

## INFLUENZA

### Background

During the last century, there were three influenza pandemics, each caused by a novel type A influenza virus of avian origin. In the 1957 and 1968 outbreaks, the responsible viruses, with components of previous human and avian viruses, emerged after viral reassortment occurred in a common host (i.e. porcine). The highly pathogenic virus of 1918 likely emerged after mutation of genetic elements from what had originally been a purely avian pathogen.

In general, influenza pandemics arise when all four of the following occur:

- A novel influenza A virus emerges as a result of an antigenic shift
- There is efficient human-to-human viral transmission
- The new virus is associated with serious morbidity and mortality
- The population has little or no immunity to the virus

Recent outbreaks of avian influenza in Asia and other parts of the world have been of great concern. Such widespread dissemination of these avian influenza viruses is unprecedented, and, in some instances, these viruses have been shown to spread from chickens directly to humans. Of even more concern are the reports of transmission, albeit inefficient, of potentially lethal infections amongst humans.<sup>1,2</sup>

The BC Centre for Disease Control has recommended that physicians be alert for any persons presenting with severe influenza-like illnesses (ILI) who have a history of travel to avian influenza-affected areas.

Physicians are to report severe ILI cases to the local medical officer of health and to collect clinical samples from these patients for viral culture as soon as possible.<sup>3</sup> Public health officials from around the world have reminded the public that the next pandemic is long overdue.



### Projections

It is estimated that an influenza pandemic in B.C, depending on the number of people infected, could result in between 18,500 hospitalizations, 6,800 deaths and 1.8 million outpatient visits. It is expected that the pandemic will last much longer than most other emergency events, and may include waves of influenza activity separated by months. The numbers of health-care workers and first responders available to work will be significantly reduced. Pandemic influenza will have an impact far beyond the health sector, and provincial planning to address the broader issues is ongoing.

### Antiviral medications/Vaccinations

Four influenza antiviral medications (amantadine, rimantadine, oseltamivir, and zanamivir) are approved for the treatment and/or prevention of influenza. However, the influenza A (H5N1) viruses identified in human patients in Asia in 2004 and 2005 were resistant to amantadine and rimantadine. This fact, and the side effect profile and need for individual dosing of amantadine have resulted in the widespread agreement to focus on oseltamivir as the drug of choice in a pandemic situation.

Monitoring of avian viruses for resistance to influenza antiviral medications is ongoing. A vaccine would probably not be available during the first wave of a pandemic, as once the pandemic strain is identified, it will take 3 to 4 months before a vaccine will be widely available. Pandemic Influenza plans have focused on the acquisition and distribution strategies of antivirals and vaccines as well as the

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establishment of priority groups to be immunized and to receive antiviral medication. Canada's contract with its domestic manufacturer calls for the manufacture of 8 million doses a month over a 4-month period, in the event of an influenza pandemic. ■

**AUTHOR:** Dr. Alicia Sarabia MD, FRCPC is a medical microbiologist at MDS Diagnostic Services in Toronto, specializing in infectious diseases.

### REFERENCES

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2. Ungchusak, K. et al. NEJM 2005; 352(4): 333-340.  
www.health.gov.on.ca/english/providers/program/emu/emu\_mn.html
3. http://www.bccdc.org

Additional information is available at:  
Public Health agency of Canada: www.phac-aspc.gc.ca/tmp-pmv/index.html  
Ministry of Health and Long-term Care:  
http://www.health.gov.on.ca/english/providers/program/emu/emu\_mn.html  
MOHLTC 24/7 Healthcare Provider Hotline: 1-866-212-2272

## CLOSTRIDIUM DIFFICILE - associated diarrhea

### OVERVIEW

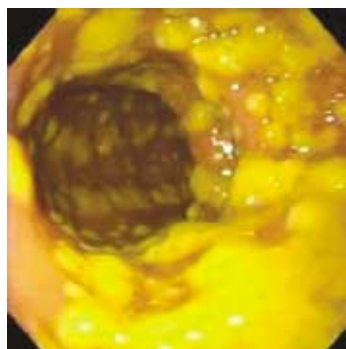
*Clostridium difficile* is the leading cause of antibiotic associated diarrhea in both inpatients and outpatients. This pathogen is also the most important cause of nosocomial diarrhea in the hospital setting, and it is a common cause of diarrhea in the community as well. *C. difficile* can cause severe diarrhea that is fatal in some patients, and it is often associated with multiple relapses requiring repeated courses of antibiotics. The organism produces spores that can survive for long periods in the environment thus facilitating transmission and nosocomial outbreaks. Recently, outbreaks of diarrhea due to a particularly virulent strain of *C. difficile* have been a problem in Eastern Canada. There is concern that this strain will spread to hospitals in other parts of Canada. The outbreaks, characterized by an increase in both mortality rates and severity of disease, suggest that *C. difficile* is a changing pathogen. It is therefore imperative to attempt to prevent the further spread of this strain by improving and adhering to infection control measures.



**Figure 1. Gram stain of *C. difficile* showing gram-positive bacilli with subterminal spores. Courtesy of Dr. Kenneth Todar, University of Wisconsin-Madison.**

### CLINICAL FEATURES

*C. difficile*-associated disease ranges from asymptomatic carriage to moderate diarrhea, pseudomembranous colitis (see Figure 2), and fulminating colitis. More than 50% of healthy neonates and infants are asymptomatic carriers compared to 0%-3% of healthy adults. Risk factors for *C. difficile*-associated diarrhea (CDAD) are antibiotic exposure (especially to second- and third-generation cephalosporins, ampicillin/amoxicillin and clindamycin), antineoplastic agents, advanced age and severe underlying disease. Symptoms manifest within one day of commencing to 10 weeks after cessation of antibiotic treatment, and include watery diarrhea, fever, anorexia, malaise, nausea and abdominal pain or cramping. Colitis can be complicated by toxic megacolon, colonic perforation, sepsis and death.



**Figure 2. Pseudomembranous colitis. Courtesy of Gregory G. Ginsberg, MD, University of Pennsylvania.**

### LABORATORY DIAGNOSIS

Although diarrhea associated with antibiotic use is suggestive of *C. difficile* infection, it is impossible, using clinical criteria alone, to distinguish *C. difficile* infection from disease caused by other enteric pathogens. Therefore, laboratory diagnosis of *C. difficile* infection is critical for effective clinical diagnosis and patient management. *C. difficile* testing is based on culture of the organism and/or detection of its toxins in stool specimens. *C. difficile* toxins may be detected by tissue culture assay or by enzyme immunoassay (EIA). Several days may be required for the tissue culture toxin assay whereas the EIA tests provide results in hours rather than days. However, both types of stool toxin assays suffer from poor sensitivity in the range of 80% or less. *C. difficile* culture is more sensitive than toxin detection, but it also requires several days from collection to reporting of results, and the possibility of carriage of non-toxigenic *C. difficile* strains not associated with disease has limited the utility of the culture approach.

The recent development of nucleic acid amplification methods offers the potential for improved laboratory diagnosis of *C. difficile* infections. Preliminary studies have demonstrated direct detection of toxigenic organisms in stool specimens by using PCR for toxin A and B genes of *C. difficile*. Toxin gene PCR can also be used to improve the culture approach by determining if isolates of the organism are toxigenic. At MDS Metro Laboratory Services in British Columbia, a combined EIA and PCR approach is used to capitalize on the benefits of the two different technologies. Stool

specimens are first screened using EIA for *C. difficile* toxins, and if the specimen is positive, results are reported and no further testing is done. EIA negative specimens are cultured, and potential *C. difficile* isolates recovered from the cultures are identified and tested for toxin A and B genes by PCR. Results are reported as positive or negative for toxigenic *C. difficile*. This approach capitalizes on both the rapid testing capability of the EIA method and the high sensitivity of the culture/PCR approach. A positive result for either test indicates *C. difficile* infection in the symptomatic patient.

## TREATMENT

The treatment of CDAD can be summarized as follows:

- Discontinue the inciting antibiotic if possible and/or change to an antibiotic with a lower risk for CDAD.
- Replace fluid and electrolyte losses.
- Avoid antiperistaltic drugs.
- Oral metronidazole (250 mg 4 times daily or 500 mg 3 times daily for 10-14 days) is the currently recommended first-line therapy.
- Oral vancomycin (125 mg 4 times daily for 10-14 days) should be reserved for patients who fail to respond to metronidazole.
- Intravenous metronidazole is indicated for patients who are unable to tolerate oral antibiotics.
- Treatment is not indicated for asymptomatic carriers.
- Test of cure following treatment is not recommended.

Recurrent CDAD has been reported in approximately 5%-35% of patients following treatment with either metronidazole or vancomycin. The drug of choice for the treatment of an initial recurrence is metronidazole. Oral vancomycin is indicated for patients who do not respond to metronidazole or who have a second relapse. A variety of treatment options are available for patients with multiple relapses but supporting evidence is limited. Tapered or pulsed dose regimens of oral vancomycin as well as combination therapy with vancomycin and rifampin have been used. Adjunctive therapies include *Saccharomyces boulardii*, *Lactobacillus GG* and cholestyramine. ■

### Summary

- *C. difficile* is the leading cause of antibiotic associated diarrhea in both inpatients and outpatients.
- Laboratory tests for *C. difficile* infection include EIA and tissue culture toxin assays as well as toxigenic culture/PCR.
- Treatment is not indicated for asymptomatic patients.
- Metronidazole is the currently recommended drug of choice.
- Test of cure after treatment is not recommended.
- Recurrent *C. difficile* diarrhea occurs in about 5%-35% of treated patients and may require repeated courses of antibiotics.
- Antibiotics should be used judiciously to prevent *C. difficile* diarrhea

**AUTHOR:** Michael Kelly MD, PhD, FRCPC is a medical microbiologist and Laboratory Director at MDS Metro in British Columbia.

Colette Pienaar MD, FCPATH is a medical microbiologist located at MDS Metro in British Columbia.

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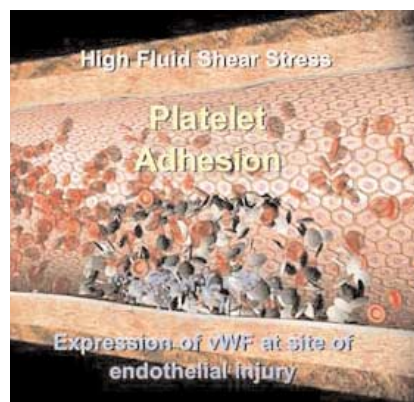
## VON WILLEBRAND DISEASE

Von Willebrand disease (vWD) is the most common congenital bleeding disorder in humans, with a reported frequency of about 1 in 100, but is symptomatic in only about one in 10,000. It was first described by von Willebrand in 1926. The disorder is caused by defective synthesis or function of the von Willebrand factor.

Von Willebrand factor (vWF) is a large adhesive glycoprotein made exclusively in vascular endothelial cells and megakaryocytes under autosomal genetic control. The molecule is synthesized as a number of subunits that polymerize to form large multimeric complexes of variable molecular weight. In the plasma, vWF multimers are combined with the factor VIII coagulant protein, and stabilize it by protecting factor VIII from proteolysis. The functional activity of vWF resides in the higher molecular weight forms of the molecule. vWF is primarily involved in the adhesion of platelets to the injured vessel wall, therefore making vWF essential for the formation of the hemostatic plug.

## CLINICAL FEATURES

Clinical manifestations of vWD are quite variable and range from a mild bleeding diathesis in many Type 1 patients to rare, life threatening hemorrhage in Type 3 and some Type 2 individuals. Due to the variable penetrance of the genes, severity and symptoms may vary among family



members. Furthermore, severity of bleeding does not always correlate with the degree of deficiency.

The most common symptoms are mucosal and superficial bleeding, including epistaxis, easy bruising, gingival bleeding, prolonged bleeding from trivial wounds, and prolonged heavy menstrual bleeding.

The risk of bleeding is increased with the use of aspirin, sodium valproate, or corticosteroids.

Table 1:

Classification of von Willebrand disease (vWD)		
<b>Type 1</b>	(>70% of cases)	partial quantitative deficiency
<b>Type 2:</b>	(15-30% of cases)	qualitative deficiency
Type 2 A	absence of high molecular weight multimers	
Type 2 B	increased affinity for platelets	
Type 2 M	qualitative deficiency with normal proportion of multimers	
Type 2 N*	multimers have decreased affinity for factor VIII	
<b>Type 3</b>	(<1% of cases)	virtually complete quantitative deficiency

\* presents as mild type of factor VIII deficiency

## DIAGNOSTIC TESTING

The laboratory diagnosis of vWD may be difficult due to the acute phase nature of the protein and also because the normal range is broad. The level of circulating vWF is dependent on ABO blood type (individuals with blood type O have lower vWF and factor VIII levels than those with other blood types), race (African-Americans have 15% higher levels than Caucasians), and age (levels increase with age). Many patients with vWD have mild disease and repeated testing may be necessary to confirm the diagnosis. Since many variants of vWD exist (see Table 1), the diagnostic yield is enhanced by performing a panel of tests to include **factor VIII coagulant activity, vWF:Ag, and vWF:RCo activity** (see Table 2).

vWF:Ag (antigen) is a quantitative measure of vWF;

vWF activity (vWF:RCo) is measured by Ristocetin cofactor assay.

Ristocetin is an antibiotic that induces platelet agglutination in the presence of functional vWF.

While the aPTT is often performed, it has low diagnostic value as it is frequently normal. The bleeding time is not recommended; however, an automated platelet function analyzer, the PFA-100, may be useful to screen patients with the most common type (Type 1) of vWD. Patients with Type 2 vWD may require further investigations to clarify the subtype.

Given the number of conditions that can increase vWF, normal values do not exclude the disorder. Female patients with vWD on oral contraceptives or who are pregnant may have normal values.

Table 2:

	APTT	FVIII activity	vWF:Ag	VWF:RCo
Type 1	N or ↑	↓	↓	↓
Type 2 A	N or ↑	N or ↓	N or ↓	↓
Type 2 B	N or ↑	N or ↓	N or ↓	↓
Type 2 M	N or ↑	N or ↓	N or ↓	↓
Type 2 N	↑	↓	N	N
Type 3	↑	↓↓	↓↓	↓↓

## TREATMENT

The type and subtype of vWD are important considerations in treatment. The general health of the patient, medications and other medical conditions must also be considered in determining the type and duration of therapy. The aim of therapeutic intervention is to bring vWF activity and factor VIII levels to 50-100% of normal. Most patients with vWD require therapy only after trauma or in preparation for surgery. The standard approach to treatment is outlined in Table 3. For Type 1 and some Type 2 vWD, DDAVP (Desmopressin) - which releases vWF from endothelial storage - is used, together with antifibrinolytic agents EACA (epsilon aminocaproic acid) or tranexamic acid. ■

Table 3:

Treatment of von Willebrand disease		
	Treatment of choice	Alternative or adjunctive therapy
<b>Type 1</b>	Desmopressin (DDAVP)	Antifibrinolytics, estrogens
<b>Type 2 (A,B,M)</b>	Factor VIII/vWF concentrates	
<b>Type 2 N</b>	Desmopressin (DDAVP)	Factor VIII/vWF concentrates
<b>Type 3</b>	Factor VIII/vWF concentrates	platelet concentrates

**AUTHOR:** **Monika Hudoba MD, FRCP(C)**  
is Hematopathologist and Hematology Discipline Head for MDS Metro

## TECHNICAL UPDATE ANNOUNCEMENTS

### SEMEN ANALYSIS

Please ensure that your patient receives and signs an instruction card provided by MDS Metro before the semen collection.

For valid test results, the instructions must be followed exactly as outlined on the instruction card. The signed card should then be returned to the Patient Service Centre along with the specimen for proper documentation.

For your convenience, copies of the instruction card are available at any MDS Metro Patient Service Centre and are also posted on our website at [www.mdsmetro.com](http://www.mdsmetro.com). Click on "Patient Instructions", then "Semen Analysis".

### HEMOGLOBINOPATHY/THALASSEMIA INVESTIGATION

Historically, hemoglobin electrophoresis was the method of choice for the investigation of hemoglobinopathies and thalassemia syndromes, but it has been replaced in most laboratories by high performance liquid chromatography (HPLC).

Hemoglobin electrophoresis is now used only as a second-line, confirmatory test for abnormal hemoglobins found by HPLC. We would like to remind physicians to request Hemoglobinopathy investigation (or briefly Hb investigation) and not request hemoglobin electrophoresis as such.

Please direct any questions to MDS Metro's Hematopathologists at (604) 431-5005 (Lower Mainland), and (250) 881-3111 (Vancouver Island).

### For more information,

**CONTACT:** **Dr. Monika Hudoba**  
Hematology Discipline Head  
MDS Metro Laboratory Services  
604-431-5005  
[monika.hudoba@mdsinc.com](mailto:monika.hudoba@mdsinc.com)