

Clostridium difficile

REPORTING INFORMATION

- Director's Journal Entry on December 28, 2005.
- January 1 through December 31, 2006, aggregate numbers of confirmed cases of *Clostridium difficile* (*C. difficile*) were reported by hospitals and long term care facilities to the local health department in which the health care facility is located.
- Only health care-associated infections were reportable during the specific time frame. Individual cases of community onset infections were not reportable.
- An outbreak, unusual incidence, or epidemic of infectious diseases of known etiology not categorized as Class A, Class B or Class C is Class C reportable in Ohio.

AGENT

Clostridium difficile is an anaerobic, spore-forming, Gram-positive bacillus that produces at least two toxins: toxin A and toxin B. It is a common cause of antibiotic-associated diarrhea (AAD), accounting for 15%-25% of all episodes of AAD.

New strain of *C. difficile* has been identified

The emergence of a new strain of *C. difficile*-associated disease associated with hospital outbreaks in several states has been reported by the Centers for Disease Control and Prevention (CDC) at scientific meetings. The new strain appears to be more virulent, with ability to produce greater quantities of toxins A and B. In addition, it is more resistant to the antibiotic group known as fluoroquinolones.

CASE DEFINITION

A case is defined as a patient with a first positive (within 6 months) laboratory diagnostic test for *Clostridium difficile* (enzyme immunoassay [EIA], cytotoxin, antigen [Ag], culture) OR pseudomembranes seen on endoscopy OR positive histology from surgical or autopsy specimen.

Health Care-Associated (Initial)

Positive laboratory diagnostic test, endoscopy or biopsy >48 hours after admission to a health care facility.

Health Care-Associated (Recurrent)

Subsequent positive laboratory diagnostic test, endoscopy or biopsy in an individual with a previous health care-associated positive within the prior 6 months. A subsequent infection which occurs >6 months after an initial infection is also classified as an initial infection.

SIGNS AND SYMPTOMS

Clinical signs and symptoms include:

- Watery diarrhea
- Fever
- Loss of appetite
- Nausea
- Abdominal pain/tenderness

DIAGNOSIS

See case definition for *C. difficile*.

As with other strains of *C. difficile*, the new virulent strain can be detected in the stool of infected patients by using laboratory tests that are commonly available in most hospitals. However, none of the commonly available tests differentiate among the various strains of *C. difficile*. Fortunately, because the prevention and control interventions for outbreaks of any strain of *C. difficile* are similar, identification of the specific strain is not imperative for controlling outbreaks.

EPIDEMIOLOGY

Source

Primarily humans; however, any surface, device or material (e.g., commodes, bathing tubs and electronic rectal thermometers) that becomes contaminated with feces may serve as a reservoir for *C. difficile* spores.

Patients at Increased Risk for *C. difficile*-Associated Disease (CDAD)

The risk for disease increases in patients with:

- antibiotic exposure
- gastrointestinal surgery/manipulation
- long length of stay in health care settings
- serious underlying illness
- immunocompromising conditions
- advanced age

C. difficile Colonization versus *C. difficile*-Associated Disease

C. difficile Colonization

- patient exhibits NO clinical symptoms
- patient tests positive for *C. difficile* organism and/or its toxin
- more common than *C. difficile*-associated disease

C. difficile-Associated Disease

- patient exhibits clinical symptoms
- patient tests positive for the *C. difficile* organism and/or its toxin

Mode of Transmission

C. difficile is shed in feces. Any surface, device or material (e.g., commodes, bathing tubs and electronic rectal thermometers) that becomes contaminated with feces may serve as a reservoir for the *C. difficile* spores. *C. difficile* spores are transferred to patients mainly via the hands of health care personnel who have touched a contaminated surface or item.

C. difficile-ASSOCIATED DISEASE PREVENTION IN HEALTH CARE SETTINGS

- Use antibiotics judiciously.
- Use Contact Precautions for patients with known or suspected *C. difficile*-associated disease:
 - Place these patients in private rooms. If private rooms are not available, these patients can be placed in rooms (cohorted) with other patients with *C. difficile*-associated disease.
 - If the institution experiences an outbreak, consider using only soap and water for hand hygiene when caring for patients with *C. difficile*-associated disease; alcohol-based hand rubs may not be as effective against spore-forming bacteria.

- Use gloves when entering patients rooms and during patient care.
- Use gowns if soiling of clothes is likely.
- Dedicate equipment whenever possible.
- CONTINUE THESE PRECAUTIONS UNTIL DIARRHEA CEASES.
- Implement an environmental cleaning and disinfection strategy:
 - Ensure adequate cleaning and disinfection of environmental surfaces and reusable devices, especially items likely to be contaminated with feces and surfaces that are touched frequently.
 - Use an Environmental Protection Agency (EPA)-registered hypochlorite-based disinfectant for environmental surface disinfection after cleaning in accordance with label instructions. Generic sources of hypochlorite (e.g., household chlorine bleach) also may be appropriately diluted and used. (Note: alcohol-based disinfectants are not effective against *C. difficile* and should not be used to disinfect environmental surfaces.)
 - Follow the manufacturer's instructions for disinfection of endoscopes and other devices.
 - Infection control practices in long term care and home health settings are similar to those practices implemented in traditional health care settings.

MANAGEMENT

Health Care Management

Treatment of *C. difficile*

In 23% of patients, *C. difficile*-associated disease will resolve within 2-3 days of discontinuing the antibiotic to which the patient was previously exposed. The infection can be treated with an appropriate course (usually 10 days) of antibiotics, including metronidazole or vancomycin (administered orally). After treatment, repeat *C. difficile* testing is not recommended if the symptoms have resolved, as patients may remain colonized.

Treatment of the Virulent Strain of *C. difficile*

The usual treatment for *C. difficile*-associated disease includes, if possible, stopping antibiotics being given for other purposes and/or treatment with metronidazole or vancomycin. In order to reduce selective pressure for vancomycin resistance in *Enterococci*, current guidelines recommend the first-line use of metronidazole over vancomycin. However, recent reports suggest that the new virulent strain may not respond as well to treatment with metronidazole despite the absence of laboratory evidence of metronidazole resistance. This may be due to increased virulence in the new strain. Depending upon the severity of the *C. difficile*-associated disease, metronidazole is likely to be the appropriate first-line therapy for most cases. Regardless of what therapy is used, patients should be carefully monitored to ensure response to therapy.

Antibiotic formulary restriction should focus on epidemiologically implicated antimicrobial agents, usually second- and third-generation cephalosporins, clindamycin or fluoroquinolones, or a combination of the three.

What is *Clostridium difficile* (*C. difficile*)?

Clostridium difficile is a bacterium that causes diarrhea and more serious intestinal conditions, such as colitis.

What are *C. difficile* diseases?

They are diseases that result from *C. difficile* infections such as colitis, more serious intestinal conditions, sepsis and rarely death.

What are the symptoms of *C. difficile* disease?

Symptoms include:

- watery diarrhea (at least three bowel movements per day for two or more days)
- fever
- loss of appetite
- nausea
- abdominal pain/tenderness

How is *C. difficile* disease treated?

C. difficile is generally treated for 10 days with antibiotics prescribed by your health care provider. The drugs are effective and appear to have few side effects.

How do people get *C. difficile* disease?

People in good health usually do not get *C. difficile* disease. People who have other illnesses or conditions requiring prolonged use of antibiotics and the elderly are at greater risk of acquiring this disease. The bacteria are found in the feces. People can become infected if they touch items or surfaces that are contaminated with feces and then touch their mouth or mucous membranes. Health care workers can spread the bacteria to other patients or contaminate surfaces through hand contact.

What should I do to prevent the spread of *C. difficile* to others?

If you are infected you can spread the disease to others. However, only people that are hospitalized or on antibiotics are likely to become ill. For safety precautions, you may do the following to reduce the chance of spread to others:

- wash hands with soap and water, especially after using the restroom and before eating
- clean surfaces in bathrooms, kitchens and other areas on a regular basis with household detergent/disinfectants

What should I do if I think I have *C. difficile* disease?

See your health care provider.

How is *Clostridium Difficile* (*C. difficile*)-associated disease changing?

Over the past 2 years, several states have reported increased rates of *C. difficile*-associated disease, noting more severe disease and an associated increase in mortality. It is not clear at this time if the population at risk (those most susceptible to acquiring *C. difficile*-associated disease) is changing.

What are the possible reasons for this change in the disease?

The increased rates and/or severity of disease may be caused by changes in antibiotic use, changes in infection control practices or the emergence of a new strain of *C. difficile*-associated disease with increased virulence and/or antimicrobial resistance.

Has a new strain of *C. difficile* been identified?

Yes. The emergence of a new strain of *C. difficile*, often associated with hospital outbreaks in several states, has been reported by the Centers for Disease Control and Prevention (CDC) at scientific meetings.

What is unique about this new strain?

The new strain appears to be more virulent, with ability to produce greater quantities of toxins A and B. In addition, it is more resistant to the antibiotic group known as fluoroquinolones.

How is the new strain detected?

Like other strains of *C. difficile*, this new strain can be detected in the stool of infected patients by using laboratory tests that are commonly available in most hospitals. However, none of the commonly available tests differentiate between the various strains of *C. difficile*. Fortunately, because the control measures for outbreaks of any strain of *C. difficile* are similar, identification of the specific strain is not imperative for controlling outbreaks.

Is treatment of this new strain different?

The usual treatment for *C. difficile*-associated disease includes, if possible, stopping antibiotics being given for other purposes and/or treatment with metronidazole or vancomycin. In order to reduce selective pressure for vancomycin resistance in *Enterococci*, current guidelines recommend the first-line use of metronidazole over vancomycin. However, recent reports suggest that the new strain may not respond as well to treatment with metronidazole, despite the absence of laboratory evidence of metronidazole resistance. This may be due to increased virulence in the new strain. Depending upon the severity of the *C. difficile*-associated disease, metronidazole is likely to be the appropriate first-line therapy for most cases. Regardless of what therapy is used, patients should be carefully monitored to be sure they are responding to therapy.

How does fluoroquinolone resistance affect management of this strain?

Increased fluoroquinolone resistance does not affect the management of infections caused by this strain. Fluoroquinolones have never been recommended for treatment of *C. difficile*-associated disease and susceptibility testing is performed only as a part of an epidemiologic investigation. However, resistance to fluoroquinolones may provide the new strain with an advantage over susceptible strains to spread within health care facilities where these antibiotics are commonly used.

What should health care facilities do in response to the emergence of the new strain?

Health care facilities should monitor the number of *C. difficile*-associated disease cases and, especially if rates at the facility increase, the severity of disease and patient outcomes.

If an increase in rates or severity is observed, health care facilities should reassess compliance with the recommended infection control measures for known cases of *C. difficile*-associated disease including the following:

- Hand hygiene using an alcohol-based hand rub or soap and water
 - If the institution experiences an outbreak, consider using only soap and water for hand hygiene when caring for patients with *C. difficile*-associated disease; alcohol-based hand rubs may not be as effective against spore-forming bacteria.
- Contact precautions
- Environmental cleaning and disinfection strategies

If compliance is optimal with continued increase in rates and disease severity, review antimicrobial use to determine whether particular antimicrobials are associated with cases of *C. difficile*-associated disease. If assistance is needed with these measures, additional help should be sought from local or state health departments and/or local infection control experts.

What is CDC doing to address this issue?

CDC efforts include:

- Collaborating with academic partners to study antimicrobial and other risk factors for *C. difficile*-associated disease for this and other strains
- Working with industry and academic partners on novel environmental disinfection strategies
- Assisting with outbreak investigations in several states

What is *Clostridium difficile* (*C. difficile*)?

C. difficile is a spore-forming, gram-positive anaerobic bacillus that produces two exotoxins: toxin A and toxin B. It is a common cause of antibiotic-associated diarrhea (AAD), accounting for 15%-25% of all episodes of AAD.

What are *C. difficile*-associated diseases?

They are diseases that result from *C. difficile* infections including:

- pseudomembranous colitis (PMC)
- toxic megacolon
- perforations of the colon
- sepsis
- death (rarely)

What are the main clinical symptoms of *C. difficile*-associated disease?

Clinical symptoms include:

- watery diarrhea
- fever
- loss of appetite
- nausea
- abdominal pain/tenderness

Which patients are at increased risk for *C. difficile*-associated disease?

The risk for disease increases in patients with:

- antibiotic exposure
- gastrointestinal surgery/manipulation
- long length of stay in healthcare settings
- serious underlying illness
- immunocompromising conditions
- advanced age

What is the difference between *C. difficile* colonization and *C. difficile*-associated disease?***C. difficile* colonization**

- patient exhibits NO clinical symptoms
- patient tests positive for *C. difficile* organism and/or its toxin
- more common than *C. difficile*-associated disease

***C. difficile*-associated disease**

- patient exhibits clinical symptoms
- patient tests positive for *C. difficile* organism and/or its toxin

Which laboratory tests are commonly used to diagnose *C. difficile*-associated disease?

- Stool culture for *C. difficile*. This is the most sensitive test available, but the one most often associated with false-positive results due to presence of non-toxigenic strains. Stool cultures for *C. difficile* also are labor intensive and require the appropriate culture environment to grow anaerobic microorganisms. Results are available within 48-96 hours of the test.

- Antigen detection for *C. difficile*. These are rapid tests (<1 hour) that detect the presence of *C. difficile* antigen by latex agglutination or immunochromatographic assays. They must be combined with toxin testing to verify diagnosis.
- Toxin testing for *C. difficile**.
 - Enzyme immunoassay (EIA) detects toxin A, toxin B or both A and B. It is a same-day assay, but less sensitive than the tissue culture cytotoxicity assay.
 - Tissue culture cytotoxicity assay detects toxin B only. This assay requires technical expertise to perform, is costly, and requires 24-48 hours for a final result. It does provide specific and sensitive results for *C. difficile*-associated disease.
 - *C. difficile* toxin is very unstable. The toxin degrades at room temperature and may be undetectable within 2 hours after collection of a stool specimen. False-negative results occur when specimens are not promptly tested or kept refrigerated until testing can be done.

How is *C. difficile* transmitted?

C. difficile is shed in feces. Any surface, device or material (e.g., commodes, bathing tubs and electronic rectal thermometers) that becomes contaminated with feces may serve as a reservoir for *C. difficile* spores. *C. difficile* spores are transferred to patients mainly via the hands of healthcare personnel who have touched a contaminated surface or item.

How is *C. difficile*-associated disease usually treated?

In 23% of patients, *C. difficile*-associated disease will resolve within 2-3 days of discontinuing the antibiotic to which the patient was previously exposed. The infection can be treated with an appropriate course (usually 10 days) of antibiotics including metronidazole or vancomycin (administered orally). After treatment, repeat *C. difficile* testing is not recommended if the symptoms have resolved, as patients may remain colonized.

How can *C. difficile*-associated disease be prevented in hospitals and other healthcare settings?

- Use antibiotics judiciously.
- Use contact precautions for patients with known or suspected *C. difficile*-associated disease:
 - Place these patients in private rooms. If private rooms are not available, these patients may be placed in rooms (cohorted) with other patients with *C. difficile*-associated disease.
 - Perform Hand Hygiene using soap and water.
 - If your institution experiences an outbreak, consider using only soap and water for hand hygiene when caring for patients with *C. difficile*-associated disease; alcohol-based hand rubs may not be as effective against spore-forming bacteria.
 - Use gloves when entering patient rooms and during patient care.
 - Use gowns if soiling of clothes is likely.
 - Dedicate equipment whenever possible.
 - CONTINUE THESE PRECAUTIONS UNTIL DIARRHEA CEASES.
- Implement an environmental cleaning and disinfection strategy:
 - Ensure adequate cleaning and disinfection of environmental surfaces and reusable devices, especially items likely to be contaminated with feces and surfaces that are touched frequently.

- Use an Environmental Protection Agency (EPA)-registered hypochlorite-based disinfectant for environmental surface disinfection after cleaning in accordance with label instructions. Generic sources of hypochlorite (e.g., household chlorine bleach) also may be appropriately diluted and used. (Note: alcohol-based disinfectants are not effective against *C. difficile* and should not be used to disinfect environmental surfaces.)
- Follow the manufacturer's instructions for disinfection of endoscopes and other devices.
- Infection control practices in long-term care and home health settings are similar to those practices implemented in traditional healthcare settings.

What can I use to clean and disinfect surfaces and devices to help control *C. difficile*?

Surfaces should be kept clean, and body substance spills should be managed promptly as outlined in the CDC "Guidelines for Environmental Infection Control in Health-Care Facilities."

Hospital cleaning products can be used for routine cleaning. Hypochlorite-based disinfectants have been used with some success for environmental surface disinfection in those patient-care areas where surveillance and epidemiology indicate ongoing transmission of *C. difficile*. Consult the aforementioned guidelines for use conditions for generic sources of hypochlorite-based products (e.g., household chlorine bleach) for disinfection of environmental surfaces.

Note: EPA-registered hospital disinfectants are recommended for general use whenever possible in patient-care areas. At present there are no EPA-registered products with specific claims for inactivating *C. difficile* spores, but there are a number of registered products that contain hypochlorite. If an EPA-registered proprietary hypochlorite product is used, consult the label instructions for proper and safe use conditions.

Running Head: INFECTION CONTROL FOR *C. DIFFICILE*-ASSOCIATED DIARRHEA

Infection Control Recommendations for

Clostridium difficile-Associated Disease in Health Care Facilities

Ohio Department of Health

December 2005

Infection Control Recommendations for *Clostridium difficile*-Associated Disease in Health Care Facilities

Clostridium difficile (*C. difficile*) is the major cause of antibiotic-associated diarrhea (AAD). *C. difficile*-associated disease (CDAD) usually affects elderly patients with other underlying diseases. The organism usually resides in the large intestine. A small proportion of the healthy adult population harbor *C. difficile*; however, normal flora usually keep it in check.

Exposure to broad spectrum antibiotics presents an increased risk for the development of CDAD. The most commonly used antimicrobial agents associated with CDAD are clindamycin, cephalosporins, ampicillin/amoxicillin and fluoroquinolones. The overuse of these antibiotics contributes to the increased incidence of *C. difficile* illness. Studies investigating *C. difficile* clinical infections reported significant changes in incidence or prevalence as a result of antibiotic practice interventions (Lautenbach, E., MD., n.d.).

Degrees of gastrointestinal involvement with *C. difficile* may range from asymptomatic colonization, to mild to severe watery diarrhea, to life-threatening disease, including perforation of the colon and death. McDonald states that *C. difficile may be emerging in a new strain with increased virulence, antimicrobial resistance or both.*

In its spore form, *C. difficile* can withstand drying and heat, and is resistant to many disinfectants. The spores can survive up to five months in the environment. *C. difficile* has been cultured in rooms of infected individuals up to 40 days post discharge. (Patient Safety Advisory, Pennsylvania Patient Safety Reporting Systems, June 2005)

Epidemiology

Agent

C. difficile is an anaerobic, spore-forming, Gram-positive bacillus that produces at least two toxins: toxin A and toxin B.

Reservoir

Human

Mode of Transmission

C. difficile is shed in feces. Any surface, device or material that becomes contaminated with feces may serve as a reservoir for *C. difficile* spores. *C. difficile* spores are transferred to patients mainly via the hands of health care personnel who have touched a contaminated surface or item (CDC Fact Sheet - Information for Health Care Providers).

Infection Control

Standard Precautions

Standard Precautions are the foundation of all infection control practices to prevent transmission of infectious agents associated with health care. The major features of Universal Precautions and Body Substance Isolation, as well as other infection control practices, are included in Standard Precautions. Consistent use of these precautions offers the greatest potential for preventing transmission of organisms. These precautions are used even when walking into an “unknown situation” and are designed to protect health care workers and patients from contact with infectious agents. Hand hygiene is the single most important measure that can be used to reduce the risk of transmitting organisms from

one person to another or from one site to another on the same person or patient, and is an essential component of Standard Precautions. Hand washing, and **not** the use of alcohol based hand rubs, is recommended for use in the presence of *C. difficile*. (APIC Text of Infection Control and Epidemiology, 2005).

Transmission-Based Precautions

In addition to Standard Precautions, Contact Precautions are recommended. Upon entering the room of a patient infected with *C. difficile*, a gown and gloves should be worn. Demonstrated environmental contamination with *C. difficile* does occur (APIC Text of Infection Control and Epidemiology, 2005).

Hand Hygiene

Even though isolation practices and hand washing products have changed since the infection control profession began in the early 1970s, the message that "hand washing is the single most important measure to prevent the transmission of infection" has remained the same.

The Ontario Best Practice Manual for Prevention and Control of Transmission of *C. difficile* within Health Care Facilities (December 2004) states: "after removal and appropriate discarding of PPE (gloves and gown), hands should immediately be washed with soap and water for at least 15 seconds." The Ontario Best Practice Manual further states that "the purpose of hand hygiene is to physically remove *C. difficile* spores through friction, lather and rinsing."

This message is reinforced in the Guideline for Hand Hygiene in Health Care Settings Recommendations of the Health Care Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force (October 25, 2002) which states:

"none of the agents (including alcohols, chlorhexidine, hexachlorophene, iodophors, para-chloro-meta-xyleneol [PCMX], and triclosan) used in antiseptic hand wash or hand rub preparations are reliably sporicidal against *Clostridium* spp. or *Bacillus* spp."

Hand Hygiene Technique

It is recommended that after glove removal, hand washing be accomplished using antimicrobial or non-antimicrobial soap and water, working up a bubbly lather with 15 seconds of vigorous friction, followed by a rinse with flowing water. The caregiver should be careful not to allow recontamination prior to leaving the patient's room.

- Wet hands with water
- Apply soap to hands
- Rub hands vigorously together for at least 15 seconds
- Cover all surfaces of hands and fingers
- Rinse hands well to remove soap residue
- Dry with a paper towel
- Use towel to turn off the faucet.

Patient Placement

Patients with *C. difficile* diarrhea are to be placed in Contact Isolation until their diarrhea has resolved. The use of private rooms or cohorting of patients with CDAD, in addition to the use of Contact Precautions has been successful in limiting transmission of *C. difficile* in hospitals (Gerding, D., MD; Johnson, S., MD; Peterson, L., MD; Mulligan, M., MD; Silva, J., MD, 1995).

Environmental Cleaning

In the Guidelines for Environmental Infection Control in Health Care Facilities (2003), the “recommended approach to environmental infection control with respect to *C. difficile* is meticulous cleaning followed by disinfection using hypochlorite-based germicides as appropriate.” The guidelines further state: “however, because no EPA-registered surface disinfectants with label claims for inactivation of *C. difficile* spores are available, the recommendation is based on the best available evidence from the scientific literature.”

Some investigators have recommended the use of a 1:10 dilution of 5.25% sodium hypochlorite (household bleach) and water for disinfection of rooms of patients with *C. difficile* when there is continued transmission. (Rutala WA, Weber EJ, 1998)

More recently, in William A. Rutala’s slide presentation (2005) at the International Association for Professionals in Infection Control and Epidemiology (APIC) Symposium in Baltimore, Maryland, he stated that: “handwashing, barrier precautions and meticulous environmental cleaning with an EPA-registered disinfectant should be effective in preventing the spread of the organism (*C. difficile*).” Additionally, he states “in units with high endemic *C. difficile* infection rates or in an outbreak setting, use dilute solutions of 5.25-6.15 % sodium hypochlorite (e.g., 1:10 dilution of bleach) for routine disinfection.”

In light of the controversy over the appropriate disinfectant to use, it would seem prudent, at least, to use a 1:10 dilution of 5.25% sodium hypochlorite (household bleach) and water for the areas most heavily contaminated (high touch items: bedrails, call bell, telephone, overbed table, bathroom doorknob, water faucets, etc.).

Dedicated Equipment

“When possible, dedicate the use of non-critical patient care equipment to a single patient (or cohort of patients infected or colonized with the pathogen requiring precautions) to avoid sharing between patients”. “If use of common equipment or items is unavoidable, then adequately clean and disinfect items before use for another patient.” (Centers for Disease Control and Prevention Guidelines for Hospital Infection Control).

If rectal temperatures are obtained, only disposable thermometers should be used with CDAD patients (Lautenbach, E, MD., n. d.).

Antimicrobial Treatment Protocol

Consider instituting a facility-wide treatment protocol, when clinically appropriate.

Oral metronidazole is considered the treatment of choice for CDAD. Oral vancomycin should be reserved for therapy only if there has been a failure to respond to metronidazole, or if the patient cannot tolerate metronidazole (Gerding, et al. 1995).

Antibiotic treatment of asymptomatic patients excreting *C. difficile* is not recommended (Gerding, et al. 1995).

Antibiotic formulary restriction should focus on epidemiologically implicated antimicrobial agents, usually second- and third generation cephalosporins, clindamycin or fluoroquinolones, or a combination of the three.

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