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The changing faces of *Clostridium difficile*: A personal reflection of the past 34 years

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ABSTRACT

Late in 1978 my boss gave me a folder with "*Clostridium difficile* (diffikilé)" written on it. Inside were a few recent and now classic papers by Bartlett, Larson and co. It was suggested that this might be an interesting research topic. So began a continuing adventure which has resulted in at least 50 publications from my group. Over the years we have made several important contributions to the field. Beginning in 1982 we showed that *C. difficile* was a common cause of community-acquired infection! During the next few years we did extensive structural studies on the bacterium. This culminated in 1984 with a fingerprinting study (by immunoblotting surface antigens), on Swedish strains supplied by Carl-Erik Nord, which was probably the first study to demonstrate that *C. difficile* was really an infectious agent. This was later reinforced with strains sent from Amsterdam by Ed Kuijper. Later in the 1980s, in a study of recurrent disease, we showed that ca. 50% of recurrences were due to infection with a different strain. During my term as chair of the European Study Group for *C. difficile*, we began to define the status of *C. difficile* infection (CDI) in Europe and develop guidance for diagnosis and treatment. Recently we utilised our extensive culture collection, with isolates from the 1970s to the present, to observe how epidemiology has been driven largely by antibiotic usage. We have now come full circle: in the early years *C. difficile* infection was caused by many different strains. Then in the period beginning in the 1990s, characterised by often-large outbreaks and poor infection control, disease was caused by a few endemic strains highlighted by the 027/NAP1/BI pandemic. Now in a much-improved local situation, we are seeing again that the majority of cases (largely sporadic) is caused by multiple types. Current studies range from molecular studies on toxin and spore production, immune responses, novel observations on CDI in children, to what is the best way of decontaminating the anaerobe laboratory.

1. Introduction

On moving back to Edinburgh in the summer of 1977 I was first introduced to anaerobes by my head of department JG (Gerry) Collee. My early work on *Bacteroides* species began immediately and I was able to use the skills developed as a post doc in Newcastle to determine the structures of these Gram-negative anaerobes. Over the next year or so Gerry suggested I also begin to look at the clostridia. Late in 1978 he handed me a manila folder with "*Clostridium difficile* (diffikilé)" written across it. Inside were a handful of then recent, now classic papers by authors such as John Bartlett and Elliot Larsen. He suggested that I should get interested in this species because he thought it might become important! Little was I to know that it would play such a big part in both my early and late research career.

2. Early days of research

One of the first things we did was to start a *C. difficile* culture collection. It began with NCTC 11223 purchased from the National Collection of Type Cultures, and this was our standard strain for many years. It was quickly followed by acquiring the collection of Hafiz, from the Oakley group in Leeds. We soon introduced a diagnostic service for the anaerobe in Edinburgh and quickly began to accumulate isolates from hospital cases of often serious pseudomembranous colitis and antibiotic-associated diarrhoea. A young ID registrar in Edinburgh, Ray Brettle, suggested we look for this emerging pathogen in cases of diarrhoea admitted from the community to the Edinburgh City Hospital, the ID hospital for the region. For the next year or so I served my apprenticeship on the bench investigating the many diarrhoeal stool samples that were submitted. This was done every third week along with the senior, experienced anaerobe expert Robert (Bob) Brown and Marie Byrne. The recently described CCFA medium [8] was used effectively and

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identification of all likely *C. difficile* isolates was by biochemical testing, gas chromatography of volatile fatty acids (now superseded by smell) and cytotoxin detection on tissue culture monolayers acquired from the nearby virology laboratory.

3. Community-acquired diarrhoea

By 1982 we were able to show that *C. difficile* was a common cause of community-acquired infection (something that has recently been rediscovered). Of the154 specimens investigated from patients admitted to the ID ward with diarrhoea, 39 were excluded as diarrhoea judged to be "non-infective". Of the remaining 115, *C. difficile* was found in 19 (16.5%) patients. Other enteric pathogens included *Salmonella* in 10%, *Campylobacter* in 13%, and dual "infection" of *C. difficile* and *Salmonella* in 3%. In the early 1980s noroviruses had not been named and viral diarrhoea could only be diagnosed easily by electron microscopy: this was not done routinely. The study was published in the British Medical Journal [3]. It has only been cited 32 times, and all but three times were pre-2000, the last in 2004!

4. Structural studies 1980s

My background was in bacterial structures and very soon we began to fractionate the cell wall of *C. difficile* and identified and analysed the teichoic acid and lipoteichoic acid analogues [15,16,23], the S-layer cell wall proteins [7,24] and flagella

5. Epidemiology

Perhaps our most important contribution to the *C. difficile* field was that we were one of the first groups (the first?) to demonstrate conclusively that it was really an infectious agent, originating outside the CDI patient and not simply being an overgrowth of a minor component of the normal colonic microbiota. Based on our structural studies we were able to define surface antigens that were amenable to analysis ("fingerprinting") by the relatively modern techniques of slab gel SDS-polyacrylamide gel electrophoresis and immunoblotting, now more commonly known as Western blotting [22]. We were keen to get our hands on strains from defined outbreaks and it was Carl-Erik Nord and colleagues from Stockholm that were the first to supply us with such a collection, which also included many strains from sporadic cases found throughout Sweden. A combination of crossed immunoelectrophoresis and SDS-PAGE/immunoblotting clearly demonstrated identity among all of the outbreak strains and differences from the other strains [17]. This was later reinforced with strains sent from Amsterdam by Ed Kuijper, suggesting that a telephone between the beds of two CDI patients could have been the vector! (Fig. 1).

During this period many more strains were collected and excellent collaborations set up with other pioneers in the field in the UK and Europe, including Peter Borriello, Jon Brazier and Soad Tabaqchali.

6. Last two decades of the millennium

From the late 1980s research funding for this uncommon, but dangerous pathogen was almost impossible to obtain and for the next two decades or so I was fully occupied working on the LPS/endotoxin of Gram-negative bacteria. This was predominantly on the LPSs of the *Enterobacteriaceae*, but we were able to investigate the LPS of *Bacteroides* species. The Anaerobe Society of the Americas recognised some of this work in 1994 and I was the first recipient of the Finegold Award [18].

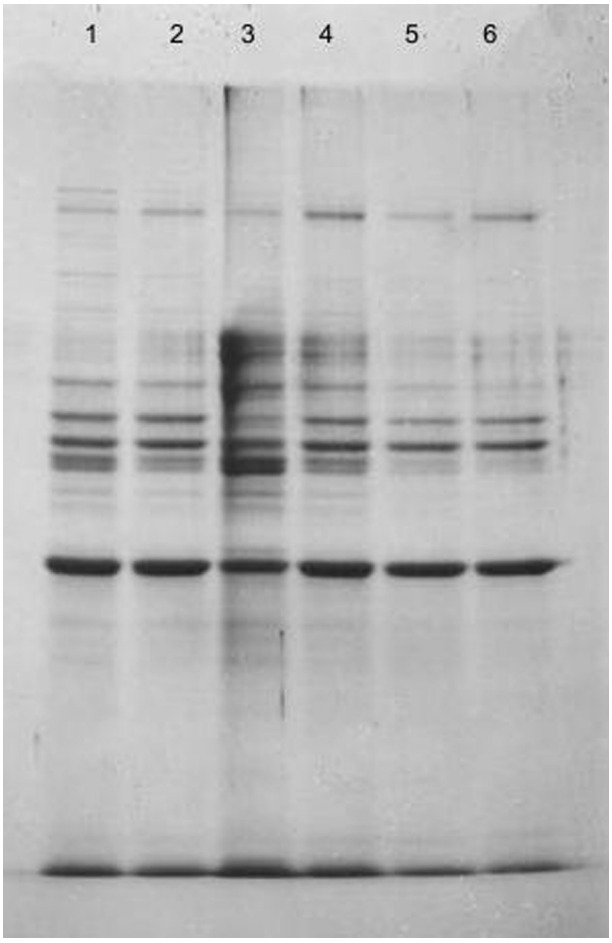

Fig. 1. SDS-PAGE of EDTA-extracted surface proteins from six isolates of *C. difficile* from two patients in a 2-bedded ward and their environment. Patient A developed CDI, and was followed a few days later by Patient B. All extracts gave an identical pattern. Source of isolates: track 1, patient A; track 2, patient B; tracks 3 and 5, the floor of the room collected 9 days apart; track 4, the shared telephone between the beds; and track 6, the floor of the corridor outside the room. Sent in April 1985 by Ed J. Kuijper, then in Amsterdam, The Netherlands.

An interest in *C. difficile* was maintained during this time as we continued to offer a typing/fingerprinting reference service. In a collaborative study with Ian McKay and John Coia on a *C. difficile* diarrhoea problem they were experiencing in an orthopaedic ward in Glasgow, we investigated recurrent infections and were able to demonstrate that ca. 50% of relapses were due to infection with a different strain [12]. Again, this was probably the first study to demonstrate this phenomenon.

We also helped in the investigation of what was possibly the largest outbreak of CDI in the UK at that time (in Manchester) demonstrating that a single strain (later recognised to be ribotype 001) was responsible for the outbreak [4].

The first of our investigations of carriage of *C. difficile* in neonates was done in this period when we sampled infants in a neonatal intensive care ward on four occasions over 12 months in 1995–1996. We showed that at our first sampling 57% (16 out of 28) of the infants were colonised, all with the same strain. This was received with some concern by the ward staff and better infection control practices were introduced. Later samplings showed a decrease to between 10 and 23% with up to 6 different types. Our conclusions at that time were that the colonisation was probably due to cross infection by handlers and the presence of *C. difficile* in the babies was an indicator of cross infection, and the levels could

be decreased by improving hand hygiene [28]. Very recently we repeated this type of study in a modern neonatal ward with extremely high infection control policies and we could only find one baby colonised on just one occasion from 130 samples (SK Taori et al, unpublished).

Late in the last century we began to promote the use of a simple fingerprinting method for typing *C. difficile* based on the S-layer proteins that could be extracted from whole bacteria with guanidine hydrochloride and analysed on SDS-PAGE. The simple two-band pattern could be given a numerical value based on the molecular masses of the S-layer proteins in kilo Daltons e.g. 50:28, 52:40 [10,14], and this was applied to a major prospective study of CDI in a geriatric population [11]. This last study forged links with John Starr, a geriatrician with long-standing an interest in *C. difficile*, and several lines of research began, including changing patterns of antimicrobial susceptibility [5,6], identification of risk factors [26], the first mathematical modelling of CDI [27], and investigations of the immune response in cases, carriers and matched controls [21].

7. Chair of the European Study Group for *C. difficile* (ESGCD)

The ESGCD of the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) was set up in 2000 thanks to the efforts of Jon Brazier, and I took over the chairmanship from Jon in 2005 to 2009. During my term of office we began to define the status of CDI in Europe e.g. Refs. [1,25] and develop guidance for diagnosis and treatment, thanks largely to the committee with Ed Kuijper and Frederic Barbut playing major roles, ably assisted by Anne Collignon, Michel Delmée, and Paola Mastrantonio and more latterly by Emilio Bouza, Jean O'Driscoll, Petra Gastmeier and Mark Wilcox.

8. Changes in epidemiology over the 34 years

Our extensive culture collection, with isolates from the 1970s to the present, has allowed us to investigate the predominant types of *C. difficile* in our region. In the early years many different types appeared to be responsible for cases of infection, and most of these appeared to be sporadic or in small outbreaks. However, by the early part of the 21st Century – there were just a few dominant strains: mainly PCR ribotype 001 locally, and characterised as epidemic strains [13]. A comprehensive investigation of almost 200 individual strains from our collection grouped into time periods has clearly shown the changing pattern of ribotypes over the years with just a few dominant strains in more recent years, and their selection no doubt driven by antibiotic resistance and outbreaks [29]. Ribotype 027 has not been a problem locally. Both locally, and in the rest of Scotland, now that outbreaks are less frequent, and antibiotic stewardship and infection control procedures are in place there appears to be a return to several different strains being responsible for CDI with no dominant strains, although ribotype 078 is currently number one [9].

9. Latest studies and final words

Finally our most recent work has been as wide-ranging as previously. Analysing which patient groups get CDI and the increasing workload experienced by both the lab and the clinical areas has been informative and somewhat frightening [20]. Ventures into the molecular biology of *C. difficile*, ably led by Prerna Vohra has moved us properly into the 21st Century [30], and we have extended our immune response studies investigating the induction of cytokines by Toxins A and B [31]. Demonstrating the true sporicidal efficiency of commonly used laboratory disinfectants has practical importance in both the research as well as the diagnostic laboratory. We tested Actichlor, MicroSol 3+, TriGene Advance, Virkon and Decon 90, and only the chlorine-releasing agent Actichlor was found to be suitable for the elimination of *C. difficile* spores from the environment, making it the agent of choice for the decontamination of laboratory surfaces, as is well recognised in the hospital [32].

Without doubt the burden of *C. difficile* infections has become recognised throughout much of the world and levels of infection are falling from unacceptably high levels – at least in the UK, but not without a huge effort. New agents for therapy such as fidaxomicin may help, but at a price [19]. Other new approaches such as active and passive immunisation, and restoration of the protective colonic microbiota by faecal transplants or designer probiotics are certainly showing promise. However, it is important to keep on top of the problem, and education of healthcare workers, as well as relatives and carers of suffers is crucial. Our recent study [2] to assess the knowledge of *C. difficile* and CDI among healthcare workers is just one of the ways forward

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References

[1] Barbut F, Mastrantonio P, Delmée M, Brazier, Kuijper E, Poxton IR. Prospective study of Clostridium difficile infections in Europe with phenotypic and genotypic characterization of the isolates. Clin Microbiol Infect 2007;13:1048–57.
[2] Brady RRW, Rodrigues MA, Harrison R, Rae C, Graham C, Poxton IR, Gibb AP. Knowledge of Clostridium difficile infection among UK health-care workers: development of a knowledge assessment tool. Scott Med J 2012;57:124–30.
[3] Brettle RP, Poxton IR, Murdoch JM, Brown R, Byrne MD, Collee JG. Clostridium difficile in association with sporadic diarrhoea. Br Med J (Clin Res Ed) 1982 Jan 23;284(6311):230–3.
[4] Cartmill TDI, Shrimpton SB, Panigrahi H, Khanna V, Brown R, Poxton IR. Nosocomial diarrhoea due to a single strain of Clostridium difficile: a prolonged outbreak in elderly patients. Age and Ageing 1992;21:245–9.
[5] Drummond LJ, McCoubrey J, Smith DGE, Starr JM, Poxton IR. Changes in sensitivity patterns to selected antibiotics in Clostridium difficile in geriatric in-patients over an 18-month period. J Med Microbiol 2003a;52:259–63.
[6] Drummond LJ, Smith DGE, Poxton IR. Effects of sub-MIC concentrations of antibiotics on growth of and toxin production by Clostridium difficile. J Med Microbiol 2003b;52:1033–8.
[7] Fagan RP, Janoir C, Collignon A, Mastrantonio Poxton IR, Fairweather NF. A proposed nomenclature for cell wall proteins of Clostridium difficile. J Med Microbiol. 2011;2011(60):1225–8.
[8] George WL, Sutter VL, Citron D, Finegold SM. Selective and differential medium for isolation of Clostridium difficile. J Clin Microbiol 1979;9:214–9.

[9] Health Protection Scotland (HPS) web site. Quarterly report on the surveillance of Clostridium difficile infection (CDI) in Scotland. April–June 2012, http://www.hps.scot.nhs.uk/haiic/newsdetail.aspx?id=506; 2012 (Accessed 22 Oct 2012).
[10] McCoubrey J, Poxton IR. Variation in the surface layer proteins of Clostridium difficile. FEMS Immunol Med Microbiol 2001;31:131–5.
[11] McCoubrey J, Starr JM, Martin HJ, Poxton IR. Clostridium difficile in a geriatric unit: prospective epidemiological study employing a novel S-layer typing method. J Med Microbiol 2003;52:573–8.
[12] McKay I, Coia JE, Poxton IR. Typing of Clostridium difficile causing diarrhoea in an orthopaedic ward. J Clin Pathol 1989;42:511–5.
[13] Mutlu E, Wroe AJ, Sanchez-Hurtado K, Brazier JS, Poxton IR. Molecular characterisation and antimicrobial susceptibility patterns of Clostridium difficile strains isolated from hospitals in South-east Scotland. J Med Microbiol 2007; 2007(56):921–9.
[14] Poxton IR, Higgins PG, Currie CG, McCoubrey J. Variation in the cell surface proteins of Clostridium difficile. Anaerobe 1999;5:213–5.
[15] Poxton IR, Byrne MD. Immunological analysis of Clostridium difficile and related species. J Gen Microbiol 1981;122:41.
[16] Poxton IR, Cartmill TDI. Immunochemistry of the cell-surface carbohydrate antigens of Clostridium difficile. J Gen Microbiol 1982;1982(15):223.
[17] Poxton IR, Aronsson Mollby, Nord C-E, Collee JG. Immunochemical fingerprinting of Clostridium difficile strains isolated from an outbreak of antibiotic-associated colitis and diarrhoea. J Med Microbiol 1984;1984(17):317–24.
[18] Poxton IR, Edmond DM. Biological activity of Bacteroides lipopolysaccharide -reappraisal. Clin Infect Dis 1985 1995;20(Suppl. 2):S149–53.
[19] Poxton IR. Fidaxomicin: a new macrocyclic, RNA polymerase-inhibiting antibiotic for treatment of Clostridium difficile infections. Future Microbiology 2010;5:539–48.
[20] Reddy S, Taori S, Poxton IR. Changes in laboratory and clinical workload for Clostridium difficile infection from 2003–2007. Clin Microbiol Infect 2010;16: 340–6.
[21] Sánchez-Hurtado K, Corretge M, Mutlu E, McIlhagger R, Starr JM, Poxton IR. The systemic antibody response to Clostridium difficile in colonised patients with and without symptoms and matched controls. J Med Microbiol 2008;57: 717–24.
[22] Sharp J, Poxton IR. An immunochemical method for fingerprinting Clostridium difficile. J Immunol Methods 1985;83:241–8.
[23] Sharp J, Poxton IR. Analysis of the membrane lipo-carbohydrate antigen of Clostridium difficile by polyacrylamide gel electrophoresis and immunoblotting. FEMS Microbiology Letters 1986;34:97.
[24] Sharp J, Poxton IR. The cell wall proteins of Clostridium difficile. FEMS Microbiology Letters 1988;55:99.
[25] Spigaglia P, Barbanti F, Mastrantonio P, Brazier JS, Barbut F, Delmée M, Kuijper E, Poxton IR. Fluoroquinolone resistance in Clostridium difficile isolates from a prospective study of Clostridium difficile infections in Europe. J Med Microbiol 2008;57:784–9.
[26] Starr JM, McCoubrey J, Martin HJ, Gibson G, Poxton IR. Risk factors for Clostridium difficile colonisation and toxin production. Age and Ageing 2003;32: 657–60.
[27] Starr JM, Campbell A, Renshaw E, Poxton IR, Gibson GJ. Spatio-temporal stochastic modelling of Clostridium difficile. J Hospital Infection 2009;71:49–56.
[28] Taffinder AJ, Beal TA, Shepherd JL, Laurenson IF, Brown R, Poxton IR. Clostridium difficile in a neonatal intensive care unit. Rev Med Microbiol 1997;8-(Suppl. 1):S61–2.
[29] Taori SK, Hall V, Poxton IR. Changes in antibiotic susceptibility and ribotypes in Clostridium difficile isolates from southern Scotland 1979–2004. J Med Microbiol 2010;59:338–44.
[30] Vohra P, Poxton IR. Comparison of toxin and spore production in clinically relevant strains of Clostridium difficile. Microbiology 2011a;157:1343–53.
[31] Vohra P, Poxton IR. Efficacy of decontaminants and disinfectants against Clostridium difficile. J Med Microbiol 2011b;60:1218–24.
[32] Vohra P, Poxton IR. Induction of cytokines in a macrophage cell line by proteins of Clostridium difficile. FEMS Immunol Med Microbiol 2012;65:96–104.