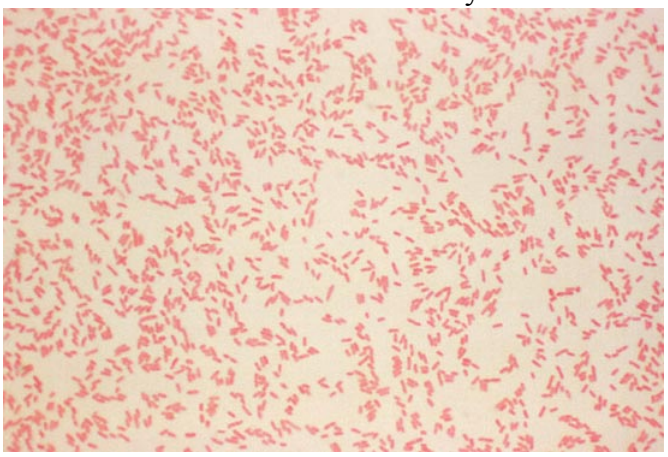
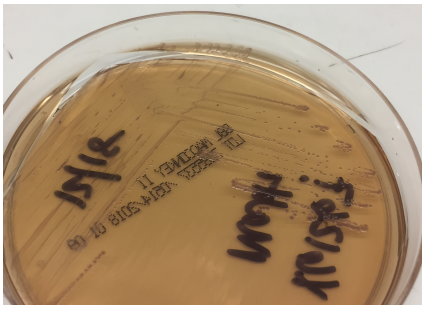
Rob Cybulski **Fellows Meeting** **10 January 2018**

You received a call from the UWMC Micro Lead on a Saturday. They had received a blood culture isolate from Northwest Hospital. The isolate was a Gram negative organism with short, plump, coccobacillus-to-rod shapes that the mass spectrometer identified to the species level with high score value (>2.0). Due to known reliability issues differentiating specific organisms of this genus (one of which is a Select Agent), the lab has a standing protocol in place to perform confirmatory testing. The confirmatory test in this case is a urease test. The urease test can come back at 24 hours as NEGATIVE, which DID NOT rule out the Select Agent but DID cause the Lead to become concerned/annoyed.

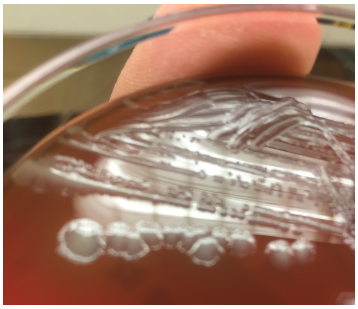


The organism grew at 35-37C on both blood (nonhemolytic) and MacConkey (non lactose fermenter), is catalase positive, oxidase negative, indole negative and ferments glucose.



(MacConkey)

(Blood)



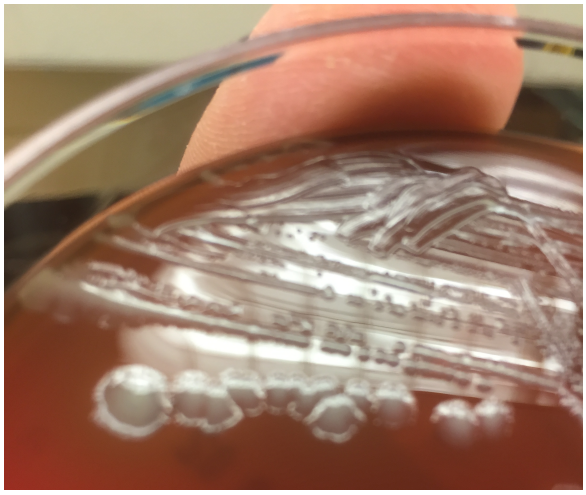
(Blood, close-up)

The patient is a 74 year old male who had been admitted from an adult care home with altered mental status (unresponsive) and suspected hepatic encephalopathy. He has Type 2 Diabetes and advanced alcoholic cirrhosis, functional quadriplegia, BPH and bladder outlet obstruction with a chronic Foley catheter and recurrent UTIs reported to contain mixed flora including Gram negative rods. His lactulose (colonic acidifier used to decrease ammonia and reduce encephalopathy in patients with cirrhosis) had recently been switched to rifaximin due to chronic diarrhea and caretaker burden (PMID 23700882). He has lived in an adult care home for 2 years. No recent travel. Once conscious again, denies exposure to raw meat, contaminated water or anyone else who is ill.

- What is the Select Agent that needs to be ruled out? What is/are the more likely organism(s) of the same genus?
- What else can be done to microbiologically rule out the Select Agent?

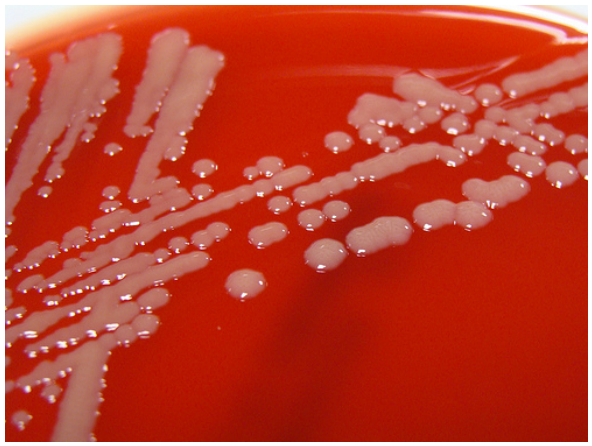
The patient's isolate was identified by mass spectrometry as *Yersinia enterocolitica*. The concern and need for rule-out has to do with *Yersinia pestis*. The concern for mis-identification of *Y. pestis* by mass spec (and prior techniques, such as automated biochemically-based identification instruments) stems from a documented issue with the instrument calling *Y.* pestis as *Y. pseudotuberculosis*, NOT *Y. enterocolitica*. So the chances of a mis-called *Y. pestis* seemed small. The Biothreat Agent mass spec library (distinct from the usual clinical library, with a greater number of spectra for Biothreat Agents and therefore greater discriminatory power) also called this isolate *Y. enterocolitica*.

BUT the technologist set up the urease slant to be sure…. And it had unfortunately made things more complicated, rather than less. *Yersinia enterocolitica* and *pseudotuberculosis* are both urease positive, whereas *Y. pestis* is urease negative. There is documentation of urease-negative *Y. enterocolitica* strains (PMID 535393), though they are rare. The other (more likely) explanation has to do with growth conditions – *Yersinia* grows optimally at 25-28C. Growth above 28C can be inconsistent, and biochemicals tested at higher temperatures can be unreliable. Specifically, if urease is tested at 35-37C and it negative, it should be repeated at 28C. (Manual of Clinical Microbiology, ASM Press, 11th Edition).



(Ours)

(Textbook *Y. pestis*)



(Textbook *Y. enterocolitica*)

Aside from urease:

- *Y. enterocolitica* and *pseudotuberculosis* are both motile at 25C, while *Y. pestis* is nonmotile.
- ~50% of *Y. enterocolitica* strains are reported as indole positive, which would also help distinguish from *Y. pestis*, which is indole negative... unfortunately, this isolate had already been tested and was indole negative.
- Sending the isolate to Molecular Micro was also a possible means of resolving the issue... MolMicro reports that they can reliably distinguish between *Y. enterocolitica* and *Y. pestis*, while having difficult with *Y. pestis*/*pseudotuberculosis* (similar to the mass spec).

Table 13-1 Biochemical reactions of *Yersinia* spp. after incubation at 35°C[a]

Species	Indole	Motility at 25°C	Urea	Ornithine	Sucrose	Rhamnose	Melibiose
Y. pestis	0	0	0	0	0	0	V
Y. enterocolitica	V	+	+	+	+	0	0
Y. pseudotuberculosis	0	+	+	0	0	+	+

[a]+, positive reaction (≥90% positive); V, variable reaction (11 to 89% positive); −, negative reaction (≤10% positive).

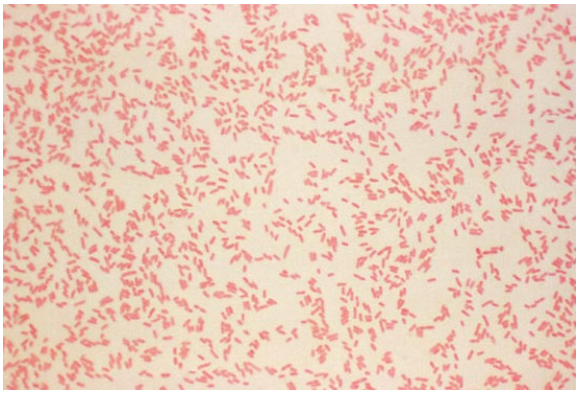
(de la Maza, Color Atlas of Medical Bacteriology, 2nd Edition)

I asked the Lead to set the urease up again, at 28C and 37C. Also asked him to set up a motility slant. Upon repeat, the Urease slant was positive:

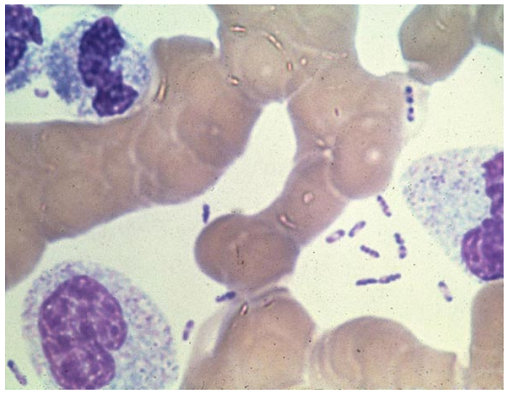
The patient's encephalopathy was attributed to his liver disease combined with dehydration. The source for his *Yersinia enterocolitica* was attributed to his urine, though it was never differentiated from other GNRs found in his multiple mixed urine cultures. He was prescribed ceftriaxone, and returned to his adult care facility after improvement.

Yersinia spp.

- 14 environmental/nonpathogenic species
- *Yersinia enterocolitica*, *pseudotuberculosis* and *pestis* – zoonotic agents
    - *Y. enterocolitica* and *pseudotuberculosis* – ingestion of contaminated food/water (urease aid survival of passage through stomach), commonly cause gastroenteritis
    - Y. pestis – bite from infected flea, causative agent of plague
- *Y. enterocolitica* and *pseudotuberculosis* diverged as much as 200 million years ago, whereas *Y. pestis* is a clone of Y. pseudotuberculosis that split as recently as 1,500 years ago (1,500-20,000)
    - 75% of genes have ≥97% identity
- Common virulence plasmid – encodes for type three secretion system (TTSS), YadA (adhesion/internalization) molecule, *Yersinia* outer membrane proteins (Yops) which interfere with host immune response
- Two *Y. pestis* specific virulence plasmids
    - Enable colonization of flea vector, mammalian pathogenesis
- *Y. pestis* also has numerous pseudogenes (including *yadA*) that contribute to its restrictive host niche (does not survive in environment like other species) – example of patho-adaptation? (PMID 17233672)



Yersinia Gram stain



Y. pestis Geimsa stain

Current Federal Select Agent List:

HHS SELECT AGENTS AND TOXINS

Abrin
Bacillus cereus Biovar *anthracis**
Botulinum neurotoxins*
Botulinum neurotoxin producing species of *Clostridium**
Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence $X_1CCX_2PACGX_3X_4X_5X_6CX_7$)[1]
Coxiella burnetii
Crimean-Congo haemorrhagic fever virus
Diacetoxyscirpenol
Eastern Equine Encephalitis virus[3]
Ebola virus*
*Francisella tularensis**
Lassa fever virus
Lujo virus
Marburg virus*
Monkeypox virus[3]
Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
Ricin
Rickettsia prowazekii
SARS-associated coronavirus (SARS-CoV)
Saxitoxin
South American Haemorrhagic Fever viruses:
- Chapare
- Guanarito
- Junin
- Machupo
- Sabia

Staphylococcal enterotoxins A,B,C,D,E subtypes
T-2 toxin
Tetrodotoxin
Tick-borne encephalitis complex (flavi) viruses:
- Far Eastern subtype
- Siberian subtype

Kyasanur Forest disease virus
Omsk hemorrhagic fever virus
Variola major virus (Smallpox virus)*
Variola minor virus (Alastrim)*
*Yersinia pestis**

OVERLAP SELECT AGENTS AND TOXINS

*Bacillus anthracis**
Bacillus anthracis Pasteur strain
Brucella abortus
Brucella melitensis
Brucella suis
*Burkholderia mallei**
*Burkholderia pseudomallei**
Hendra virus
Nipah virus
Rift Valley fever virus
Venezuelan equine encephalitis virus[3]

USDA SELECT AGENTS AND TOXINS

African horse sickness virus
African swine fever virus
Avian influenza virus[3]
Classical swine fever virus
Foot-and-mouth disease virus*
Goat pox virus
Lumpy skin disease virus
Mycoplasma capricolum[3]
Mycoplasma mycoides[3]
Newcastle disease virus[2,3]
Peste des petits ruminants virus
Rinderpest virus*
Sheep pox virus
Swine vesicular disease virus

USDA PLANT PROTECTION AND QUARANTINE (PPQ) SELECT AGENTS AND TOXINS

Peronosclerospora philippinensis (Peronosclerospora sacchari)
Phoma glycinicola (formerly Pyrenochaeta glycines)
Ralstonia solanacearum
Rathayibacter toxicus
Sclerophthora rayssiae
Synchytrium endobioticum
Xanthomonas oryzae

Soviet Union Biological Weapons Program
(AFTER the 1972 Biological Weapons Convention)

- *Bacillus anthracis* (anthrax)
- *Yersinia pestis* (plague)
- *Francisella tularensis* (tularemia)
- *Burkholderia mallei* (glanders)
- *Brucella* spp (brucellosis)
- *Coxiella burnetii* (Q-fever)
- Venezuelan equine encephalitis virus (VEE)
- Botulinum toxin (botulism)
- Staphylococcal enterotoxin B
- Smallpox
- Marburg virus