>	<b>INFECTION CONTROL PROCEDURES: ADOPTED (RECOMMENDED)</b>
26) Kamthan AG <i>et al</i> , 1992 <sup>36</sup>	Case reports	4 (49)	Oncology	Culture Toxin	Chemotherapy	None stated
27) Johnson S <i>et al</i> , 1992 <sup>73</sup>	Randomised placebo controlled to compare Vancomycin and Metronidazole	30 (67)	Medical Surgical	Culture Toxin	None stated	(No treatment of carriers)
28) Cartmill TDI <i>et al</i> , 1994 <sup>8</sup>	Outbreak over 6 months	175 (73)	Elderly Medical	Culture Toxin	Antibiotics	Antibiotic review Isolation – enteric precautions Handwashing staff/patients Environmental cleaning

## APPENDIX IV

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Local hospital infection control guidelines.

## **APPENDIX V**

### **Recommended infection control procedures to prevent transmission of *C. difficile* infection in clinical areas**

#### **Hand Washing**

1. Poor hand washing has frequently played a key role in the spread of infection in hospitals, in *C. difficile* as in other infections.
2. After handling a patient or completing any task involving blood, excretions and secretions and contact with soiled equipment, staff should always wash and dry hands thorough using soap and water. The equipment provided for washing and drying should be such as to encourage staff to use them regularly.
3. Staff should ensure that patients' hands are cleaned with soap and water after using the lavatory and before meals. Soap and water in a bowl or disposable wipes should be used at the bedside of immobile patients.

#### **Other Measures**

1. Staff should wear a plastic apron and non-sterile latex or vinyl gloves whenever exposure to body fluids and excretions from a patient is anticipated.
2. All equipment that has come into patient contact should be decontaminated according to the Disinfection/Sterilisation Policy. Mattresses and pillows should have impermeable covers. Staff must understand the importance of the care to be taken in cleaning equipment.
3. Urine and faeces in bedpans should be disposed of if possible down a lavatory attached to the room. If this is not available, bedpans should be taken to the washer or macerator outside the room. They should be hand-held until placed in the washer or macerator and not placed temporarily on any surfaces. The washer or macerator should be left open when not in operation so that its handles do not become contaminated by opening it whilst holding a soiled bedpan.
4. A supply of detergent and warm water should be readily available at all times, and staff should understand the importance of thorough and rigorous cleaning.
5. Following the discharge of the patient the room and its contents should be cleaned thoroughly. Special attention should be paid to removing all faecal soiling, and in particular to cleaning of furniture, fittings and horizontal surfaces. Mattress and pillow covers should be cleaned, and replaced if torn. Medical equipment should be decontaminated according to the local policy.

### **Movement of patients with *C. difficile* infection**

1. Patients with *C. difficile* infection should not be transferred to other wards in the hospital, except for purposes of isolation or cohort nursing. Visits to other departments should be kept to a minimum. When this is necessary, either for investigation or treatment, prior arrangements should be made with the Senior Staff of that department, so that the infection control policy for the department can be applied.
  - (a) Infected patients should be seen at the end of the working session and should only be sent for when the department is ready to see them; they should not be left in a waiting area with other patients.
  - (b) All procedures should be planned in advance to keep equipment and staff to a minimum and to ensure adequate supplies of cleaning materials. Disposable equipment should be used whenever possible; non-disposable equipment should preferably be sterilisable.
  - (c) Staff should wear disposable aprons and gloves and meticulous infection control procedures should be employed.
  - (d) The patient should return to the ward immediately after the procedure. Equipment and surfaces should be appropriately cleaned or disinfected. Linen, waste etc should be bagged and disposed of according to the local policy.

## APPENDIX VI

### Culture of *C. difficile* from faeces and the environment

#### Selective medium

Cycloserine-Cefoxitin Supplement (Unipath Ltd) final concentrations 250mg/L and 8mg/L respectively.

Agar base eg Fastidious Anaerobe Agar (FAA) base (Lab M Ltd) 23g

Blood 25ml

Distilled water 500ml

#### *Optional ingredients*<sup>133</sup>

p-hydroxyphenylacetic acid (Sigma H-4377) 0.5g

Cholic acid sodium salt (Sigma C-1254) 0.5g (This enhances spore germination which may be of particular value in environmental studies.)

Egg-yolk emulsion (two yolks emulsified in 20ml sterile saline) instead of blood supplementation.

#### Alcohol Shock Method<sup>117</sup>

Prepare a 50% suspension of stool sample in absolute (74 O.P.) alcohol. Mix and leave at room temperature to stand for 20-30 minutes. Remove approximately 0.5ml of deposit layer with disposable pipette and inoculate agar or broth media.

#### Culture Procedure

Inoculate faeces or deposit from the alcohol shock suspension onto selective agar. Incubate plates anaerobically at 37°C. Examine after overnight incubation. Check suspect colonies for typical odour, lack of lecithinase or lipase activity (if egg-yolk used), UV fluorescence (optimum at 48h) and agglutination with Microcreeen latex reagent (Mercia Diagnostics Ltd Guildford, Surrey). If plates are negative after overnight incubation reincubate. Examine at 48h and discard if negative.

#### Cytotoxin detection in stools and on isolates

Make an approximate 1:10 dilution in sterile phosphate buffered saline. Mix well, centrifuge (approx 3000rpm for 5 min) and/or filter (0.45m) to give a clear supernate. Add an appropriate volume of test material to a monolayer of cells, e.g., 2-3 drops for tubes and 10-20 l for microtitration plate wells. Ideally, another monolayer of cells is inoculated with an equal volume of *C. Sordellii* or *C. difficile* antitoxin (1:50). Incubate at 37°C overnight, then examine for a cytopathic effect which is also neutralised with antitoxin. In practice, when large numbers of stools are being proceeded this neutralisation step can be saved to confirm positives only. None immune serum should be used as a negative control.

Toxigenicity studies of isolates can be performed either by filtration of a 48h broth culture or from a plate by removing a plug of agar adjacent to a colony and placing it in the tissue culture tubes as described above.

## **Enzyme Immunoassay Kits for Toxins A or B in stools**

Follow instructions as provided from manufacturer. Toxin production of isolates can be tested by making a suspension of colonies and proceeding as if it were a stool sample.

## **Environmental Sampling**

Environmental sampling should be directly onto solid selective media; it is a practical advantage if the selective medium also has differential properties. The medium found useful in practice is detailed above. Once inoculated, agar plates can be kept in air until the end of sampling (e.g. for at least a working day) because of spore survival. Inoculated media should be examined daily for up to three days.

### *Surface, fabric and air sampling*

One of the most useful general methods of air sampling is use of a high volume airborne particle slit sampler (Casella London Ltd, Milton Keynes). This can be adapted to sample particles from surfaces by addition of a collecting head, wide-bore tubing and a collection nozzle onto the solid medium of choice in a 14cm Petri dish. If three of the four sampling fabrics (bedding, curtains etc), surfaces (floors, bedframes etc). It should not be used in areas where there is a very high accumulation of dust or particles as these may block the open slit; in these cases methods listed below are more applicable.

### *Surface sampling by swab or contact plate*

A swab moistened with a mild surfactant (e.g., 0.9% saline with 0.2% Tween 80) can be used to sample areas with high levels of particulates, wet areas, (e.g. bedpan macerator seals) and areas where dissolution is needed (e.g., dried spills). The fluid can then be inoculated directly onto selective agar and/or into a selective enrichment broth. Contact plates containing selective agar may also be used for sampling surfaces.

### *Dust*

Large accumulations of dust can be placed in surfactant solutions (as above) and the solution then put onto well-dried selective agar.

### *Hands*

Fingers can be sampled by gentle application to the surface of selective agar.

### *Laundry process*

Laundry process can be sampled by two methods. Spread of *C. difficile* between items in the laundry process can be detected by introducing a small (approx. 50 x 50cm) sterile fabric test piece to the washload. This can be recovered, placed into surfactant

solution, as above, and the liquid put onto selective agar. Alternatively, freshly laundered fabric can be pressed onto selective agar with a sterile-gloved hand.

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