Introduction to Disease Block

2020 CSIE ICE Facilitator Guide

ICE Student Online "Virtual" Case

REQUIRED READING:

To access some of the resources below, please make sure to login to the library's website first, in order for the links to load.

Lecture Notes you might find helpful (see Introduction to Disease block schedule):

- "A Pediatrician's Approach to Immunizations" (2-part lecture by Dr. Sicilio), February 27 and 28
- "Immunological Memory; Vaccination" (1-hour lecture by Dr. Alaniz), February 28

Links and Review Articles:

Review of vaccines: CDC Manual of Vaccine Preventable Disease; review Chapter 10

Wessels MR, Brigham KS, DeMaria A. Case records of the Massachusetts General Hospital. Case 6.-2015. A 16-year-old boy with coughing spells. 2015. NEJM 372:765-773.

Wood N, McIntrye P. 2008. Pertussis: review of epidemiology, diagnosis, management and prevention. Rev 9:201-212.Cherry JD. 2012. NEJM 367:785-787.

Additional References:

Texas State Immunization Schedule (Others will be presented throughout the case).

Normal laboratory values for children: Normal Child Laboratory Values

Part A: ----------Wednesday: Case Information ----------

Part A Learning Objectives:

1. Become familiar with the diseases against which vaccines are recommended by the Texas Department of State Health Services.

Review of vaccine schedule: Review the immunization schedule recommended by the Texas Department of State Health Services.

Chief Complaint: "Daniel still has a cough."

ID and source of information: Daniel is an 8-year-old boy with mild intermittent asthma and seasonal allergies, who presents to his pediatrician accompanied by his father, who together with Daniel, provided the history.

History of Present Illness (HPI): Two weeks ago, Daniel had presented to his pediatrician accompanied by his mother and then 21 day old brother for evaluation of a persistent cough. His mother had reported that Daniel had a 1 week history of runny nose, nasal congestion and low grade fever with an initially occasional cough that had increased in frequency by the time of the clinic visit. He had no ill contacts at home, but several classmates at school had similar symptoms. He had trialed cetirizine 5 mg daily without improvement. He was empirically started on nebulized albuterol every 4 hours as needed and a 5 day course of prednisone 25 mg twice daily. Today, his father reports that the albuterol and steroids seemed to have helped his nasal symptoms, which have resolved, but his cough persists. He also reports that Daniel had a severe coughing spell last night with post-tussive emesis and difficulty breathing that prompted him to deliver 3 albuterol nebulizer treatments every 20 minutes according to Daniel's "Asthma Action Plan" until he was convinced that the episode had resolved.

Daniel's mother had called the pediatrician's office earlier that evening to report that his 5 week old brother had developed a few days of nasal congestion, then bouts of coughing and tachypnea with more "spitting up" than usual. She was instructed to take the baby to the emergency department of the local hospital, where the baby was admitted to the inpatient pediatric service.

Daniel's father denies noting audible wheeze, fevers, chills, or night sweats.

Daniel has no complaints apart from the persistent cough, but commented that he felt afraid last night at one point, because it was "hard to breathe."

Birth History: Daniel was born full term via spontaneous vaginal delivery to his then 28-year-old Gravida 1, Para 0, Ab 0 mother after an uncomplicated pregnancy with prenatal care. Birth weight was 3520 gm. Discharged on day of life #2. Normal newborn screen.

Past Medical History: Briefly hospitalized at 3 months old with *respiratory syncytial virus* (*RSV*) bronchiolitis. Diagnosed with mild intermittent asthma at age 2 years. Respiratory viruses trigger respiratory distress and protracted cough responsive to steroids and bronchodilators required on a once or twice a year basis.

Past Surgical History: None.

Immunizations: Immunizations up to date (IUTD). Review the recommended immunization schedule.

Allergies: Seasonal allergies based on his symptoms of rhinitis every spring. He has not been skin tested.

Medications: Antihistamines and beta-agonists on an as needed basis.

Family History: Daniel's mother and maternal aunt suffer from asthma and allergic rhinitis. His father has hypertension, but neither he, nor the paternal family, has respiratory problems.

Social History: Daniel lives with his parents and 5 week old brother. Neither parent smokes. They have an outdoor dog. Daniel is doing well in the third grade at school.

Review of Systems: A complete review of systems is performed. The pertinent positives are discussed in the HPI.

Question 1: List at least 10 of the **infectious diseases and their corresponding pathogens** against which children are routinely vaccinated in Texas: ***(e.g. Gastroenteritis; Rotavirus)***.

Answer 1:

1. Hepatitis; Hepatitis B virus (HBV)
2. Gastroenteritis; Rotavirus
3. Diphtheria; *Corynebacterium diphtheriae*
4. Tetanus; *Clostridium tetani*
5. Pertussis; *Bordetella pertussis*
6. Epiglottitis, pneumonia, and meningitis; *Haemophilus influenzae* type b
7. Pneumonia and meningitis; *Streptococcus pneumoniae*
8. Poliomyelitis; Poliovirus
9. Influenza; Influenza virus
10. Measles; Measles virus
11. Mumps (epidemic parotitis); Mumps virus
12. Rubella (German measles); Rubella virus
13. Hepatitis; Hepatitis A
14. Meningitis and sepsis; *Neisseria meningitidis*
15. Cervical carcinoma; Human papillomavirus (HPV)

Question 2: If Daniel received all of his DTaP vaccinations at the youngest suggested ages according to the Texas schedule, how old was he when he received each of the 5 doses and what is the interval between his last vaccine dose and his current illness?

Answer 2: 2 months; 4 months; 6 months; 15 months; 4 years. Interval is 4 years.

---Part B: --Information below available as soon as the above questions are answered----------

Part B Learning Objectives:

1. Demonstrate understanding of the basic components and descriptors in the physical examination of the lungs.

2. Demonstrate a basic understanding of the differential diagnosis of prolonged cough in a child.

Required videos and reading:

- Review of terminology describing breath sounds: Bates 9.4: Examining the Posterior Thorax
- Review of pediatric lung examination: Examining the thorax and lungs of a child; first 1:10 only.
- Wessels MR, Brigham KS, DeMaria, A. Case 6: A 16-year-old Boy with Coughing Spells. 2015; NEJM 372:765-773

Physical Examination:

Vitals: Weight 25 kg (48th%), height 128 cm (50th%), BMI 15.8 (50th%), temperature 37.0°C (98.6°F), blood pressure 100/60 mmHg, heart rate 112 beats/min, respiratory rate 24/min, oxygen saturation 100% on room air.

General: Well-developed, well-nourished, school-age boy, who coughs occasionally during the visit, but is in no obvious respiratory distress. Hear Daniel's cough at this website: cough example

HEENT: The sclera are anicteric and conjunctivae are pink and moist. Extraocular movements are intact and pupils are equal, round, and reactive to light. Tympanic membranes are dull with serous effusions behind both. Nose with crusting and boggy mucosa over the anterior turbinates. No tenderness to palpation over the sinuses. Normal dentition. The oral mucosa, hard and soft palate and tongue are normal in appearance. The posterior oropharynx had a cobblestone appearance. Uvula is midline and tonsils are 1+ without edema or exudate.

NECK: Supple and symmetric without masses or tenderness.
CHEST: Normal anteroposterior (AP) diameter and contour.
LUNGS: The inspiration to expiration ratio is 1:1. The lungs are well aerated and clear to auscultation. Examining the thorax and lungs of a child; first 1:10 only.
CARDIOVASCULAR: There is a regular rate and rhythm without any murmurs, gallops, rubs. Peripheral pulses are 2+ and symmetric.
ABDOMEN: Scaphoid, soft and nontender with active bowel sounds. The liver edge is mildly tender and extends approximately 2 cm below the right costal margin. The spleen is not palpable. There are no inguinal or umbilical hernias noted.
LYMPH NODES: Shotty anterior cervical lymphadenopathy is palpable bilaterally with a single 1 cm anterior cervical node on the right. No lymphadenopathy is appreciated in the axillae or groin.
SKIN: Inspection of the skin reveals no rashes.
MUSCULOSKELETAL: Gait is normal. There is no joint tenderness or effusions noted. Muscle strength and tone are normal.
EXTREMITIES: No cyanosis, clubbing or edema is noted. Skin turgor is normal.
NEUROLOGIC: Alert and oriented x 3. Normal affect; chatty and engaging. Normal deep tendon reflexes with no pathological reflexes. Sensation to touch is normal.

Question 3: Use the terminology explained in the Bates Visual Guide to Physical Examination Thorax and Lungs: Examining the Posterior Thorax, list:

1. Another term for the "clear" breath sounds heard
2. The term that describes the sounds that are heard in an asthma exacerbation (which were absent in Daniel)
3. List the 4 steps to assessing the respiratory system based on the Bates Visual Guide to Physical Examination Head-To-Toes Assessment: Child Thorax and Lungs video

Answer 3:

1. Vesicular
2. Wheezes
3. 4 Steps: Inspection or observation; Palpation; Percussion; Auscultation

Case Continuation:

Daniel has a history of mild intermittent asthma with exacerbations triggered by respiratory viruses requiring courses of steroids and bronchodilators. His symptoms during his first visit seemed consistent with an asthma exacerbation due to a respiratory virus and standard therapy is prednisone 2 mg/kg/day divided q12 h and albuterol q4 h as needed. Here is an example of an Asthma Action Plan: NIH Asthma Action Plan

Question 4: List 4 likely **non-infectious** and 4 **infectious causes** of prolonged cough as shown in the Wessles case report.

Answer 4: See table below: 4 non-infectious: asthma, GERD, foreign body aspirations, upper airway couch syndrome. 4 infectious: RSV, pertussis, TB, influenza

Table 1. Some Causes of Prolonged Cough in Children.

Noninfectious causes
Asthma
Gastroesophageal reflux disease
Upper-airway cough syndrome
Smoking (active or passive)
Nonasthmatic eosinophilic bronchitis
Cystic fibrosis
Foreign-body aspiration
Extrinsic airway compression
Interstitial lung disease
Infectious causes
Chronic sinusitis with upper-airway cough syndrome
Pyogenic bacterial pneumonia
Prolonged bacterial bronchitis
Tuberculosis
Mycoplasma pneumoniae infection
Chlamydophila pneumoniae infection
Pertussis
Respiratory viral infections (influenza, adenovirus, rhinovirus, respiratory syncytial virus, parainfluenza virus)

---Part C: --Information below available as soon as the above questions are answered----------

Learning Objectives Part C:

1. Demonstrate understanding of the epidemiology, diagnosis, management and prevention of pertussis.
2. Identify factors related to the resurgence in pertussis.

Read the following articles and consider the approach that the pediatrician took with Daniel to diagnose and treat his case of pertussis. The pediatrician may have ordered the IgE to ascertain the contribution of allergic inflammation to an asthma exacerbation, which was the initial diagnosis. Pertussis infections have not been linked epidemiologically with asthma exacerbations, although one study found lower lung function in asthmatics with concurrent pertussis based on PCR testing [1] and *B. pertussis* can evoke a phenotype typical of the inflammation associated with allergic asthma in a mouse model [2]. Had Daniel wheezed during his initial visit, the likelihood of a delayed diagnosis of pertussis would have been even more likely [3].

Required reading:

- Wessels MR, Brigham KS, DeMaria A. Case records of the Massachusetts General Hospital. Case 6.-2015. A 16-year-old boy with coughing spells. 2015. NEJM 372:765-773.
- Wood N, McIntrye P. 2008. Pertussis: review of epidemiology, diagnosis, management and prevention. Rev 9:201-212.Cherry JD. 2012. NEJM 367:785-787.
- Cherry JD. Epidemic Pertussis in 2012 — The Resurgence of a Vaccine-Preventable Disease. *NEJM.* 367:785-787.

Case Continuation:

Serum Laboratory Analysis: Results available later the same day.

Electrolytes

Sodium (Na^+)	142 mEq/L
Potassium (K^+)	4.1 mEq/L
Chloride (Cl^-)	105 mEq/L
Bicarbonate (HCO_3^-)	24 mEq/L
Blood urea nitrogen (BUN)	20 mg/dL
Creatinine (Cr)	0.8 mg/dL
Glucose (Glu)	95 mg/dL

Complete blood count (CBC) with differential (diff)

White blood cell (WBC)	20.3 k/dL
Hemoglobin (Hgb)	12.6 g/dL
Hematocrit (Hct)	37%

Platelet count (Plt)	295 k/dL
Neutrophils	32%
Bands	0%
Lymphocytes	58%
Monocytes	4%
Eosinophils	5%
Basophils	1%

Question 5: What feature of the laboratory studies available to the pediatrician the day Daniel was seen accompanied by his father should have prompted consideration of pertussis? [Hint: review this

Laboratory Diagnosis of Pertussis in the US]

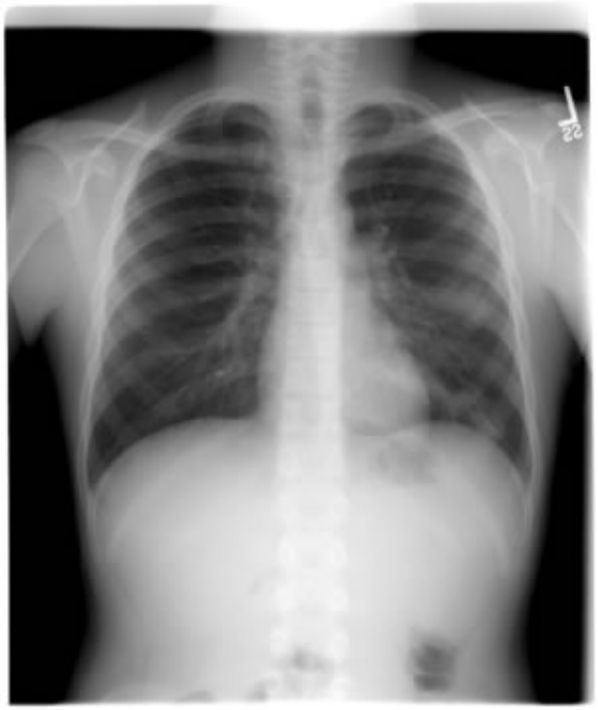
Answer 5: Leukocytosis and especially, lymphocytosis [A WBC count of > 20,000 cells/mm^3 with a lymphocyte count of > 10,000 cells/mm^3 should be diagnosed as pertussis and immediately treated with azithromycin.]

Additional Testing:

Pulmonary Function Testing: Did not meet ATS criteria due to coughing during exam.

ATS Spirometry Criteria; see Table 5 p325 only

Radiographic Studies: Upright PA chest x-ray available the same day. Read as normal.



Question 6: Which age group has the highest incidence of pertussis and list the 3 most common continents with pertussis?

Answer 6: Infants (< 6 months). Continents: Asia, Africa and South America

Question 7: Name the 3 stages of classic pertussis and list symptoms during each stage and typical duration.

Answer 7:

1. Catarrhal – non-specific prodromal coryzal illness, mild cough, lasting 1–2 weeks
2. Paroxysmal – spasmodic cough, post-tussive vomiting and inspiratory whoop lasting 4–6 weeks
3. Convalescent – symptoms slowly improving over 1–2 weeks.

Extend your thinking: In the context of the natural history of a pertussis infection, think about the assumption that Daniel's father made that the albuterol and steroids helped Daniel's rhinorrhea and that the albuterol helped abort the coughing fit that prompted the visit with Daniel's pediatrician. Do you agree with the assumptions that Daniel's father made? To come up with your assessment, think about the following questions: Does albuterol help rhinorrhea? Do steroids? Did the albuterol change the duration of the coughing fit?

Answer to the above questions: Although Daniel has a history of allergic rhinitis and steroids, but not albuterol, may alleviate those symptoms, most likely the rhinorrhea represented the catarrhal phase of the pertussis infection and resolved on its own. Similarly, the albuterol Daniel received did not likely impact the duration of the coughing paroxysm that prompted the clinic visit. Recall that pertussis has not been linked to asthma exacerbations that result in bronchospasm, which may be relieved by albuterol.

Question 8: List the 3 major laboratory tests available to aid in the diagnosis of pertussis and estimates of the sensitivity and specificity of each test at the time of Daniel's second visit (3 weeks of symptoms). [This resource might be helpful: Laboratory Diagnosis of Pertussis in the US]

Answer 8:

1. Culture – sensitivity 1-60% (preferred answer 1-3%), specificity 100%
2. Serology – PT IgG sensitivity 36-76%, specificity 95-99%
3. PCR – sensitivity 70-100%, specificity 86-100% (preferred answer <100%, because the test may have false positives)

Note: If the PCR for IS1001 had been positive, what organism might be causing Daniel's symptoms? *B. holmesii* (also a cause of clinical pertussis) or a mixed infection with *B. pertussis* and *B. parapertussis*.

Treatment: Daniel is started on macrolides, namely, azithromycin 10 mg/kg in a single dose day 1, then 5 mg/kg per day (maximum 500 mg) on days 2 through 5, and sent home. He will follow up in 2 weeks.

Question 9: Name 6 possible explanations for the current increase in cases for patients < 5 months and > 10 years in developed countries.

Answer 9:

1. duration of protection and waning immunity following infection and vaccination
2. incomplete protection from vaccination
3. infection source for infants
4. infection source for adolescents
5. strain polymorphism
6. increased diagnosis and reporting

What you have learned from this case…

You learned about Daniel, an 8-year-old boy with mild intermittent asthma, who presents with a 3-week history of cough unresponsive to steroids and beta-agonists and recently complicated by post-tussive emesis. His infant brother was admitted with similar symptoms. You learned about immunization schedules and routine vaccinations

in the state of Texas. You also reviewed your physical exam skills for the thorax and lung (for a child). After performing a history and physical, you are able to generate a differential diagnosis list of cough in a child to include both infectious and non-infectious causes. Additional studies were obtained such as a largely unremarkable chest x-ray. Spirometry testing as not performed due to his cough. A CBC showed marked leukocytosis suggesting pertussis, which was confirmed by PCR and serology, but not culture. Lastly, after reviewing the epidemiology and features of pertussis, you identify several factors related to the resurgence in pertussis.

STUDENTS IN THE WHITE SUBGROUPS: Please prepare a 4-5 sentence summary (~ 30 seconds to 1 minute) of this entire clinical case. This is often asked of students to do in the clinical setting after performing a full H&P. You will each present your summary at the live session.

Student Expectations

- Students are expected to arrive on time, in professional dress, white coat and badge.
- Students are expected to actively participate, show professional behavior such as appropriate listening skills and refraining from disrupting the session (please see the **student evaluation** rubric for more details).
- Students are expected to have prepared for the live session by reviewing the online "virtual" case and answering all the questions within each part, as well as reviewing the suggested lectures, and completing the required reading (see page 1-2, above) to prepare them for the live session.

ICE Assessment

- The CSIE ICE comprises 3.0% of the overall block grade in the following way:
  - 1.5% for completing all the questions from Parts A, B, C of the online "virtual" case
  - 1.5% from the student evaluation form (provided by you as the facilitator)

Live Session Facilitator Guide

General Timeline:

8:00-8:30 am - Facilitator Meeting with Content Expert to answer questions

8:30-10:20 am - Meet with your group

10:20-10:30 am - Release students and complete student evaluations

10:30-11:00 am - Faculty debriefing

Recommended timeline for the group activities

- **Get started** (5 minutes)
- **Activity 1** (20 minutes): Online Virtual Case Summary Presentation and Debriefing
- **Activity 2** (50-60 minutes): Practice Clinical Cases
- **Activity 3** (20-30 minutes): High Yield Microbiology and Immunology Content

Get started (Start at 8:30 am: 5-10 minutes)

- Start the live session by introducing yourself.
- Meet the students and let them introduce themselves.
- Take this time to check attendance utilizing the provided student roster with their pictures/names. This will help in completeing the student evaluation form for grading.
- If you are familiar with the students, take a few minutes to check in on them and how they are doing.
- Let the pre-designated student/students connect the computer to the AV system to log on and bring up the google doc forms to be used in the session

Activity 1 – Online "Virtual" Case Summary and Debriefing (Start no later than 8:40 am: 20 minutes)

PART 1 – Online Virtual Case Summary (10 minutes)

Objective: To concisely present a clinical case, which is often done during rounds in the clinical setting.

Instructions:

- Ask each student in the "white" group to provide a 4-5 sentence summary (~ 30 seconds to 1 minute per student). This is often asked of students in the clinical setting after performing a full H&P. The summary should at least include the following:
    - Presenting symptoms
    - Important (pertinent) history and physical exam findings
    - Pertinent laborotory and other diagnostic test findings
    - Diagnosis
    - Treatment
    - Outcome

  Example: Daniel is an 8 year-old boy with mild intermittent asthma, who presents with a 3-week history of cough unresponsive to steroids and beta-agonists and recently complicated by post-tussive emesis. His infant brother was admitted with similar symptoms. Daniel coughed during the exam, which was largely unremarkable, as was a chest x-ray. He could not perform spirometry due to his cough. A CBC showed marked leukocytosis suggesting pertussis, which was confirmed by PCR and serology, but not culture.

PART 2 – Online Virtual Case Debriefing (10 minutes or less)

Objective: Review, debrief, and reinforce the contents of the online virtual case.
Instructions:

- This is a brief review of the student online "virtual" case. Consider just opening it up to specific student questions rather than going through the entire case or every question. If there are no questions, move on to activity 2. If there are questions, please ask the "AV student" to bring up the case on eCampus (PDF) so you can review their question. The answers are in your Facilitator Guide PDF document (see also paper copy in your maroon folder).

Activity 2 – Clinical Cases (Start no later than 9:00 am: 50-60 minutes)

Objective: This exercise consists of a series of 8 cases that highlight the clinical courses of patients battling infections with toxin-secreting bacterial pathogens. Again, the students have been introduced to the basic science of bacterial toxins in lectures prior to this CSIE and have a general understanding of the cellular pathophysiology induced by toxin classes. The primary goal of the CSIE is to link that cellular pathophysiology to the manifestations exhibited by infected patients. Additionally, the students will consider the vaccinogenic potential of the toxins, that is, they will determine if vaccination with formulations containing these toxins provide protective immunity against the corresponding pathogens. Alternatively, they may determine if the toxins have the potential for passive immunotherapy.

Instructions:

1) Divide students into 4 groups of similar size.
2) Each group will work on **2 cases** (there will be no bonus case).
3) Project **Activity 2 Handouts A-D** in Google docs.
    a. Group 1: activity handout A, group 2: activity handout B, group 3: activity handout C, etc.
4) Allow the groups 10-15 minutes to work through the cases and answer the provided questions. Encourage them to use online resources.
5) Rotate between groups and guide them if they are struggling (using the answers provided to you).
6) Regroup and go through answers, allowing all four groups to present their work (allow each group to present their two cases, e.g.: group A presents cases 1-2, group B presents cases 3-4, group C presents cases 5-6; group D presents cases 7-8).
7) Allow 45-50 minutes for presentation and discussion of the cases.

Group A Cases (Case 1 and 2)

Case 1: *Vibrio cholera* "cholera"

Mr. Lucarelli is a 48-year-old man, who presented to his local emergency room with watery diarrhea, dehydration, loss of 10 kg of body weight, hypotension and severe oligoanuric renal failure one day after returning from a two-week vacation in Havana, Cuba. He reported no direct contact with sick individuals there. He did not seek medical advice before travelling. While in Cuba, he drank tap water and ate fruits and vegetables washed with tap water. On the last day of his stay, he ate raw seafood including sea urchin and crabs, which he caught himself, along the coast of Havana. The following day, during the flight home, he developed watery diarrhea, severe weakness, tachycardia, muscle cramps, dizziness, abdominal pain, nausea and vomiting. His travel companion had consumed the same meals during their stay in Cuba, except for the raw seafood. She did not develop any symptoms.

Questions:

1. Identify the bacteria and associated toxin(s) that is (are) responsive for Mr. Lucarelli's symptoms. What studies would you like to perform to make the diagnosis? (Feel free to ask your facilitator for laboratory results.)

2. Describe the mechanism of action of this (these) toxins including the structure/function relationship, receptor(s) and target(s). How does this mechanism of action relate to the disease pathophysiology?

3. What is the vaccinogenic potential of this (these) toxin(s)?

Answers: see below including table.

Studies: Blood tests showed leukocytosis (white blood cell count of 16,810/µL; norm: 4,000-11,000/µL), high serum creatinine level (5.69 mg/dL; norm: 0.50–1.30 mg/dL), metabolic acidosis (pH: 7.16; norm: 7.35–7.45), low bicarbonate (11.3 mmol/L (norm: 22–26 mmol/L), hypokalemia (2.7 mEq/L; norm: 3.50–5.00 mEq/L).

Vibrio cholerae was isolated from stool samples taken on the first day of hospitalization. The isolate was shipped to the CDC for serogrouping and was confirmed as V. cholerae O1.

PCR was performed in order to discriminate between the classical, El Tor, and Haitian type of ctxB allele (encoding cholera toxin B subunit). The strain was positive for the Haitian type of ctxB allele.

Pathogen	Toxin[ref] (abbrev)	Mechanism	Receptor	Structure	Target/Pathology	Vaccinogenic potential
Vibrio cholera	Cholera toxin[1] (Ctx)	ADP-ribosylase	GM1	AB_5	Gs a-subunit, activates adenylate cyclase; activates CFTR (Cl^-), intestinal fluid secretion	Oral, inactivated whole cell + recombinant B subunit, protective antibody

Source: Mascarello M, Deiana ML, Maurel C, Lucarelli C, Luzzi I, Luzzati R. Cholera with severe renal failure in an Italian tourist returning from Cuba, July 2013. Euro Surveill. 2013; 18(35):20572.

Case 2: *Corynebacterium diphtheria* "diphtheria"

Grace is a 9-year-old girl, who was referred to the local emergency room with a 5-day history of low-grade fever, cough and sore throat. On the first day of illness, she had been taken to her pediatrician, who diagnosed her with pharyngitis and prescribed an unknown oral antibiotic and acetaminophen. By the third day of illness, her fever had gradually declined and the sore throat had resolved. However, she subsequently experienced neck swelling, dyspnea and dysphagia. Her parents also noted a "harsh" breathing sound that prompted them to contact her pediatrician, who referred her to the ER. In the ER, she was found to be afebrile with normal blood pressures, but tachycardic and tachypneic with a room air oxygen saturation of 75%. Her tonsils were inflamed and had white patches and her neck examination revealed bilateral neck tissue swelling that was soft, tender and without fluctuation or rash. She had inspiratory stridor and poor air entry, but no adventitious sounds. She had received her HepB vaccine at birth, but none others since then.

Questions:

1. Identify the bacteria and associated toxin(s) that is (are) responsive for Grace's symptoms. What studies would you like to perform to make the diagnosis? (Feel free to ask your facilitator for laboratory results.)

2. Describe the mechanism of action of this (these) toxins including the structure/function relationship, receptor(s) and target(s). How does this mechanism of action relate to the disease pathophysiology?

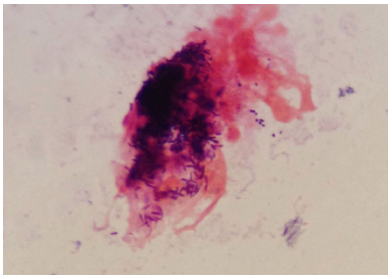
3. What is the vaccinogenic potential of this (these) toxin(s)?

Answers: see below including table.

Studies:

CBC showed a leukocytosis of 30.3k cells/μL and chemistries showed an elevated creatinine of 1.7 mg/dL.

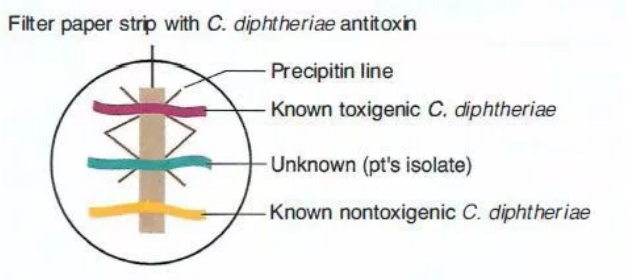
A throat swab Gram stain showed Gram-positive bacilli and culture showed *Corynebacterium diphtheriae*. The swab of a white plaque caused bleeding. The Elek test for detection of toxigenic *Corynebacterium* strains was positive.



Elek test is an **in vitro immunoprecipitation (immunodiffusion) test** to determine whether or not a strain of *C. diphtheriae* is toxigenic. A test strip of filter paper containing diphtheria antitoxin is placed in the center of the agar plate.

Strains to be tested (patient's isolate), known positive and negative toxigenic strains are also streaked on the agar's surface in a line across the plate and at a right angle to the antitoxin paper strip.

Antitoxin diffuses away from the strip of filter paper whereas toxin produced by toxin-producing strains diffuse away from growth. At the zone of equivalence a precipitin line is formed.



PCR for the *tox* gene was also positive for subunits A and B, although one need only perform either the Elek test or PCR to make the diagnosis.

Pathogen	Toxin[ref] (abbrev)	Mechanism	Receptor	Structure	Target/Pathology	Vaccinogenic potential
Coryne-bacterium diphtheria	Diphtheria toxin[2] (DTx)	ADP-ribosylase	HB-EGF	AB	EF-2, inhibits proteins synthesis	DPT, Quinvaxem, pentivalent protective antibody

Source: Washington CH, Issaranggoon na ayuthaya S, Makonkawkeyoon K, Oberdorfer P. A 9-year-old boy with severe diphtherial infection and cardiac complications. BMJ Case Rep. 2014; pii: bcr2014206085.

Group B Cases (Case 3 and 4)

Case 3: *Escherichia coli* O157:H7 "colitis"

Courtney is a 7-year-old girl vacationing in Seattle with her parents, who presented to an emergency room near her hotel with a four-day history of diarrhea, crampy abdominal pain, nausea and vomiting. Initially, she had watery diarrhea, but two days later, the diarrhea became grossly bloody. Her mother also had nonbloody diarrhea. Her father had no symptoms. Courtney and her mother recalled eating lunch a week ago at a burrito restaurant, while her father attended a business meeting. On examination in the ER, her temperature was noted to be 38.0˚C. Her abdomen was soft to palpation, but diffusely tender with hyperactive bowel sounds. After sending samples to the laboratory, she was started on empiric antibiotics and loperamide, then admitted to the inpatient service. She received intravenous fluids overnight, but by morning she had decreased urinary frequency. She also reported feeling very tired, and appeared pale. The antibiotics and loperamide were discontinued.

Questions:

1. Identify the bacteria and associated toxin(s) that is (are) responsive for Courtney's symptoms. What studies would you like to perform to make the diagnosis? (Feel free to ask your facilitator for laboratory results.)

2. Describe the mechanism of action of this (these) toxins including the structure/function relationship, receptor(s) and target(s). How does this mechanism of action relate to the disease pathophysiology?

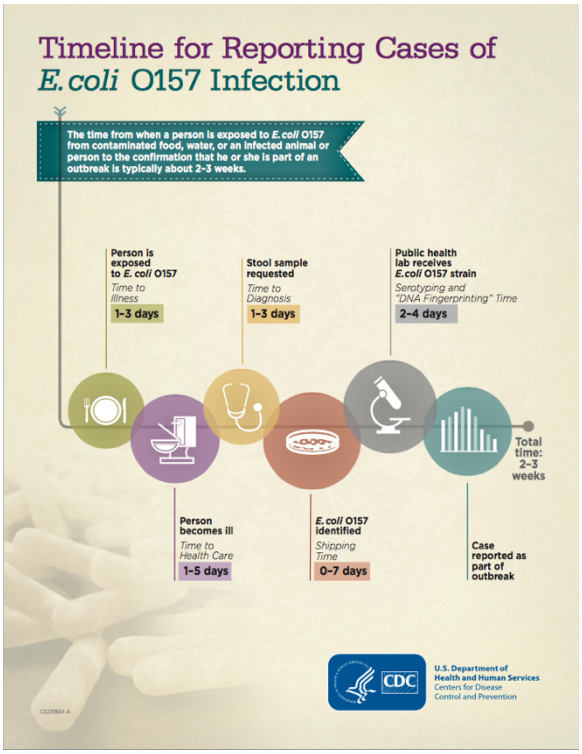
3. What is the vaccinogenic potential of this (these) toxin(s)?

Answers: see below including table.

Studies: Microscopic investigation of the stool sent from the ER revealed leukocytes and erythrocytes. The initial diagnosis was infectious colitis. After taking a stool culture, empiric antibiotic treatment (metronidazole and trimethoprim/sulfamethoxazole) was started.

Stool culture plates on sorbitol-MacConkey agar revealed sorbitol-negative colonies with biochemical properties of *E. coli.* Strains presumptively identified as *E. coli* O157:H7 were sent to a reference laboratory, then reported to the CDC.

The antibiotics and loperamide were stopped after the strains were confirmed to be *E. coli* O157:H7 using serotyping by agglutination test using *E. coli* O and H antisera set and verotoxin production was determined by the Vero cell assay. Verotoxin typing was done using a VTEC-RPLA verotoxin detection kit. Verotoxin 2 (vt 2) was positive, but verotoxin 1 (vt 1) was negative.



Pathogen	Toxin[ref] (abbrev)	Mechanism	Receptor	Structure	Target/Pathology	Vaccinogenic potential
Escherichia coli O157:H7	Shiga-like toxin[3] (Stx)	N-glycosyl-transferase	Gb3/4	AB_5	28S RNA, blocks protein synthesis; selective for glomerulus and vascular endothelium	Humanized Mab therapy Experimental CRM vaccine

Sources: Erdoğan H1, Erdoğan A, Levent B, Kayali R, Arslan H. Enterohemorrhagic Escherichia coli O157:H7: case report. Turk J Pediatr. 2008; 50(5):488-491 and http://www.cdc.gov/ecoli/reporting-timeline.html

Case 4: *Clostridium tetani* "tetanus"

Mrs. Wheeler is a 77-year-old woman with advanced Alzheimer's disease and type 2 diabetes, who was admitted to a geriatrics unit with fever. She lived at home with a caregiver and had been bedridden for 2 months. She had a stage 4 sacral pressure ulcer that had recently been debrided and treated with oral antibiotics. On admission, chest radiograph showed left lung opacity consistent with pneumonia and she was started on intravenous antibiotics. A week after admission she developed intermittent generalized rigidity, opisthotonus, flexion of the upper limbs, extension of the lower limbs, trismus and risus sardonicus. She would suddenly adopt these abnormal postures, often after sensory stimulation such as noise, light, or touch. She was not taking neuroleptics or other drugs that can cause Parkinsonism. She received all of her childhood immunizations, but her son confirmed that she had not received booster vaccinations for 30 years.

Questions:

1. Identify the bacteria and associated toxin(s) that is (are) responsive for Mrs. Wheeler's symptoms. What studies would you like to perform to make the diagnosis? (Feel free to ask your facilitator for laboratory results.)

2. Describe the mechanism of action of this (these) toxins including the structure/function relationship, receptor(s) and target(s). How does this mechanism of action relate to the disease pathophysiology?

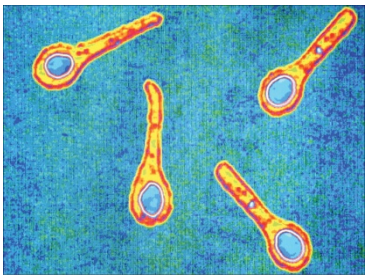
3. What is the vaccinogenic potential of this (these) toxin(s)?

Answers: see below including table.

Studies:

A head CT showed no acute lesions, and cerebrospinal fluid and serum ionized calcium were normal.

Tetanus can be found on PCR and bacterial culture, but the diagnosis is essentially clinical. The CDC states that "tetanus is a clinical syndrome without confirmatory laboratory tests."



False-color transmission electron micrograph of *Clostridium tetani*, a Gram positive, spore-forming, anaerobic bacillus.

Pathogen	Toxin[ref] (abbrev)	Mechanism	Receptor	Structure	Target/Pathology	Vaccinogenic potential
Clostridium tetani	Tetanus toxin[4] (TeNT)	Protease	VAMP	AB	inhibits GABA neurotransmitter	DPT, Quinvaxem, pentivalent protective antibody

Source: Verde F, Riboldi G, Zappa C, Ferretti M, Silani V, Mari D, Priori A. An old woman with pressure ulcer, rigidity, and opisthotonus: never forget tetanus! Lancet. 2014; 384(9961):2266.

Group C Cases (Case 5 and 6)

Case 5: *Clostridium botulinum* "botulism"

Adam is a 6-month-old infant boy, who presented with a 3-day history of increasing hypotonia and a 1-day history of dehydration. Three days prior to admission, his mother noted decreased suck while breast-feeding. The following day, he took 1 hour to take his usual amount of breast milk that typically took him 15 minutes. That evening, his parents noted generalized weakness with decreased movement, difficulty sitting up and inability to breast feed. The next morning, he was increasingly floppy with poor head control. His parents noted a gurgling sound in the back of his throat and took him to his pediatrician, who noted severe hypotonia and dehydration and sent him to a local emergency room. On review of symptoms, the ER physician noted that Daniel demonstrated constipation 4 weeks previously with only two small stools in the last 6 days. His diet consisted of breast milk, occasional sweet potatoes and rice cereal. Physical exam showed generalized hypotonia with head lag.

Questions:

1. Identify the bacteria and associated toxin(s) that is (are) responsive for Adam's symptoms. What studies would you like to perform to make the diagnosis? (Feel free to ask your facilitator for laboratory results.)

2. Describe the mechanism of action of this (these) toxins including the structure/function relationship, receptor(s) and target(s). How does this mechanism of action relate to the disease pathophysiology?

3. What is the vaccinogenic potential of this (these) toxin(s)?

Answers: see below including table.

Studies:

Spinal tap showed normal CSF.

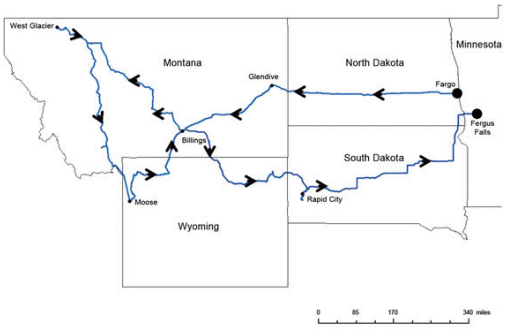
Stool specimen on admission was positive in a mouse lethality test. PCR tests for *C. bolulinum* toxin genes exist, but have not been shown to be more sensitive than the mouse lethality test.

Pathogen	Toxin[ref] (abbrev)	Mechanism	Receptor	Structure	Target/Pathology	Vaccinogenic potential
Clostridium botulinum	Botulinum toxin[5] (BT)	Zinc metallo-protease	Pre-synaptic receptors	AB	Cleavage of SNARE family at axon terminal; prevents release of acetylcholine, blocks impulse, flaccid paralysis	Antitoxin trivalent and heptavalent, equine, Fab + Fc and F(ab')2

Source: Gilligan PH, Shapiro DS, Miller MB. (2014) Cases in Medical Microbiology and Infectious Diseases 4th Edition. Washington, DC. ASM Press. Case 52, pp 363-368.

Case 6: *Bacillus anthracis* "anthrax"

Mr. Jones is a 61-year-old Florida resident with mild Type 2 diabetes, who was admitted to a community hospital in Minnesota with fever, malaise, headache, cough, shortness of breath, and chest pain after a 3-weeks summer road trip with his wife. They had driven through North Dakota, Montana, Wyoming, and South Dakota stopping to walk through national parks, collect loose rocks, and purchase elk antlers. They had driven through herds of bison and burros, frequently stopping while animals surrounded their vehicle. The couple had no contact with dead animals, but reported dusty conditions while driving through the herds. Mr. Jones had not traveled abroad during the past year or been exposed to tanneries, wool or goat hair mills, bone meal, African drums, or illicit drugs. He crafted metal and stone jewelry and knives with elk antler handles in a home workshop. His wife experienced no symptoms.



Route traveled by Mr. and Mrs. Jones.

Questions:

1. Identify the bacteria and associated toxin(s) that is (are) responsive for Mr. Jones' symptoms. What studies would you like to perform to make the diagnosis? (Feel free to ask your facilitator for laboratory results.)

2. Describe the mechanism of action of this (these) toxins including the structure/function relationship, receptor(s) and target(s). How does this mechanism of action relate to the disease pathophysiology?

3. What is the vaccinogenic potential of this (these) toxin(s)?

Answers: see below including table.

Studies:

A blood culture drawn at the community hospital grew a *Bacillus* sp that was submitted to the Minnesota Department of Health to submit for speciation.

Laboratories that are a part of the Laboratory Response Network (LRN) can test patient specimens for *Bacillus anthracis*. LRN labs are strategically located across the United States and abroad, each playing a role in their state's or territory's overall emergency response plan.

The identification of *B. anthracis* was confirmed by PCR and gamma phage. Gamma phage specifically lyses vegetative cells of *Bacillus anthracis* and serves as part of the basis for identification of isolates from agar cultures. CDC typed the isolate as GT59 using multiple-locus variable-number tandem repeat analysis specific for 8 loci. CDC also sequenced the strain and performed whole-genome single nucleotide polymorphism analysis.

The isolate was related to strains with genotypes generally associated with imported animal products and most closely related to a strain obtained from a 1965 investigation of a case of cutaneous anthrax in a worker at a New Jersey gelatin factory, which used bone imported from India. No exposure source was identified after extensive testing of the couple's vehicle and their collected rocks and antlers. Animal anthrax is sporadic or enzootic in the states in which the couple traveled. Bioterrorism was not suspected.

Pathogen	Toxin[ref] (abbrev)	Mechanism	Receptor	Structure	Target/Pathology	Vaccinogenic potential
Bacillus anthracis	Anthrax toxin[6] (LF/EF)	protease/ adenylate cyclase	TEM8, CMG2	A_2B	MPKK1, MAPKK2, proteasome activation; cytokine release, shock; fluid release, edema	Biotharx, PA + alum protective antibody

Source: Griffith J, Blaney D, Shadomy S, Lehman M, Pesik N, Tostenson S, Delaney L, Tiller R, DeVries A, Gomez T, Sullivan M, Blackmore C, Stanek D, Lynfield R; Anthrax Investigation Team. Investigation of inhalation anthrax case, United States. Emerg Infect Dis. 2014; 20(2):280-283.

Group D Cases (Case 7 and 8)

Case 7: *Clostridium difficile "C. diff"*

Mr. Nguyen is a 74-year-old man with diabetes, coronary artery disease and thyroid cancer, who was admitted a month ago with chest pain, shortness of breath and cough. Chest radiograph was consistent with a right middle and left lower lobe pneumonia. He received a 14-day course of IV levofloxacin and was discharged to a skilled nursing facility. Three weeks later, he had the onset of loose, watery stools, which increased in frequency to > 10 times a day. He complained of diffuse abdominal pain, nausea and vomiting. Three weeks into his diarrheal disease course, he was readmitted to the hospital with continued diarrhea and abdominal pain accompanied by fever, chills, malaise, myalgias and dizziness. He had had a 5-lb weight loss since his prior admission, yet his abdomen was severely distended.

Questions:

1. Identify the bacteria and associated toxin(s) that is (are) responsive for Mr. Nguyen's symptoms. What studies would you like to perform to make the diagnosis? (Feel free to ask your facilitator for laboratory results.) What is the typical treatment?

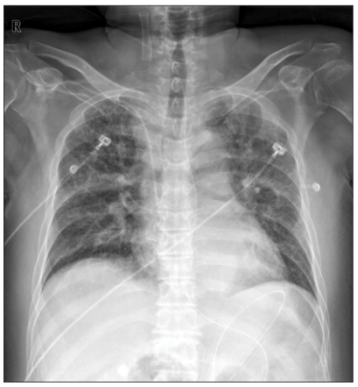
2. Describe the mechanism of action of this (these) toxins including the structure/function relationship, receptor(s) and target(s). How does this mechanism of action relate to the disease pathophysiology?

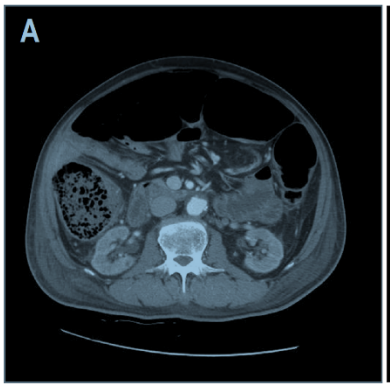
3. What is the vaccinogenic potential of this (these) toxin(s)?

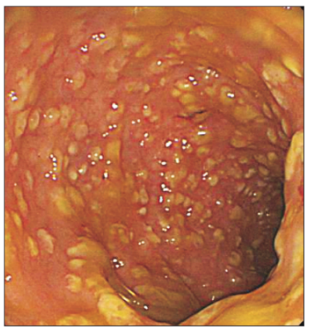
Answers: see below including table.

Studies:

Chest x-ray on first admission







Abdominal CT on second admission (A. transverse, B. coronal): Abdominal CT showed marked dilation of the entire colon by up to 7 cm in diameter in the transverse colon.

Abdominal x-ray on second admission showed marked dilation of the colon.

Sigmoidoscopy showed multiple yellowish plaques in the sigmoid colon. Stool was heme negative. Stool culture typically not performed in this situation, but was sent and showed *Clostridium difficile*.

A test for *C. difficile* toxins A and B was performed (two possibilities: 1. PCR for the toxin genes or 2. Immunochromatography) and was positive. An immunochromographic test for the *C. difficile* cell wall antigen GDH was positive.

Pathogen	Toxin[ref] (abbrev)	Mechanism	Receptor	Structure	Target/Pathology	Vaccinogenic potential
Clostridium difficile	*C. diff* toxin[7] (TcdA/B)	Glucosyl-transferase	Carbo-hydrates Gala1-3GlcNac	ABCD	Rho family GTPases, disrupts cell cytoskeleton; tight junction disruption, pseudomembranous colitis	Phase I/II clinical trials, hMab phase III

Sources:

1. *Gilligan PH, Shapiro DS, Miller MB. (2014) Cases in Medical Microbiology and Infectious Diseases 4th Edition. Washington, DC. ASM Press. Case 26, pp 190-196.*
2. *Gweon TG, Lee KJ, Kang DH, Park SS, Kim KH, Seong HJ, Ban TH, Moon SJ, Kim JS, Kim SW. A case of toxic megacolon caused by clostridium difficile infection and treated with fecal microbiota transplantation. Gut Liver. 2015; 9(2):247-250.*
3. *Voth DE, Ballard JD.* Clostridium difficile *Toxins: Mechanism of Action and Role in Disease. Clin Microbiol Rev. 2005; 18(2): 247–263.*

Case 8: *Staphylococcus aureus* "toxic shock syndrome"

Elizabeth is a previously healthy 15-year-old young woman, who presented initially to her family physician complaining of perineal irritation and abdominal pain. She subsequently presented to a community hospital 4 days later with a history of worsening abdominal pain, diarrhea, headache, and nausea and vomiting, as well as a decreased level of consciousness. She had completed her last day of menses a week prior to presenting to the hospital and reported using tampons. On initial examination, she was lethargic but rousable, and had signs of dehydration. Her heart rate was 122 bpm, blood pressure was 80/60 mmHg, and temperature was 36.6°C. Her perineum was erythematous with mild swelling. Her laboratory investigations indicated a leukocytosis, an elevated creatinine, elevated liver enzymes, a decreased platelet count, and arterial blood gases consistent with a compensated metabolic acidosis. Her β-HCG and monospot tests were negative. She was diagnosed with severe dehydration secondary to gastroenteritis, admitted for fluid resuscitation, and started on intravenous antibiotics. Over the next few hours, her vital signs improved, but she exhibited a significant decrease in her platelet count. Seventeen hours after admission, a fine, faint, erythematous rash was seen over her trunk and extremities. The erythroderma faded, leaving scarlatiniform lesions on her arms and legs that desquamated 7 days after hospital admission.

Questions:

1. Identify the bacteria and associated toxin(s) that is (are) responsive for Elizabeth's symptoms. What studies would you like to perform to make the diagnosis? (Feel free to ask your facilitator for laboratory results.)

2. Describe the mechanism of action of this (these) toxins including the structure/function relationship, receptor(s) and target(s). How does this mechanism of action relate to the disease pathophysiology?

3. What is the vaccinogenic potential of this (these) toxin(s)?

Answers: see below including table.

Studies:

Blood, urine, and pharyngeal cultures were all negative. Cervical swabs and urine PCR for Chlamydia and gonorrhea were negative. However, vaginal swabs were positive at 24 hours for methicillin-sensitive *Staphylococcus aureus*. Molecular analysis of the vaginal swab specimen for TSST-1 was performed and was positive.

TSS clinical criteria. An illness with the following clinical manifestations:

- Fever: temperature greater than or equal to 102.0°F (greater than or equal to 38.9°C)
- Rash: diffuse macular erythroderma
- Desquamation: 1-2 weeks after onset of rash
- Hypotension: systolic blood pressure less than or equal to 90 mm Hg for adults or less than fifth percentile by age for children aged less than 16 years
- Multisystem involvement (three or more of the following organ systems):
  - Gastrointestinal: vomiting or diarrhea at onset of illness
  - Muscular: severe myalgia or creatine phosphokinase level at least twice the upper limit of normal
  - Mucous membrane: vaginal, oropharyngeal, or conjunctival hyperemia
  - Renal: blood urea nitrogen or creatinine at least twice the upper limit of normal for laboratory or urinary sediment with pyuria (greater than or equal to 5 leukocytes per high-power field) in the absence of urinary tract infection
  - Hepatic: total bilirubin, alanine aminotransferase enzyme, or asparate aminotransferase enzyme levels at least twice the upper limit of normal for laboratory
  - Hematologic: platelets less than 100,000/mm^3
  - Central nervous system: disorientation or alterations in consciousness without focal neurologic signs when fever and hypotension are absent

Pathogen	Toxin[ref] (abbrev)	Mechanism	Receptor	Structure	Target/Pathology	Vaccinogenic potential
Staphylo-coccus aureus	Toxic shock syndrome toxin[8] (TSST1)	superantigen	TcR/MHCII	AB domains	Ligate TcR-MHCII, activate T cell release of inflammatory cytokines (IL1, IL2, TNF), shock	rTSST1, CRM clinical trials protective antibody

Sources:

- *LeRiche T1, Black AY, Fleming NA. Toxic shock syndrome of a probable gynecologic source in an adolescent: a case report and review of the literature. J Pediatr Adolesc Gynecol. 2012; 25(6):e133-137.*
- *Rasigade JP, Thomas D, Perpoint T, Peyramond D, Chidiac C, Etienne J, Vandenesch F, Lina G, Ferry T. T-cell response to superantigen restimulation during menstrual toxic shock syndrome. FEMS Immunol Med Microbiol. 2011; 62(3):368-371.*
- *https://wwwn.cdc.gov/nndss/conditions/toxic-shock-syndrome-other-than-streptococcal/case-definition/2011/*

Activity 3 – High Yield Microbiology and Immunology Content (Start by 9:50 to 10:00 am: 20-30 minutes)

Vaccines and the Immune System

Objective: As a complement to the CSIE, which focuses in part on immunization schedules, students will review high yield immunology content in the context of vaccination.

Pedagogical Strategy: Students will work in small groups to design a hypothetical vaccine, thereby synthesizing and applying their understanding of immunology, clinical skills, and the benefits and limitations of immunization.

Instructions for Facilitators:

- Divide students into 4 small subgroups (A-D) of 4 students each *(or alternatively into 2 groups of 8 students each)*.
- Students should read the Lassa virus example below and then design a hypothetical vaccine based on one of the following vaccine strategies:
    - Live-attenuated- Groups A and B
    - Fractional (protein or polysaccharide subunit)- Groups C and D
- Allow students about 10 minutes to discuss in their groups.
- Each subgroup should then present their answers to the entire group (5-10 minutes total)

Lassa Virus Vaccine

Lassa virus is an arenavirus that causes Lassa hemorrhagic fever. Currently endemic to West Africa, it was first noticed in three missionary nurses in Nigeria in 1969 and was isolated and characterized at the Yale Arbovirus Research Unit (YARU) in 1970 after killing two YARU nurses and one laboratory technician. In 2017, the Coalition for Epidemic Preparedness Innovations (CEPI) raised almost $500 million to develop promising vaccine candidates targeted against three emerging infectious diseases (EID), including Lassa.

Instructions to Students: Based on your assigned vaccine strategy, evaluate its advantages and disadvantages, considering effectiveness of eliciting immunity in individuals, safety, and the practical aspects of administering vaccines in the rural areas and/or the developing world. For your chosen strategy, list the real world examples of that vaccine strategy currently used in the USA.

Vaccine Strategy	**How is an immune response engaged and elicited?** *Include details about the innate, T cell-mediated, and humoral responses.* **Provide Real World Examples**	Advantages	Disadvantages

ANSWER GUIDE FOR FACILITATORS

Vaccine Strategy	**How is an immune response engaged and elicited?** *Include details about the innate, T cell-mediated, and humoral responses.* **Provide Real World Examples**	**Advantages**	**Disadvantages**
Group A/B: Live-attenuated Retains ability to replicate and produce immunity, but usually does not cause illness	*Closely mimics an actual infection* ***Innate****: LA-virus replicates in the vaccinated person; PAMPs, such as viral nucleic acids, from the LA-virus activate innate cells, which then activate and license pathogen-specific T cells; strong innate response due to the live pathogen, and therefore a strong adaptive response (see Advantages →)* ***T cell-mediated****: Pathogen-specific T cells proliferate, form memory T cells, and activate pathogen-specific B cells* ***Humoral****: Pathogen-specific B cells proliferate, undergo class-switch recombination and somatic hypermutation to generate antibodies that are most appropriate and effective at fighting future infection, and form memory B cells*	*Strong cellular and humoral immune responses; lifelong immunity (immunological memory) with few doses*	*Cannot be administered to individuals with weakened immune systems; must stay refrigerated*

	Real World Examples: • MMR • RV • Varicella		
Group C/D: Fractional (protein or polysaccharide subunit) Proteins or polysaccharides from a pathogen, combined with an adjuvant	*Elicits immunity to subunit(s) from a pathogen; requires an adjuvant to activate the innate system* ***Innate**: The adjuvant is required to activate innate cells, which then activate and license subunit-specific T cells* ***T cell-mediated**: Subunit-specific T cells proliferate, form memory T cells, and activate subunit-specific B cells* ***Humoral**: Subunit-specific B cells proliferate, undergo class-switch recombination and somatic hypermutation to generate antibodies that are most appropriate and effective at fighting future infection, and form memory B cells* ***Real World Examples**: (protein subunits)* • Flu • HepB • DTaP (Pertussis) • HPV *Examples (polysaccharide subunits)* • PCV HiB	*Can be given to immunologically compromised patients; can produce immunity without the use of the live-attenuated or inactivated pathogen*	*Must predict which subunits will be the most important for raising effective immunity. For example, the annual flu vaccine contains proteins that epidemiologists predict will be prevalent and important in anti-flu immunity for that year.*