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The Crux

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CONTENTS

1 Editorial
2 Disease Diagnosis
9 Trouble Shooting
10 Bouquet
11 Interpretation
14 Tulip News

Editorial

Cholera is an infection of the small intestine by some strains of the bacterium *Vibrio cholerae*. Symptoms may range from none, to mild, to severe. The classic symptom is large amounts of watery diarrhea that lasts a few days. Vomiting and muscle cramps may also occur. Diarrhea can be so severe that it leads within hours to severe dehydration and electrolyte imbalance. This may result in sunken eyes, cold skin, decreased skin elasticity, and wrinkling of the hands and feet. The dehydration may result in the skin turning bluish. Symptoms start two hours to five days after exposure.

Cholera is caused by a number of types of *Vibrio cholerae*, with some types producing more severe disease than others. It is spread mostly by water and food that has been contaminated with human feces containing the bacteria. Insufficiently cooked seafood is a common source. Humans are the only animal affected. Risk factors for the disease include poor sanitation, not enough clean drinking water, and poverty. There are concerns that rising sea levels will increase rates of disease. Cholera can be diagnosed by a stool test. A rapid dipstick test is available but is not as accurate.

Cholera affects an estimated 3–5 million people worldwide and causes 58,000–130,000 deaths a year as of 2010. While it is currently classified as a pandemic, it is rare in the developed world. Children are mostly affected. Cholera occurs as both outbreaks and chronically in certain areas. Areas with an ongoing risk of disease include Africa and south-east Asia. While the risk of death among those affected is usually less than 5%, it may be as high as 50% among some groups who don't have access to treatment. Historical descriptions of cholera are found as early as the 5th century BC in Sanskrit. The study of cholera by John Snow between 1849 and 1854 led to significant advances in the field of epidemiology. The **"DISEASE DIAGNOSIS"** segment delves deep into Clinico-diagnostic aspects of CHOLERA.

"TROUBLE SHOOTING" talks about three simple yet important procedures conducted in a clinical Laboratory, the exact **procedures and timings may vary from lab to lab.**

"INTERPRETATION" portion highlights colony description as seen on bacteriological media, basic understanding is provided.

"BOUQUET" is as integral to this communiqué as the other items are. Enjoy!

ISO 9001

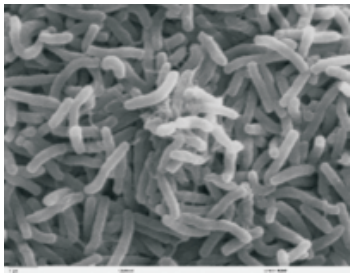
Tulip Group

DISEASE DIAGNOSIS

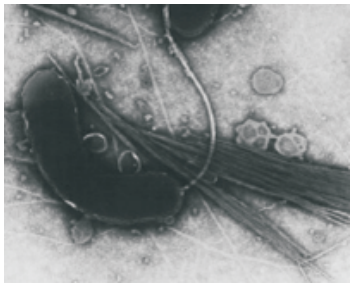
CHOLERA

Background

Cholera is an intestinal infection caused by *Vibrio cholerae* (see the images below). The hallmark of the disease is profuse secretory diarrhea. Cholera can be endemic, epidemic, or pandemic. Despite all the major advances in research, the condition still remains a challenge to the modern medical world. Although the disease may be asymptomatic or mild, severe cholera can cause dehydration and death within hours of onset.



Scanning electron microscope image of *Vibrio cholerae* bacteria, which infect the digestive system.



Electron microscopic image of *Vibrio cholera*.

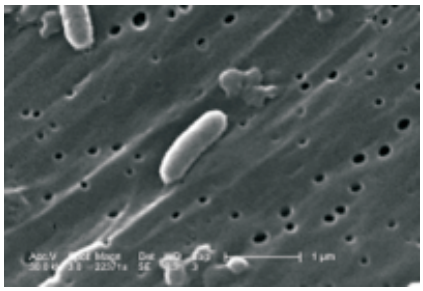
Cholera is transmitted by the fecal-oral route. In the advanced nations because of effective water and sanitation systems, cholera is not a major threat. Nevertheless, both clinicians and members of the general public, especially travelers, should be aware of how the disease is transmitted and what can be done to prevent it. Definitive diagnosis is not a prerequisite for the treatment of patients with cholera. The priority in management of any watery diarrhea is replacing the lost fluid and electrolytes and providing an antimicrobial agent when indicated.

Historical background

Cholera is an ancient disease. Throughout history, populations all over the world have sporadically been affected by devastating outbreaks of cholera. Records from Hippocrates (460-377 BCE) and the Indian peninsula describe an illness that might have been cholera. The 19th century English physician John Snow provided the first demonstration that the transmission of cholera was significantly reduced when uncontaminated water was provided to the population. During a recurrent epidemic of cholera in London in 1854, Snow identified water from the Broad Street pump as the likely source of the disease; removal of the pump handle contained the epidemic. Although not the first description, the discovery of the cholera organism is credited to German bacteriologist Robert Koch, who independently identified *V.cholerae* in 1883 during an outbreak in Egypt. The genus name refers to the fact that the organism appears to vibrate when moving. Since 1817, 7 cholera pandemics have occurred. The pandemics originated from cholera's endemic reservoir in the Indian subcontinent. The first 6 occurred from 1817-1923 and were probably the result of *V. cholerae* O1 of the classic biotype. Of these 6 pandemics, 5 affected Europe and 4 reached the United States, causing more than 150,000 deaths in 1832 and 50,000 deaths in 1866. The seventh pandemic of cholera, and the first in the 20th century, began in 1961; by 1991, it had affected 5 continents. The pandemic continues today. This seventh pandemic was the first recognized to be caused by the El Tor biotype of *V. cholerae* O1. The pandemic originated from the Celebes Islands, Indonesia, and affected more countries and continents than the previous 6 pandemics. A new strain of cholera, *V. cholerae* serogroup O139 (Bengal) emerged in the fall of 1992 and caused outbreaks in Bangladesh and India in 1993. Disease from this strain has become endemic in at least 11 countries. Cholera has been rare in industrialized nations for the past century; however, the disease is still common in other parts of the world, including the Indian subcontinent and sub-Saharan Africa. Epidemics occur after war, civil unrest, or natural disasters when water and food supplies become contaminated with *V. cholerae* in areas with crowded living conditions and poor sanitation.

Pathophysiology

V. cholerae is a comma-shaped, gram-negative aerobic or facultatively anaerobic bacillus that varies in size from 1-3 µm in length by 0.5-0.8 µm in diameter (see the image below). Its antigenic structure consists of a flagellar H antigen and a somatic O antigen. The differentiation of the latter allows for separation into pathogenic and nonpathogenic strains. Although more than 200 serogroups of *V. cholerae* have been identified, *V. cholerae* O1 and *V. cholerae* O139 are the principal ones associated with epidemic cholera.



This scanning electron micrograph (SEM) depicts a number of *Vibrio cholerae* bacteria of the serogroup 01

Currently, the El Tor biotype of *V. cholerae* O1 is the predominant cholera pathogen. Organisms in both the classical and the El Tor biotypes are subdivided into serotypes according to the structure of the O antigen, as follows: Serotype Inaba - O antigens A and C, Serotype Ogawa - O antigens A and B, Serotype Hikojima - O antigens A, B, and C. The clinical and epidemiologic features of disease caused by *V. cholerae* O139 are indistinguishable from those of disease caused by O1 strains. Both serogroups cause clinical disease by producing an enterotoxin that promotes the secretion of fluid and electrolytes into the lumen of the small intestine. To reach the small intestine, however, the organism has to negotiate the normal defense mechanisms of the GI tract. Because the organism is not acid-resistant, it depends on its large inoculum size to withstand gastric acidity. The infectious dose of *V. cholerae* required to cause clinical disease varies by the mode of administration. If *V. cholerae* is ingested with water, the infectious dose is 10^3-10^6 organisms. When ingested with food, fewer organisms (10^2-10^4) are required to produce disease. The use of antacids, histamine receptor blockers and

proton pump inhibitors increases the risk of cholera infection and predisposes patients to more severe disease as a result of reduced gastric acidity. The same applies to patients with chronic gastritis secondary to *Helicobacter pylori* infection or those who have undergone a gastrectomy. *V. cholerae* O1 and *V. cholerae* O139 cause clinical disease by producing an enterotoxin that promotes the secretion of fluid and electrolytes into the lumen of the small intestine. The enterotoxin is a protein molecule composed of 5 B subunits and 2 A subunits. The B subunits are responsible for binding to a ganglioside (monosialosyl ganglioside, GM1) receptor located on the surface of the cells that line the intestinal mucosa. The activation of the A1 subunit by adenylate cyclase is responsible for the net increase in cyclic adenosine monophosphate (cAMP). cAMP blocks the absorption of sodium and chloride by the microvilli and promotes the secretion of chloride and water by the crypt cells. The result is watery diarrhea with electrolyte concentrations isotonic to those of plasma. Fluid loss originates in the duodenum and upper jejunum; the ileum is less affected. The colon is usually in a state of absorption because it is relatively insensitive to the toxin. However, the large volume of fluid produced in the upper intestine overwhelms the absorptive capacity of the lower bowel, resulting in severe diarrhea. Unless the lost fluid and electrolytes are replaced adequately, the infected person may develop shock from profound dehydration and acidosis from loss of bicarbonate. The enterotoxin acts locally and does not invade the intestinal wall. As a result, few neutrophils are found in the stool. The O139 Bengal strain of *V. cholerae* has a very similar pathogenic mechanism except that it produces a novel O139 lipopolysaccharide (LPS) and an immunologically related O-antigen capsule. These 2 features enhance its virulence and increase its resistance to human serum in vitro and occasional development of O139 bacteremia.

Etiology

Cholera can be an endemic, epidemic, or a pandemic disease. Initiation and maintenance of epidemic and pandemic disease by *V. cholerae* result from human infection and poor sanitation with assistance from human migration and seasonal warming of coastal waters. Owing to the relatively large infectious dose, transmission occurs almost exclusively via contaminated water or food. *V. cholerae* O1 has been shown to survive in crabs boiled for 8 minutes, but not in crabs boiled for 10 minutes. Transmission via direct person-to-person contact is rare. Certain environmental and host factors appear to play a role in the spread of *V. cholerae*.

Environmental factors

V. cholerae is a saltwater organism, and its primary habitat is the marine ecosystem where it lives in association with plankton. Cholera has 2 main reservoirs, humans and water. *V. cholerae* is rarely isolated from animals, and animals do not play a role in transmission of disease. Primary infection in humans is incidentally acquired. Risk of primary infection is facilitated by seasonal increases in the number of organisms, possibly associated with changes in water temperature and algal blooms. Secondary transmission occurs through fecal-oral spread of the organism through person-to-person contact or through contaminated water and food. Such secondary spread commonly occurs in households but can also occur in clinics or hospitals where patients with cholera are treated. Infection rates predictably are highest in communities in which water is not potable and personal and community hygiene standards are low.

Host factors

Malnutrition increases susceptibility to cholera. Because gastric acid can quickly render an inoculum of *V. cholerae* noninfectious before it reaches the site of colonization in the small bowel, hydrochlorhydria or achlorhydria of any cause (including *Helicobacter pylori* infection, gastric surgery, vagotomy, use of H2 blockers for ulcer disease) increases susceptibility. The incidence of cholera appears to be twice as high in people with type O blood. The reason for this increased susceptibility is unknown. Infection rates of household contacts of cholera patients range from 20-50%. Rates are lower in areas where infection is endemic and individuals, especially adults, may have preexisting vibriocidal antibodies from previous encounters with the organism. For the same reason, adults are symptomatic less frequently than children, and second infections rarely occur or are mild. An attack of the classic biotype of *V. cholerae* usually results in the generation of antibodies that protect against recurrent infection by either biotype. Those who have had El Tor cholera are not protected against further attacks. Attacks of *V. cholerae* 01 do not lead to immunity against *V. cholerae* 0139. Asymptomatic carriers may have a role in transfer of disease in areas where the disease is not endemic. Although carriage usually is short-lived, a few individuals may excrete the organisms for a prolonged period.

Epidemiology

International statistics

The number of patients with cholera worldwide is uncertain because most cases go unreported. Likely contributory factors are as follows: Most cases occur in remote areas of developing countries where definitive diagnosis is not possible, Reporting systems often are nonexistent in such areas, The stigma of cholera, which has direct adverse effects on commercial trade and tourism, discourages reporting, Many countries with endemic cholera do not report at all. In 1990, fewer than 30,000 cases were reported to the WHO. Reported cases increased more than 10-fold with the beginning of the Latin American epidemic in 1991. In 1994, the number of cases (384,403) and countries (94) reporting cholera was the largest ever registered at the WHO. Even Europe experienced a 30-fold increase in cholera from 1993-1994, with reported cases increasing from 73 to 2,339 and deaths increasing from 2 cases to 47. According to the WHO, the number of cases surged again in 2005. From 2005 to 2008, 178,000-237,000 cases and 4000-6300 deaths were reported annually worldwide. However, the actual global burden is estimated to be 3-5 million cases and 100,000-130,000 deaths per year. The 2008 outbreak in Zimbabwe lasted longer than a year, with more than 98,000 cases and more than 4000 deaths. Outbreaks in Guinea and Yunnan province in China contributed to this increase. The *V. cholerae* O139 serogroup (also known as Bengal), which emerged from Madras, India in October 1992, has spread throughout Bangladesh and India and into neighboring countries; thus far, 11 countries in Southeast Asia have reported isolation of this serogroup. Some experts regard this as an eighth pandemic. In mid-October 2010, a cholera epidemic broke out in Haiti, which has been worsened by heavy rains in 2011. As of June 20, 2011, 363,117 cases of cholera and 5,506 deaths have been reported. The epidemic is the first in Haiti in at least a century, and the source may have been a United Nations peacekeeping team from Nepal that came to Haiti after the catastrophic earthquake that hit the Caribbean nation on January 12, 2010. Analyses performed by US and Haitian laboratories indicate that the strain involved in the outbreak is *V .cholerae* El Tor O1 from the ongoing seventh pandemic predominant in South Asia . This

may have consequences beyond Haiti, since this strain is more hardy and virulent, with an increased resistance to antibiotics.

Age-related differences in incidence

In nonendemic areas, the incidence of infection is similar in all age groups, although adults are less likely to become symptomatic than children. The exception is breastfed children, who are protected against severe disease because of less exposure and because of the antibodies to cholera they obtain in breast milk.

Prognosis

Before the development of effective regimens for replacing fluids and electrolyte losses, the mortality in severe cholera was more than 50%. Mortality is higher in pregnant women and children. Mortality rates are lowest where intravenous therapy is available. Average case fatality rates for Europe and the Americas continue to hover around 1%. At the Treatment Center of the International Center for Diarrheal Disease Research, Bangladesh, less than 1% of patients with severe dehydration die. In Africa, a marked decline in case fatality rates has occurred since 1970; however, Africa continues to have the highest reported case fatality rates (approximately 4% in 1999) compared with the rest of the world. Low case fatality rates have been achieved in South America, presumably because of the availability of adequate treatment facilities and trained personnel.

Patient Education

Education in environmental control is critical for the prevention of cholera. The source of *V. cholerae* in nature is human excrement, and the most common vehicle of infection is water. Environmental control must focus on keeping these elements apart. In the developed world, much has been done in public health planning and in the engineering of water conservation and sewage disposal. However, in developing countries, contamination of water by human excrement is a daily hazard. Members of these populations experience a constant cycle of infection, excretion, and reinfection. Education about the sterilization of water and hand-washing techniques is critical but difficult. Contamination via food is also an important consideration. The source of this contamination is impure water used to wash or flush vegetables and fruit. Water contamination occurs via sewage or soil that is used to fertilize crops. In this situation, training food handlers is necessary.

CLINICAL PRESENTATION

History

After a 24 to 48 hour incubation period, symptoms begin with the sudden onset of painless watery diarrhea that may quickly become voluminous and is often followed by vomiting. The patient may experience accompanying abdominal cramps, probably from distention of loops of small bowel as a result of the large volume of intestinal secretions. Fever is typically absent. However, most *Vibrio cholerae* infections are asymptomatic, and mild to moderate diarrhea due to *V. cholerae* infection may not be clinically distinguishable from other causes of gastroenteritis. An estimated 5% of infected patients will develop cholera gravis, ie, severe watery diarrhea, vomiting, and dehydration.

Diarrhea

Profuse watery diarrhea is a hallmark of cholera. Cholera should be suspected when a patient older than 5 years develops severe dehydration from acute, severe, watery diarrhea (usually without vomiting) or in any patient older than 2 years who has acute watery diarrhea and is in an area where an outbreak of cholera has occurred. Stool volume during cholera is more than that of any other infectious diarrhea. Patients with severe disease may have a stool volume of more than 250 mL/kg body weight in a 24 hour period. Because of the large volume of diarrhea, patients with cholera have frequent and often uncontrolled bowel movements. The stool may contain fecal material early in the course of clinical illness. The characteristic cholera stool is an opaque white liquid that is not malodorous and often is described as having a “rice water” appearance (i.e. in color and consistency, it resembles water that has been used to wash or cook rice).

Vomiting

Vomiting, although a prominent manifestation, may not always be present. Early in the course of the disease, vomiting is caused by decreased gastric and intestinal motility; later in the course of the disease it is more likely to result from acidemia.

Dehydration

If untreated, the diarrhea and vomiting lead to isotonic dehydration, which can lead to acute tubular necrosis and renal failure. In patients with severe disease, vascular collapse, shock, and death may ensue. Dehydration can develop with remarkable rapidity, within hours after the onset of symptoms. This contrasts with disease produced by infection from any other enteropathogen. Because the dehydration is isotonic, water loss is proportional between 3 body compartments, intracellular, intravascular, and interstitial.

Physical Examination

Clinical signs of cholera parallel the level of volume contraction. The amount of fluid loss and the corresponding clinical signs of cholera are as follows: 3-5% loss of normal body weight - Excessive thirst. 5-8% loss of normal body weight - Postural hypotension, tachycardia, weakness, fatigue, dry mucous membranes or dry mouth. >10% loss of normal body weight - Oliguria; glassy or sunken eyes; sunken fontanelles in infants; weak, thready, or absent pulse; wrinkled "washerwoman" skin; somnolence; coma.

Assessment for Dehydration

The World Health Organization has classified dehydration in patients with diarrhea into the following 3 categories, to facilitate treatment (see Table 1): Severe. Some (previously termed moderate, in the WHO criteria). None (previously termed mild, in the WHO criteria). Children without clinically significant dehydration (< 5% loss of body weight) may have increased thirst without other signs of dehydration. In children with some (i.e. moderate) dehydration, cardiac output and vascular resistance are normal, and changes in interstitial and intracellular volume are the primary manifestations of illness. Skin turgor is decreased, as manifested by prolonged skin tenting in response to a skin pinch (the most reliable sign of isotonic dehydration), and a normal pulse. For the skin pinch, it is important to pinch longitudinally rather than horizontally and to maintain the pinch for a few seconds before releasing the skin. The skin pinch may be less useful in patients with marasmus (severe wasting), kwashiorkor (severe malnutrition with edema), or obesity. In adults and children older than 5 years, other signs of severe dehydration include tachycardia, absent or barely palpable peripheral pulses, and hypotension. Tachypnea and hypercapnia also are part of the clinical picture and are attributable to the metabolic acidosis that invariably is present in patients with cholera who are dehydrated.

Metabolic and systemic manifestations

After dehydration, hypoglycemia is the most common lethal complication of cholera in children. Hypoglycemia is a result of diminished food intake during the acute illness, exhaustion of glycogen stores, and defective gluconeogenesis secondary to insufficient stores of gluconeogenic substrates in fat and muscle. Cholera causes bicarbonate loss in stools, accumulation of lactate because of diminished perfusion of peripheral tissues, and hyperphosphatemia. Acidemia results when respiratory compensation is unable to sustain a

normal blood pH. Hypokalemia results from potassium loss in the stool, with a mean potassium concentration of approximately 3.0 mmol/L. Because of the existing acidosis, however, children often have normal serum potassium concentrations when first observed, despite severe total body potassium depletion. Hypokalemia develops only after the acidosis is corrected and intracellular hydrogen ions are exchanged for extracellular potassium. Hypokalemia is most severe in children with preexisting malnutrition who have diminished body stores of potassium and may be manifested as paralytic ileus. Rehydration therapy with bicarbonate-containing fluids can also produce hypocalcemia by decreasing the proportion of serum calcium that is ionized. Chvostek and Trousseau signs are often present, and spontaneous tetanic contractions can occur.

Pediatric patients

In pediatric patients, the primary signs are similar to those in adults. However, children with severe cholera may present with signs that are rarely seen in adults. A child with cholera is usually very drowsy, and coma is not uncommon. In addition, pediatric patients may have convulsions that appear to be related, in part, to hypoglycemia because patients exhibit some response to intravenous dextrose. Another significant difference from the adult presentation is that children are often febrile.

Cholera sicca

Cholera sicca is an old term describing a rare, severe form of cholera that occurs in epidemic cholera. This form of cholera manifests as ileus and abdominal distention from massive outpouring of fluid and electrolytes into dilated intestinal loops. Mortality is high, with death resulting from toxemia before the onset of diarrhea and vomiting. The mortality in this condition is high. Because of the unusual presentation, failure to recognize the condition as a form of cholera is common.

Table: Assessment for Dehydration

Table 1. Assessment of the Patient With Diarrhea for Dehydration (based on WHO classification)

Sensorium	Eyes	Thirst	Skin Pinch	Decision
Abnormally sleepy or lethargic	Sunken	Drinks poorly or not at all	Goes back very slowly (>2 sec)	If the patient has 2 or more of these signs, severe dehydration is present
Restless, irritable	Sunken	Drinks eagerly	Goes back slowly (< 2 sec)	If the patient has 2 or more signs, some dehydration is present
Well, alert	Normal	Drinks normally, not thirsty	Goes back quickly	Patient has no dehydration

Differential Diagnosis

Diagnostic Considerations

Although other differential diagnosis of gastroenteritis may be considered, the clinical picture of cholera is unlikely to be confused with any other disease. This is especially true in adults, in whom no other infectious disease causes such profound dehydration so quickly.

Differential Diagnoses

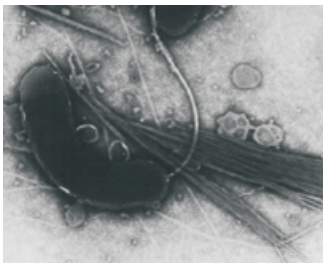
Escherichia Coli Infections. Gastroenteritis. Rotavirus.

Approach Considerations

Definitive diagnosis is not a prerequisite for the treatment of patients with cholera. The priority in management of any watery diarrhea is replacing the lost fluid and electrolytes and providing an antimicrobial agent when indicated. According to World Health Organization (WHO) standard case definition, a case of cholera is suspected when the following conditions are met: In an area where the disease is not known to be present, a patient aged 5 years or older develops severe dehydration or dies from acute watery diarrhea. In an area with a noted cholera epidemic, a patient aged 5 years or older develops acute watery diarrhea, with or without vomiting. In endemic areas, biochemical confirmation and characterization of the isolate are usually unnecessary. However, these tasks may be worthwhile in areas where *Vibrio cholerae* is an uncommon isolate. If identification of the organism is required, direct microscopic examination of stool (including dark-field examination) is indicated, along with Gram stain, culture, and serotype and biotype identification. Polymerase chain reaction (PCR) tests for identifying *V. cholerae* have been developed. These have a high degree of sensitivity and specificity. At present, however, such tests are used for screening of food samples.

Stool Examination

Vibrio cholerae is a gram-negative curved bacillus that is motile by means of a single flagellum. Laboratory diagnosis is required not only for identification but also for epidemiological purposes (see the image below).



Electron microscopic image of *Vibrio cholera*.

Although observed as a gram-negative organism, the characteristic motility of *Vibrio* species cannot be identified on a Gram stain, but it is easily seen on direct dark-field examination of the stool.

Stool Culture

V. cholerae is not fastidious in nutritional requirements for growth. However, it does need an adequate buffering system if fermentable carbohydrate is present because viability is severely compromised if the pH is less than 6, often resulting in autosterilization of the culture. Many of the selective media used to differentiate enteric pathogens do not support the growth of *V. cholerae*.

Routine differential media

Colonies are lactose-negative, like all other intestinal pathogens, but sucrose-positive. When plated onto triple-sugar iron agar to screen for *Salmonella* and *Shigella* species, the organism gives the nonpathogenic pattern of an acid (yellow) slant and acid butt because of fermentation of the sucrose contained in triple-sugar iron agar. Unlike other Enterobacteriaceae, *V. cholerae* is oxidase-positive; hence, in countries where selective media are not available and cholera is not endemic, *V.*

cholerae should be suspected if any motile, oxidase-positive, gram-negative rod isolated on routine differential media from the stool of a patient with diarrhea produces an acid reaction on triple sugar iron agar.

Alkaline enrichment media

As *Vibrio* has the ability to grow at a high pH or in bile salts, which inhibit many other Enterobacteriaceae, peptone water (pH 8.5-9) or selective media containing bile salts (e.g. thiosulfate-citrate-bile-sucrose-agar [pH 8.6]) are recommended to facilitate isolation and lab diagnosis. On thiosulfate-citrate-bile-sucrose-agar, the sucrose-fermenting *V. cholera* grow as large, smooth, round yellow colonies that stand out against the blue-green agar.

Serotyping and Biotyping

Specific antisera can be used in immobilization tests. A positive immobilization test result (i.e. cessation of motility of the organism) is produced only if the antiserum is specific for the *Vibrio* type present; the second antiserum serves as a negative control. *Vibrio* antisera may be unavailable in countries where cholera is not endemic. In endemic regions, this is an excellent quick method of identification, even in small laboratories. Classic and El Tor biotypes also can be identified using the same method. This is useful for epidemiologic studies.

Hematologic Tests

The major hematologic derangements in patients with cholera derive from the alterations in intravascular volume and electrolyte concentrations. Hematocrit, serum-specific gravity, and serum protein are elevated in dehydrated patients because of resulting hemoconcentration. When patients are first observed, they generally have a leukocytosis without a left shift.

WORK UP

Metabolic Panel

Serum sodium is usually 130-135 mmol/L, reflecting the substantial loss of sodium in the stool. Serum potassium usually is normal in the acute phase of the illness, reflecting the exchange of intracellular potassium for extracellular hydrogen ion in an effort to correct the acidosis. Hyperglycemia may be present, secondary to systemic release of epinephrine, glucagon, and cortisol due to hypovolemia. Patients have elevated blood urea nitrogen and creatinine levels consistent with prerenal azotemia. The extent of elevation depends on the degree and duration of dehydration. A reduced bicarbonate level (< 15 mmol/L) and an elevated anion gap occur as a result of increases in serum lactate, protein, and phosphate levels. The arterial pH is usually low (approximately 7.2). Calcium and magnesium levels are usually high as a result of hemoconcentration.

Approach Considerations

Rehydration is the first priority in the treatment of cholera. Rehydration is accomplished in 2 phases: rehydration and maintenance. The goal of the rehydration phase is to restore normal hydration status, which should take no more than 4 hours. Set the rate of intravenous infusion in severely dehydrated patients at 50-100 mL/kg/hr. Lactated Ringer solution is preferred over isotonic sodium chloride solution because saline does not correct metabolic acidosis. The goal of the maintenance phase is to maintain normal hydration status by replacing ongoing losses. The oral route is preferred, and the use of oral rehydration solution (ORS) at a rate of 500-1000 mL/hr is recommended.

Treatment Guidelines

The World Health Organization (WHO) guidelines for the management of cholera are practical, easily understood, and readily applied in clinical practice (see Table 7). These guidelines can be used for the treatment of any patient with diarrhea and dehydration. Diagnosis of cholera is not required to initiate hydration therapy.

Cholera Cots

In areas where cholera is endemic, cholera cots have been used to assess the volume of ongoing stool losses. A cholera cot is a cot covered by a plastic sheet with a hole in the center to allow the stool to collect in a calibrated bucket underneath. Use of such a cot allows minimally trained health workers to calculate fluid losses and replacement needs. The volume of stool is measured every 2-4 hours, and the volume of fluid administered is adjusted accordingly. In the initial phase of therapy, urine losses account for only a small proportion of fluid losses, and the amount of fluid in the bucket is an adequate reflection of stool losses. With rehydration, urine should be collected separately, so that a vicious circle of increasing urine output and overhydration can be avoided.

Rehydration

The WHO has provided recommendations for fluid replacement in patients with dehydration (see Table 2). The recommendations include recommendations for fluid replacement for severe hydration, some dehydration, and no dehydration.

Severe dehydration

Administer intravenous (IV) fluid immediately to replace fluid deficit. Use lactated Ringer solution or, if that is not available, isotonic sodium chloride solution. If the patient can drink, begin giving oral rehydration salt solution (ORS) by mouth while the drip is being set up; ORS can provide the potassium, bicarbonate, and glucose that saline solution lacks. For patients older than 1 year, give 100 mL/kg IV in 3 hours-30 mL/kg as rapidly as possible (within 30 min) then 70 mL/kg in the next 2 hours. For patients younger than 1 year, administer 100 mL/kg IV in 6 hours-30 mL/kg in the first hour then 70 mL/kg in the next 5 hours. Monitor the patient frequently. After the initial 30 mL/kg has been administered, the radial pulse should be strong and blood pressure should be normal. If the pulse is not yet strong, continue to give IV fluid rapidly. Administer ORS solution (about 5 mL/kg/h) as soon as the patient can drink, in addition to IV fluid. Reassess the hydration status after 3 hours (infants after 6 h), using Table 1. In the rare case that the patient still exhibits signs of severe dehydration, repeat the IV therapy already given. If signs of some dehydration are present, continue as indicated below for some dehydration. If no signs of dehydration exist, maintain hydration by replacing ongoing fluid losses. *Routes for parenteral rehydration:* Accessing a peripheral vein is relatively easy, despite the severe dehydration. If a peripheral vein is not readily accessible, scalp veins have been used for initial rehydration. As the vascular volume is reestablished, a larger needle or catheter can be introduced in a peripheral vein. Intraosseous routes have been used successfully in young children when veins cannot be accessed. The intraperitoneal route has been tried, but is not recommended. ORS solution can be administered via nasogastric tube if the patient has some signs of dehydration and cannot drink or if the patient has severe dehydration and IV therapy is not possible at the treatment facility. *Overhydration:* A risk of overhydration exists with intravenous fluids; it usually first manifests as puffiness around the eyes. Continued excessive administration of intravenous fluids can lead to pulmonary edema and has been observed even in children with normal cardiovascular reserve. Thus, it is important to monitor patients who are receiving intravenous rehydration hourly. Serum-specific gravity is an additional measure of the adequacy of rehydration.

Some dehydration

Administer ORS solution according to the amount recommended in Table 3. WHO ORS contains the following: Sodium – 75 mmol/L, Chloride – 65 mmol/L, Potassium – 20 mmol/L, Bicarbonate – 30

mmol/L, Glucose – 111 mmol/L. A homemade equivalent is 6 teaspoons of sugar and one half teaspoon of salt in a liter of water; a half cup of orange juice or some mashed banana can provide potassium. Use the patient's age only when weight is unknown. The approximate amount of ORS required (in mL) also can be calculated by multiplying the patient's weight (in kg) times 75. If the patient passes watery stools or wants more ORS solution than shown, give more. Monitor the patient frequently to ensure that the ORS solution is taken satisfactorily and to identify patients with profuse ongoing diarrhea who require closer monitoring. Reassess the patient after 4 hours, using Table 1. In the rare case where signs of severe dehydration have appeared, rehydrate for severe dehydration, as above. If some dehydration is still present, repeat the procedures for some dehydration and start to offer food and other fluids. If no signs of dehydration are present, maintain hydration by replacing ongoing fluid losses. Most patients absorb enough ORS solution to achieve rehydration, even when they are vomiting. Vomiting usually subsides within 2-3 hours, as rehydration is achieved. Urine output decreases as dehydration develops and may cease. It usually resumes within 6-8 hours after starting rehydration. Regular urinary output (ie, every 3-4 h) is a good sign that enough fluid is being given.

No signs of dehydration

Patients who have no signs of dehydration when first observed can be treated at home. Give these patients ORS packets to take home, enough for 2 days. Demonstrate how to prepare and give the solution. The caretaker should give the patient the amount of ORS solution shown in Table 4. Instruct the patient or the caretaker to return if any of the following signs develop: Increased number of watery stools, Eating or drinking poorly, Marked thirst, Repeated vomiting, Any signs indicating other problems (eg, fever, blood in stool).

Maintenance of Hydration

Maintain hydration of patients presenting with severe or some dehydration. Replace ongoing fluid losses until diarrhea stops. When a patient who has been rehydrated with IV fluid or ORS solution is reassessed and has no signs of dehydration, continue to administer ORS solution to maintain normal hydration. The aim is to replace stool losses as they occur with an equivalent amount of ORS solution. See Table 5. The amount of ORS solution required to maintain hydration varies greatly among patients, depending on the volume of stool passed. It is highest in the first 24 hours of treatment and is especially large in patients who present with severe dehydration. In the first 24 hours, the average requirement of ORS solution in such patients is 200 mL/kg, but some patients may need as much as 350 mL/kg. Continue to reassess the patient for signs of dehydration at least every 4 hours to ensure that enough ORS solution is being taken. Patients with profuse ongoing diarrhea require more frequent monitoring. If signs of some dehydration are detected, the patient should be rehydrated as described earlier, before continuing with treatment to maintain hydration. A few patients, whose ongoing stool output is very large, may have difficulty in drinking the volume of ORS needed to maintain hydration. If these patients become tired, vomit frequently, or develop abdominal distension, ORS solution should be stopped and hydration should be maintained intravenously with lactated Ringer solution or isotonic sodium chloride solution, administering 50 mL/kg in 3 hours. After this is done, resuming treatment with ORS solution is usually possible. Keep the patient under observation, if possible, until diarrhea stops or is infrequent and of small volume. This is especially important for any patient presenting with severe dehydration. If a patient must be discharged from the hospital before diarrhea has stopped, show the caretaker how to prepare and give ORS solution, and instruct the caretaker to continue to give ORS solution, as above. Also instruct the caretaker to return the patient to the hospital if any signs of danger appear.

Antibiotic Treatment

An effective antibiotic can reduce the volume of diarrhea in patients with severe cholera and shorten the period during which *V. cholerae* O1 is excreted. In addition, it usually stops the diarrhea within 48 hours, thus shortening the period of hospitalization. Whenever possible, antibiotic therapy should be guided by susceptibility reports. Antibiotic treatment is indicated for severely dehydrated patients who are older than 2 years. Begin antibiotic therapy after the patient has been rehydrated (usually in 4-6 h) and vomiting has stopped. No advantage exists to using injectable antibiotics, which are expensive. No other drugs should be used in the treatment of cholera. Antimicrobial agents typically are administered for 3-5 days (see Table 6). However, single-dose therapy with tetracycline, doxycycline, furazolidone, or ciprofloxacin has been shown effective in reducing the duration and volume of diarrhea. Because single dose doxycycline has been shown to be as effective as multiple doses of tetracycline, this has become the preferred.

Fluid Replacement for Dehydration

Table 2. Fluid Replacement for Dehydration

Severe dehydration	Intravenous (IV) drips of Ringer Lactate or, if not available, normal saline and oral rehydration salts as outlined below	● 100 mL/kg in 3-h period (in 6 h for children < 1 y) ● Start rapidly (30 mL/kg within 30 min, then slow down) ● Total amount for first 24 h: 200 L/kg
Some dehydration	Oral rehydration salts (amount in first 4 h)	● Infants < 4 mo (< 5 kg): 200–400 mL ● Infants 4–11 mo (5–7.9 kg): 400–600 mL ● Children 1–2 y (8–10.9 kg): 600–800 mL ● Children 2–4 y (11–15.9 kg): 800–1200 mL ● Children 5–14 y (16–29.9 kg): 1200–2200 mL ● Patients >14 y (≥30 kg): 2200–4000 mL
No dehydration	Oral rehydration salts	● Children < 2 y: 50–100 mL, up to 500 mL/day ● Children 2–9 y: 100–200 mL, up to 1000 mL/day ● Patients >9 y: As much as wanted, up to 2000 mL/day

Oral Rehydration During First 4 Hours

Table 3. Approximate Amount of Oral Rehydration Solution to Administer in the First 4 Hours (Open Table in a new window)

Age	< 4 mo	4-11 mo	12-23 mo	2-4 y	5-14 y	≥15 y
Weight	< 5 kg	5-7.9 kg	8-10.9 kg	11-15.9 kg	16-29.9 kg	≥30 kg
ORS solution in mL	200-400	400-600	600-800	800-1200	1200-2200	2200-4000

Oral Rehydration for Home Administration

Table 4. Estimate of Oral Rehydration Solution Packets to Be Administered at Home

Age	Amount of Solution After Each Loose Stool	ORS Packets Needed
< 24 mo	50-100 mL	Enough for 500 mL/d
2-9 y	100-200 mL	Enough for 1000 mL/d
≥10 y	As much as is wanted	Enough for 200 mL/d

Oral Replacement Solution for Hydration Maintenance

Table 5. Oral Replacement Solution for Maintenance of Hydration

Age	Amount of Solution After Each Loose Stool
< 24 mo	100 mL
2-9 y	200 mL
≥10 y	As much as is wanted

Antimicrobial Therapy for Cholera

Table 6. Antimicrobial Therapy Used in the Treatment of Cholera*

Antibiotic	Single Dose (PO)	Multiple Dose (PO)
Doxycycline†	7 mg/kg; not to exceed 300 mg/dose‡	2 mg/kg bid on day 1; then 2 mg/kg qd on days 2 and 3; not to exceed 100 mg/dose
Tetracycline†	25 mg/kg; not to exceed 1 g/dose‡	40 mg/kg/d divided qid for 3 d; not to exceed 2 g/d
Furazolidone	7 mg/kg; not to exceed 300 mg/dose	5 mg/kg/d divided qid for 3 d; not to exceed 400 mg/d
Trimethoprim and sulfamethoxazole	Not evaluated	< 2 months: Contraindicated ≥2 months: 5-10 mg/kg/d (based on trimethoprim component) divided bid for 3 d; not to exceed 320 mg/d trimethoprim and 1.6 g/d of sulfamethoxazole
Ciprofloxacin§	30 mg/kg; not to exceed 1 g/dose‡	30 mg/kg/d divided q12h for 3 d; not to exceed 2 g/d
Ampicillin	Not evaluated	50 mg/kg/d divided qid for 3 d; not to exceed 2 g/d
Erythromycin	Not evaluated	40 mg/kg/d erythromycin base divided tid for 3 d; not to exceed 1 g/d

* Antimicrobial therapy is an adjunct to fluid therapy of cholera and is not an essential component. However, it reduces diarrhea volume and duration by approximately 50%. The choice of antibiotics is determined by the susceptibility patterns of the local strains of *V. cholerae* O1 or O139.

† Tetracycline and doxycycline can discolor permanent teeth of children younger than 8 years. However, the risk is small when these drugs are used for short courses of therapy, especially if used in a single dose.

‡ Single-dose therapy of these drugs has not been evaluated systematically in children, and recommendations are extrapolated from experience in adults.

§ Fluoroquinolones (eg, ciprofloxacin) are not approved in some nations for use in persons younger than 18 years. When given in high doses to juvenile animals, they cause arthropathy. Clinical experience indicates that this risk is very small in children when used for short courses of therapy.

WHO Guidelines for Cholera Management

Table 7. WHO Guidelines for Cholera Management

Steps in the treatment of a patient with suspected cholera are as follows:

1. Assess for dehydration (see Table 1)
2. Rehydrate the patient and monitor frequently, then reassess hydration status
3. Maintain hydration; replace ongoing fluid losses until diarrhea stops
4. Administer an oral antibiotic to the patient with severe dehydration
5. Feed the patient

More detailed guidelines for the treatment of cholera are as follows:

- Evaluate the degree of dehydration upon arrival
- Rehydrate the patient in 2 phases; these include rehydration (for 2-4 h) and maintenance (until diarrhea abates)
- Register output and intake volumes on predesigned charts and periodically review these data
- Use the intravenous route only (1) during the rehydration phase for severely dehydrated patients for whom an infusion rate of 50-100 mL/kg/h is advised, (2) for moderately dehydrated patients who do not tolerate the oral route, and (3) during the maintenance phase in patients considered high stool purgers (i.e. >10 mL/kg/h)
- During the maintenance phase, use oral rehydration solution at a rate of 800-1000 mL/h; match ongoing losses with ORS administration
- Discharge patients to the treatment center if oral tolerance is greater than or equal to 1000 mL/h, urine volume is greater than or equal to 40 mL/h, and stool volume is less than or equal to 400 mL/h.

Diet

Resume feeding with a normal diet when vomiting has stopped. Continue breastfeeding infants and young children. Malnutrition after infection is not a major problem, as it is after infection with *Shigella* species or rotavirus diarrhea. The catabolic cost of the infection is relatively low, anorexia is neither profound nor persistent, and intestinal enzyme activity remains intact after infection; hence, intestinal absorption of nutrients is near normal. There is no reason to withhold food from cholera patients.

TROUBLESHOOTING

Hanging Drop Procedure

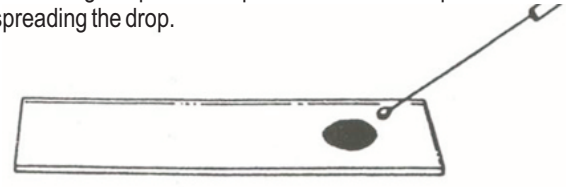
1. Hold a clean coverslip by its edges and carefully dab Vaseline on its corners using a toothpick. If too much Vaseline is used, it will be squeezed toward the center and mix with the drop or squeeze out the edges and get on the objective lens of the microscope.
2. Place a loopful of the culture to be tested in the center of the prepared coverslip.
3. Turn the clean concavity slide upside down (concavity down) over the drop on the coverslip so that the Vaseline seals the coverslip to the slide around the concavity.
4. Turn the slide over so the coverslip is on top and the drop can be observed banging from the coverslip over the concavity.
5. Place the preparation in the microscope slide holder and align it using the naked eye so an edge of the drop is under the low power objectives.
6. Turn the objective to its lowest position using the coarse adjustment and CLOSE THE DIAPHRAGM.
7. Look through the eyepiece and raise the objective slowly using the coarse adjustment knob until the edge of the drop is observed as an irregular line crossing the field.
8. Move the slide to make that line (the edge of the drop) pass through the center of the field.
9. Without raising or lowering the tube, swing the high dry objective into position (Be sure the high dry objective is clean).
10. Observe the slide through the eyepiece and adjust the fine adjustment until the edge of the drop can be seen as a thick, usually dark line.
11. Focus the edge of the drop carefully and look at each side of that line for very small objects that are the bacteria. The cells will look either like dark or slightly greenish, very small rods or spheres. Remember the high dry objective magnifies a little less than half as much as the oil immersion objective.
12. Adjust the light using the diaphragm lever to maximize the visibility of the cells.
13. Observe the cells noting their morphology and grouping and determine whether true motility can be observed.
14. Brownian movement should be visible on slides of all the organisms, but two should also show true motility.
15. Wash the depression slide and after soaking in lysol buckets.

NOTE: The bacteria are still alive in a hanging drop slide. Slides made from possible pathogens should be soaked in lysol for 5-10 minutes with the coverslip pulled aside to expose the drop before they are washed.

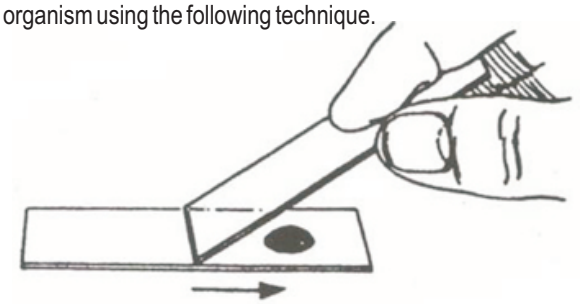
Negative Stain Procedure

To conduct a proper negative stain the following procedure should be followed:

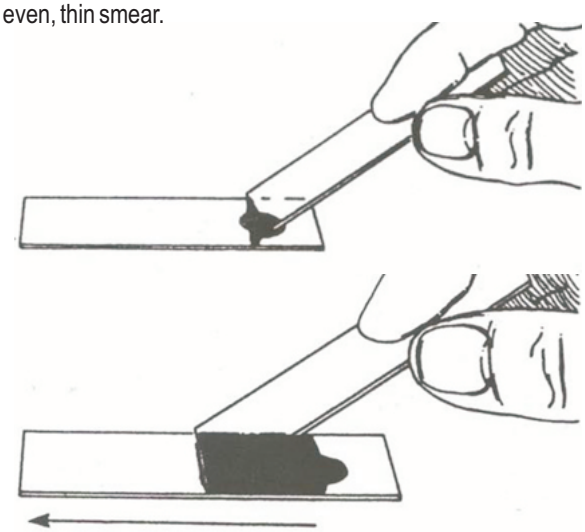
1. Place a very small drop (more than a loop full--less than a free falling drop from the dropper) of nigrosin near one end of a well-cleaned and flamed slide.
2. Remove a small amount of the culture from the slant with an inoculating loop and disperse it in the drop of stain without spreading the drop.



3. Use another clean slide to spread the drop of stain containing the organism using the following technique.



4. Rest one end of the clean slide on the center of the slide with the stain. Tilt the clean slide toward the drop forming an acute angle and draw that slide toward the drop until it touches the drop and causes it to spread along the edge of the spreader slide. Maintaining a small acute angle between the slides, push the spreader slide toward the clean end of the slide being stained dragging the drop behind the spreader slide and producing a broad, even, thin smear.



5. Allow the smear to dry without heating.

6. Focus a thin area under oil immersion and observe the unstained cells surrounded by the gray stain.

Flagella Stain Procedure:

Procedure:

A. Bacterial Suspension

From an agar slant culture: Suspend a loopful of bacteria in 2 ml distilled water to obtain an opalescent suspension. Allow the suspension to stand undisturbed for 15 to 20 minutes while flagella are regenerated and extended.

B. Slides

Select new or unscratched slides. Clean slides with cleaning powder as directed. Flame one of the slides and allow it to cool. Make a heavy wax line on the flamed side along the margin of one end, along both sides to within about one inch of the other end, and across the slide to complete the rectangle. Handle the slide by the unmarked end only. The wax line creates a retaining wall to allow a pool of stain to surround the cells during the 15 to 15 minute staining

period.

C. Preparation of smear

Handling the suspension carefully remove a large loopful of the suspension and place it at one end of the rectangular area. Tilt the slide to permit the suspension to run down to the other end of the slide. If the drop fails to run, add another drop. Air dry the film. Do not heat. Place the slide on a horizontal staining rack.

D. Staining procedure

Add about 1 ml. of the Flagella Stain solution (one dropper full) to the smear and allow to stain for 10-15 minutes. The solution should not be allowed to flow outside the waxed area. Flood off the stain by adding tap water to the slide while it remains on the rack. Do not tip the slide before this is done. Drain and flood the slide with carbol fuchsin for one minute. Rinse by flooding. Drain and air dry. Do not blot.

E. Examination of slide

With the naked eye identify the line made by the drop as it ran down the slide. Position the slide so that the edge of the run is under the objective. Focus the edge of the run under low power, as oil, and examine it using the oil immersion objective. Identify cells as distinguished from dye and debris which will adhere to the slide because of the mordant. Cells will be small rods and have regular outlines. They will be more plentiful near the lower end of the run and may look larger than usual because of the mordant. Once cells are identified follow along the edge of the run, examining cells until some are found with flagella attached. Flagella are much longer than the cell and often look like faint hairs. Ideally, cells should be located which are isolated enough to determine unequivocally the arrangement of the flagella and, for the Pseudomonas, the number of flagella per cell. Draw the cells and their flagella.

BOUQUET

Wisdom Whispers

Kindness is in our power, even when fondness is not.

Some reflections don't have Names and some reflections are just for Names.

While the right to talk may be the begging of freedom, the necessity of listening is what makes that right important.

You were born an original, don't die a copy.

Success without a positive attitude is called Luck. Success with a positive attitude is called Achievement.

He who is able to manage his own self truly understands management.

The road to man's liberation is barred by three gates: lust, hatred and greed.

In every mistake you make, in every fall you encounter, there is a lesson of vital importance if you search for it.

Minds are locked-up stores, only questions open them. If you change the way you look at things, the things you look at change.

Be cautious of every deed which is performed in secret but is embarrassing to perform in public. There is a big difference between a human being and being human. Only a few really understand it.

Without respect there is no love. Without trust there is no reason to continue.

In Lighter Vein

There is a black man, a white man, and a Mexican man on a plane that is too heavy to fly and they are about to crash. They each have to throw something off the plane to save them from crashing. The black man throws out his Jordan shoes and says, "We have too many in our country." The Mexican tosses out his lawn mower and says, "We have too many in our country." The white man puts his item down, grabs the Mexican, throws him out the window and says, "We have too many in our country."

Q: How do Chinese people name their babies?

A: They throw them down the stairs to see what noise they make.

Reaching the end of a job interview, the Human Resources Officer asks a young engineer fresh out of the Massachusetts Institute of Technology, "And what starting salary are you looking for?" The engineer replies, "In the region of $125,000 a year, depending on the benefits package." The interviewer inquires, "Well, what would you say to a package of five weeks vacation, 14 paid holidays, full medical and dental, company matching retirement fund to 50% of salary, and a company car leased every two years, say, a red Corvette?" The engineer sits up straight and says, "Wow! Are you kidding?" The interviewer replies, "Yeah, but you started it."

Brain Teasers

1. **The following: Acetic acid, picric acid, formalin, potassium dichromate, ethyl alcohol are referred to as**
   A. Dehydrants
   B. Infiltrates
   C. Stains
   D. Fixatives
2. **Dehydration is usually accomplished by placing tissue in**
   A. Xylene
   B. Paraffin
   C. Toulene or Benzene
   D. Various concentrations of ethyl alcohol
3. **Slicing a paraffin block containing a piece of tissue is called**
   A. Clearing
   B. Embedding
   C. Microtomy
   D. Flotation
4. **The range of thickness with paraffin technique is**
   A. 1-2 microns
   B. 20-25 microns
   C. 3-10 microns
   D. 15-20 microns

ANSWERS: 1. D, 2. D, 3. C, 4. C

INTERPRETATION

Colony Description

To accurately describe a colony the following traits should be taken into account:

Size: A mm ruler should be held under a plate and used to measure the diameter of several representative colonies, these measurements should then be averaged and recorded.

General Shape: The shape of a colonly will have one of three general shapes when viewed from above; round, irregular and spreading, and concentric.

Margin: The margin should be observed from above also and will represent the outside edge of the general shape. The margin will be one of the following; entire, undulate lobate or filamentous

Elevation: The elevation of a colony should be observed from the side of the plate and will be characterized in the following ways; effuse (like water on a flat surface), flat (discrete colony with a flat top), convex (water on wax paper), umbonate (with a nipple in the center), or umbilicate (sunken center)

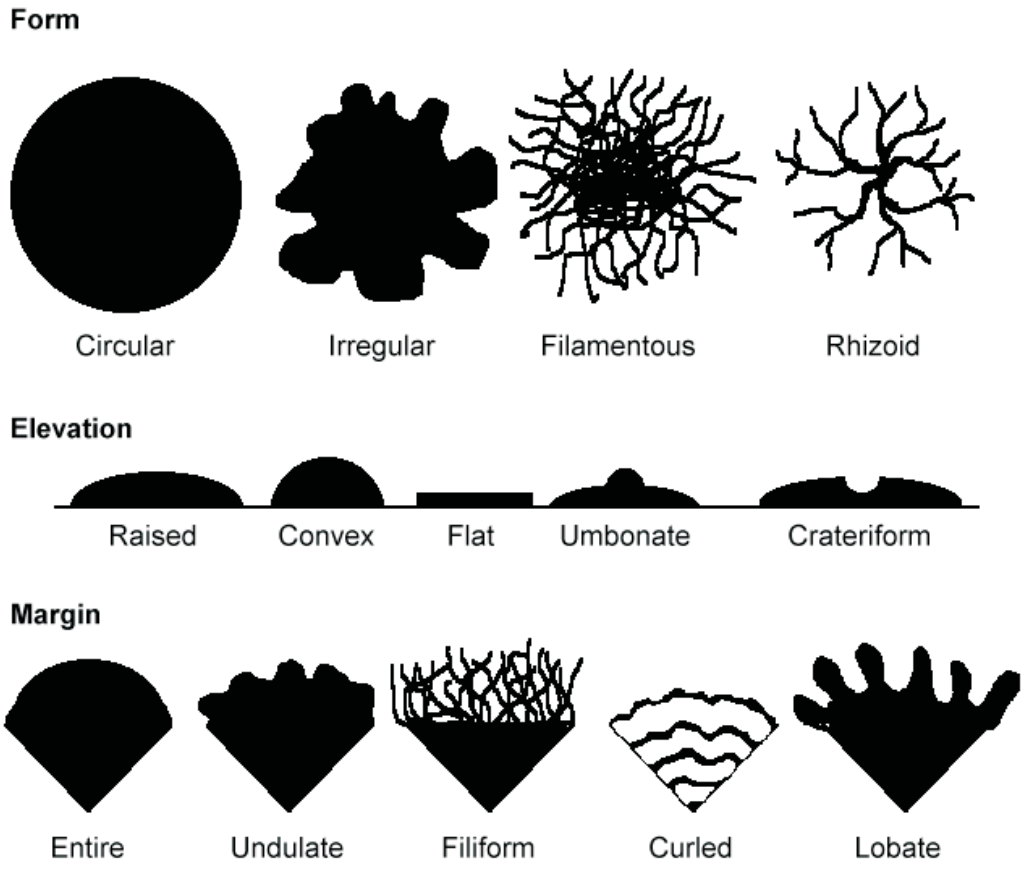
Surface: The surface of the colony should be observed from the top and will have one of the following characteristics; smooth, fliamentous, powdery, wrinkled or ringed.

Density: This quality dexcribes if the colony is opaque or translucent

Pigment: There are three ways a colony may be pigmented; (1) Non pigmented colonies may be described as colorless, white, or off-white, (2) Nondiffusible pigment means that just the cell is pigmented, while (3) Diffusible/water soluble pigments will color the agar around the colony

Interpreting Plates

Please ensure you have JavaScript enabled in your browser. If you leave JavaScript disabled, you will only access a portion of the content we are providing. Bacteria grow tremendously fast when supplied with an abundance of nutrients. Different types of bacteria will produce different-looking colonies, some colonies may be colored, some colonies are circular in shape, and others are irregular. The characteristics of a colony (shape, size, pigmentation, etc.) are termed the colony morphology. Colony morphology is a way scientists can identify bacteria. In fact there is a book called Bergey's Manual of Determinative Bacteriology (commonly termed Bergey's Manual) that describes the majority of bacterial species identified by scientists so far. Although bacterial and fungi colonies have many characteristics and some can be rare, there are a few basic elements that you can identify for all colonies: Form - What is the basic shape of the colony? For example, circular, filamentous, etc. Elevation - What is the cross sectional shape of the colony? Turn the Petri dish on end. Margin - What is the magnified shape of the edge of the colony? Surface - How does the surface of the colony appear? For example, smooth, glistening, rough, dull (opposite of glistening), rugose (wrinkled), etc. Opacity - For example, transparent (clear), opaque, translucent (almost clear, but distorted vision, like looking through frosted glass), iridescent (changing colors in reflected light), etc. Chromogenesis (pigmentation) - For example, white, buff, red, purple, etc. Please note that 3 additional elements of morphology should be examined only in a supervised laboratory setting: consistency, emulsifiability, and odor. Refer to the diagram below for illustrated examples of form, elevation, and margin:

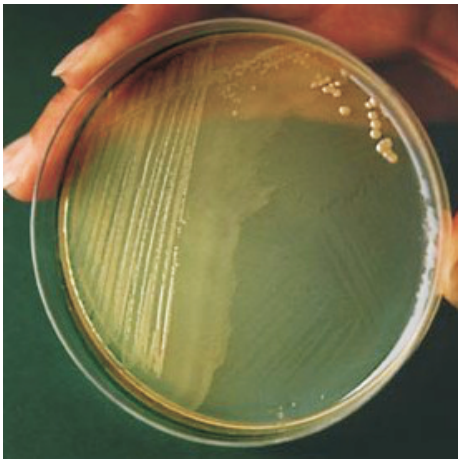


What Can Grow on a Nutrient Agar Plate?

Bacteria

Each distinct circular colony should represent an individual bacterial cell or group that has divided repeatedly. Being kept in one place, the resulting cells have accumulated to form a visible patch. Most bacterial colonies appear white, cream or yellow in color and fairly circular in shape.
For example:

Bacillus subtilis



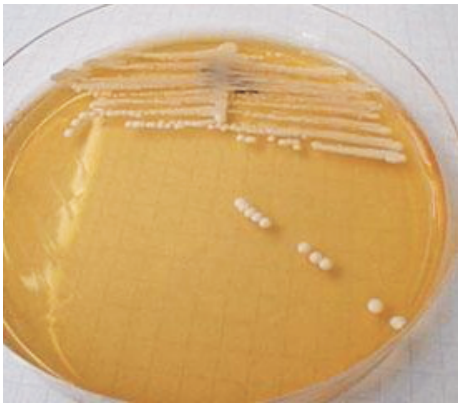
Proteus vulgaris

Staphylococcus aures

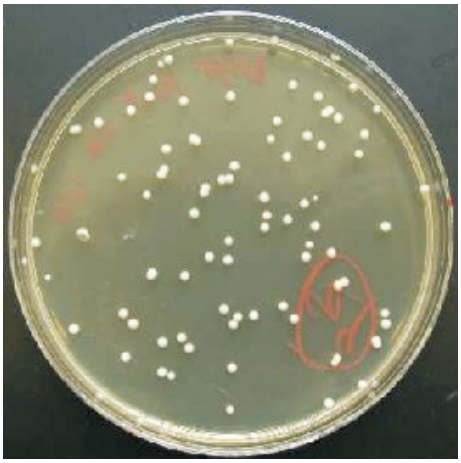
Streptococcus pyogenes

Yeasts

Yeast, a type of fungi (plural for fungus), is found in many places from nature, to research labs and even everyday kitchens for baking. Yeast colonies generally look similar to bacterial colonies. Some species, such as *Candida*, can grow as white patches with a glossy surface.
For example:



Candida Albicans
is a type of yeast that can grow on the surface of skin

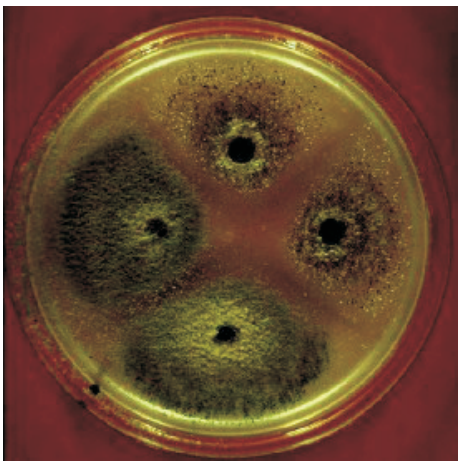


Round yeast colonies

Pink yeast colonies

Other Fungi

Moss green colonies, a white cloud, or a ring of spores can be attributed to the growth of *Aspergillus*, which is common in such fungal infections as athlete's foot. Here is an example of what *Aspergillus* looks like:

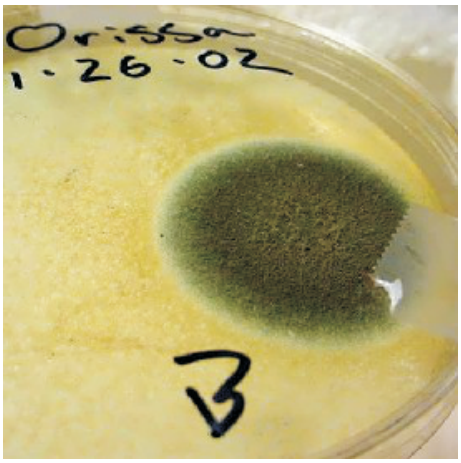


Aspergillus

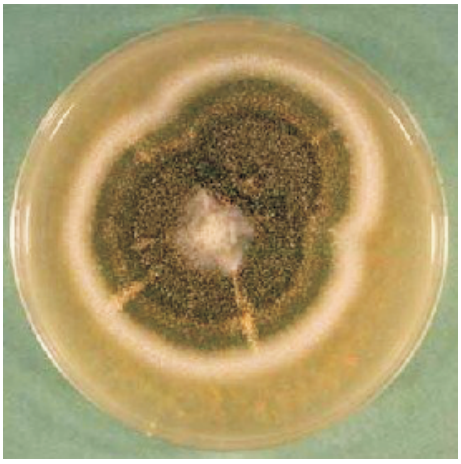
Finally, whenever a thorough, visual identification is not possible, examples of additional tests are gram stains, growths on selective media, and enzymatic tests.

Molds

Molds are actually fungi, and they often appear whitish grey, with fuzzy edges. They usually turn into a different color, from the center outwards. Two examples of molds are shown below:



Green Mold (*Trichoderma harzianum*)



Black Mold (*Aspergillus nidulaus*)

TULIP NEWS

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Turbodyne SC™

Turbidimetry • Nephelometry

Dynamic Metamorphosis to

HbA1c
MA
Cystatin C
RF
CRP
ASO
IgE
D-Dimer...

Simple Test Protocol

Insert Smart Card

Insert R1 + Sample & read

Insert R2 read results

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