Nebraska Public Health Laboratory Newsletter

A publication of the Nebraska Public Health Laboratory (NPHL) at the University of Nebraska Medical Center
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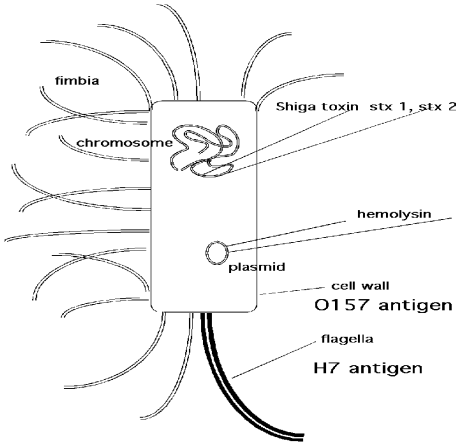
Introduction:

This issue of the Nebraska Public Health Laboratory (NPHL) Newsletter is focused on a topic of importance to all clinical laboratories, the diagnosis of enteric pathogens, *Salmonella, Shigella, Campylobacter*, and toxin producing *E. coli. E. coli* O157:H7 has not only impacted the physical health of Nebraskans but also the state's economy. The clinical laboratory serves a vital role in detecting *E. coli* O157:H7, a cause of hemorrhagic enterocolitis (HEC) and also hemolytic uremic syndrome which most commonly appears in children.

One of the most important questions facing the laboratory is whether it is cost-effective to add a screening plate to detect *E. coli* 0157:H7, since its occurrence is considered rare. A second question is which of the available assays is most effective and should screening for toxins be routinely performed? We are beginning a research study this spring to investigate these issues and provide a factual basis for making decisions on data obtained in Nebraska. A local study is necessary because the prevalence of *E. coli* O157:H7 varies significantly in northern vs. southern climates. It is interesting to note that *E. coli* O157:H7 was first recognized in the U.S. in 1982 and the third national major outbreak occurred in a Nebraska nursing home in 1984.

The research study is being conducted by Dr. Paul Fey in collaboration with Dr. Tom Safranek, State Epidemiologist at the Department of Health and Human Services. This study seeks to obtain stool samples from patients experiencing diarrhea and will compare screening agar methods with ELISA methods for detection of toxin and PCR methods for detection of specific genes. NPHL will also provide confirmation of *E. coli* 0157:H7, as well as assay for the presence of toxin production in non-0157 strains recovered from patients with hemorrhagic enterocolitis. We greatly appreciate the cooperation of laboratories across the state and we will share with you results of the study as it becomes available.

Steven H. Hinrichs



Schematic representation of Escherichia coli showing key structural elements including the flagella, which are the source of H antigen, and cell wall which is the source of somatic O antigen.

Escherichia coli O157:H7

by Paul D. Fey, Ph.D.

Escherichia coli is an important pathogen which can cause a variety of diseases including diarrhea, urinary tract infections, bacteremia, pneumonia, and meningitis. Most strains of *E. coli* are found surviving as avirulent commensals in the large bowel of mammals. However, specific virulence traits can be acquired from plasmids and other transferable elements which allow the organism to invade or attach to specific cellular types or to produce toxins. A loose correlation exists between strains of *E. coli* that cause specific disease and a serological classification scheme first described by Kauffman in 1944. This scheme is based on two highly variable cellular antigens, the somatic (O) antigen and the flagellar (H) antigen. Strains with a similar O antigen are of the same serogroup, while those strains with a similar O antigen and H antigen are of the same serotype. Additionally, strains can be characterized by the type of virulence genes they possess. Strains which possess the same virulence factors are said to be of the same virotype. Using serotyping and virotyping in conjunction, six different groups have evolved from those strains of *E. coli* that cause diarrhea. The first five

***E. coli* O157:H7**

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Salmonella Typing

by Peter C. Iwen, M.S.

Nomenclature

The salmonellae are a heterogenous group of bacteria in the genus *Salmonella* of the family *Enterobacteriaceae.* The taxonomy and nomenclature of *Salmonella* have changed over the years and are still evolving. Currently, the CDC recognizes **two species** which are divided into **seven subspecies**: *S.enterica* (six subspecies) and *S.bongori* (one subspecies). The subspecies are divided into over **50 serogroups** based on somatic (O) antigens present. The serogroups are further divided into over **2300 serotypes** based on flagellar (H) antigens. The CDC now recommends that all organisms identified as *Salmonella* be reported by genus and serotype (or serogroup) omitting the reference to species. *Salmonella* serotypes are recognized with antigenic formulas listed in the document called the Kauffman-White Scheme. Updating

***Salmonella* Typing**

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groups include enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC),enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC), and enteropathogenic *E. coli* (EPEC). These groups cause diarrheal disease by various combinations of virulence traits and mechanisms including the liberation of enterotoxins (ETEC), adherance to intestinal epithelial cells (EAEC, DAEC, and EPEC) and invasion of intestinal epithelial cells (EIEC). The sixth group, enterohemorrhagic *E. coli* or EHEC, is a more serious pathogen which not only causes bloody diarrhea but may also cause other serious diseases such as hemolytic uremic syndrome (HUS).

EHEC was first recognized as an emerging pathogen in 1982 when an outbreak of diarrhea and HUS occured following ingestion of undercooked hamburgers from a fast-food restaurant. An epidemiological investigation found that the etiologic agent causing the disease was *E. coli* O157:H7, a serotype not previously recognized as a human pathogen. Since that time, large foodborne outbreaks caused by *E. coli* O157:H7 have occurred as well as sporadic cases of disease. The CDC has recently reported *E. coli* O157:H7 as the fourth most prevalent bacterial diarrheal pathogen behind *Campylobacter* sp., *Salmonella* sp., and *Shigella* sp.

EHEC are ingested from contaminated water or food and appear to colonize the lower intestine. A small infectious dose is required; studies have shown that as few as 200 bacteria are sufficient to cause disease. The major virulence factor of EHEC is a bacteriophage-encoded shiga-toxin. This toxin, in part, mediates diarrheal disease and presumably HC and HUS as well. There are two different types of immunologically distinct shiga-toxins produced in EHEC called Stx1 and Stx2. Stx1 is identical to the shiga-toxin produced by *Shigella dysenteriae.* Other virulence factors include an outer membrane protein called intimin, which is important in colonization of the intestine, and an enterohemolysin, encoded on a 60 Mda plasmid called pO157. Enterohemolysin may contribute to virulence by lysing red blood cells and therefore providing a source of iron to the bacterium.

Even though *E. coli* O157:H7 is the major serotype isolated from patients with HC and HUS, other serotypes have recently been isolated which express Stx1, Stx2, intimin and enterohemolysin. The most common non-O157:H7 serotypes isolated from humans with diarrheal disease are O26:H11, O103:H2, O111:NM, and O113:H21. Additionally, investigators have found Stx-producing *Citrobacter freundii* and *Enterobacter cloacae* from patients with HUS. Livestock animals, including cattle, sheep, pigs, chickens and goats, are the major reservoir of Stx-producing *E. coli.* However, since the majority of these isolates are not of the O157:H7 serotype and do not contain other virulence factors such as *eae* or enterohemolysin, the virulence of these isolates is not known.

Treatment of patients with HUS is at best controversial. Some studies have suggested that EHEC infected patients who were treated with antibiotics had a greater chance of developing HUS than those patients who were not. Therefore, treatment is mostly supportive. However, screening for and detection of shiga-toxin producing *E. coli* is necessary for the detection of outbreaks so that appropriate infection control measures can be administered. Detection of shiga-toxin producing *E. coli* is difficult in the clinical microbiology laboratory due to the large number of avirulent *E. coli* present in fecal specimens. However, since *E. coli* O157:H7 do not ferment sorbitol as strongly as other commensal *E. coli,* selective and differential media such as sorbitol-MacConkey agar (SMAC) or cefixime-tellurite-sorbitol-MacConkey agar (CT-SMAC) can be used to screen stool samples for this bacterium. Since other serotypes of EHEC ferment sorbitol strongly, they will not be detected using SMAC or CT-SMAC agars. To circumvent this problem, investigators have developed ELISA and PCR assays that will detect shiga toxin (both Stx1 and Stx2) from fecal samples. However, due to the expense of these assays, they are not widely used in clinical microbiology laboratories across the country. Therefore, the prevalence of non-*E. coli* O157:H7 EHEC is not known in most parts of the United States.

If your laboratory is not screening stool specimens for *E. coli* O157:H7, the NPHL suggests that you add SMAC or CT-SMAC to your routine stool culture procedure for a period of six months (preferably during the spring and summer). We do not suggest that you add a SMAC or CT-SMAC plate only to stools which are visibly bloody since blood in stools may not be visually evident. After the six month period, an assessment can be made as to whether continuing to screen for *E. coli* O157:H7 is appropriate for your laboratory. Our opinion is that even one case of diagnosed *E. coli* O157:H7 infection is worth the cost of the added SMAC or CT-SMAC agar plate.

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this scheme is the responsibility of the WHO Collaborating Centre for Reference and Research on *Salmonella*, which is located at the Pasteur Institute , Paris, France. Most *Salmonella* serotypes isolated from humans and warm-blooded animals belong to ***Salmonella enterica* subspecies 1 in O-serogroups A, B, C1, C2, D, E1, E2, E3, and E4**. Additionally, a majority of the isolates reported in the West North Central Region of the United States (Iowa, Kansas, Minnesota, Nebraska, North Dakota, and South Dakota) during 1996, were one of five serotypes included in O-serogroups B, C1, C2, or D1. It is impractical for most laboratories to perform even a limited typing of *Salmonella* because of the large number of reagents required. The NPHL offers testing available to confirm the most common serogroups in our region.

Salmonella serogroup

Bacterial identification systems, such as the Vitek™, API™, and MicroScan™, are reliable in the biochemical identification of *Salmonella* to the genus level. These systems however, do not identify the salmonellae into serogroups or serotypes. To identify *Salmonella* serogroups, numerous O-grouping antisera along with control antigens, are necessary. The NPHL has antisera to

Salmonella Typing

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perform agglutination testing and recognize the following O-serogroups: Groups A, B, C1, C2, D, and E. In addition, antiserum to detect the capsular or virulence (Vi) antigen is also available to screen for *Salmonella* serotype (Group D). At the NPHL, serogrouping is routinely performed on all biochemically recognized salmonellae for confirmation and reporting.

***Salmonella* serotype**

Typing of *Salmonella* for specific identification into serotypes requires an assortment of antisera such as single factor O-antisera as well as phase 1 and phase 2 H-typing antisera. In 1996, the most common *Salmonella* serotypes reported from the West North Central Region of the U.S. in descending order were ***Salmonella* serotype Enteritidis (Group D1), *Salmonella* serotype Typhimurium (Group B), *Salmonella* serotype Heidelberg (Group B), *Salmonella* serotype Newport (Group C2), and *Salmonella* serotype Braenderup (Group C1).** These serotypes accounted for more than 75% of the isolates reported from this region. The NPHL is developing protocols to identify these common serotypes when epidemiological investigations are warranted. Susceptibility testing is also available to detect multidrug resistant *Salmonella* serotype Typhimurium Definitive Type 104 (DT104). This isolate shows resistance to ampicillin, chloramphenicol, sulfonamides, streptomycin, and tetracycline, but generally is sensitive to trimethoprim and fluoroquinolones. Isolates submitted to NPHL which require additional typing are sent to the CDC for specific serotyping.

Conclusion

For epidemiological purposes, the NPHL has available reagents necessary to confirm and serogroup most isolates of *Salmonella* suspected throughout our region. Clinical laboratories are encouraged to submit all isolates of *Salmonella* to the NPHL for typing. This testing is performed without charge and the only requirement for submission of the isolate is completion of the "Special Microbiology Requisition Form". For more information on the typing of *Salmonella* at the NPHL,

Typing of *Salmonella*

Availability: Monday - Thursday by 0900

Results: 48 hours

Specimen: Organism on culture media

Transport: Room temperature

Submission: Submit organism accompanied with a "Special Microbiology Requisition Form". A copy of the requistion form can be obtained by FAX by calling Peter Iwen at (402) 559-7774.

Comment: Typing generally includes the recognition of the O-serogroup for *Salmonella.* Protocols to perform a limited specific serotyping are being developed.

A Reminder.....

The following enteric diseases are required by Nebraska State law to be reported to the Nebraska Department of Health:

Campylobacteriosis
Cholera
Cryptosporidiosis
***E. coli* O157:H7 infection**
Giardiasis
Shigellosis
Salmonellosis
Yersiniosis

Address Change

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Campylobacter Enteritis

Campylobacter is one of the most common causes of food borne diseases in the United States, causing approximately 2 million cases of gastroenteritis each year. Most illnesses associated with *Campylobacter* infection are sporadic. Common source outbreaks occur, and most have been traced to unpasteurized milk and contaminated drinking water. In comparison, most sporadic cases, are associated with improper handling and preparing of poultry. *Campylobacter* has been found in up to 88% of broiler chicken carcasses in the United States. The infectious dose of *Campylobacter* is low; ingestion of only 500 organisms can result in human illness. Therefore, contamination of food by raw chicken is an efficient mechanism for transmission of this organism. Culture of stool using a specialized medium and culture condition with subsequent biochemical testing, are the common methods used to isolate and identify *Campylobacter jejuni*. Stools and/or suspected isolates may be submitted to the NPHL to detect or confirm *Campylobacter*.

*[Condensed from an article which appeared in the **Morbidity and Mortality Weekly Report**; February 27 1998/Vol.47/No.7)]*

Enterohemorrhagic *E. coli P*revalence Study

by Paul D. Fey, Ph.D.

The Nebraska Public Health Laboratory (NPHL) in conjunction with the Office of Epidemiology at the Nebraska Health and Human Services System (NHHSS) is conducting a study this spring and summer to determine the prevalence of *Escherichia coli* O157:H7 and other enterohemorrhagic *E. coli* (EHEC) in the state of Nebraska. We plan to collect approximately 500 diarrheal stool specimens from nine participating laboratories throughout Nebraska. Once the specimens arrive at

Prevalence Study

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the NPHL, we will perform three different tests to detect EHEC. First, the stool specimen will be plated to cefixime-tellurite sorbitol MacConkey agar to screen for the phenotypic properties of *E. coli* O157:H7. This screen will not detect other EHEC serotypes, as they are known to ferment sorbitol. Secondly, stool specimens will be tested for the presence of shiga-toxin by the Meridian Premier EHEC enzyme immunoassay kit. Lastly, DNA will be extracted from the stool specimens and shiga toxin and other EHEC virulence genes will be detected using multi-plex PCR. If EHEC are detected by any of these three methods, the strain will be isolated and serotyped using type specific anti-sera. Results from this study will give Nebraska health care providers and microbiologists essential information on the frequency of this group of organisms and optimal methods for detection.

EHEC study

Participating laboratories:

Omaha
UNMC
Bergan Mercy Medical Center
North Platte
Great Plains Medical Center
Grand Island
St. Francis Medical Center
Lincoln
Quest Laboratories
Kearney
Good Samaritan Hospital
Hastings
Mary Lanning Hospital
McCook
Community Hospital
Beatrice
Beatrice Community Hospital

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Identification of Inactive *E. coli*

by Steven H. Hinrichs, M.D.

Escherichia coli and *Shigella* species are genetically closely related and a specific situation exists which may provide a diagnostic problem for the clinical laboratory. This situation occurs when a lactose-negative, non-motile organism is recovered and identified by commercial assays as *Shigella*. species. We have encountered examples of mistaken identification by automated systems, although biochemical reactions did not follow the usual pattern and the data base indicated a low probability. Serotyping was helpful in identifying the organism as an inactive *E. coli*, also formerly called Alkalescens/Dispar. This biogroup of *E. coli* is typically lactose-negative on screening media, however is generally not thought to be pathogenic. The issue may be brought to the laboratory's attention when the clinician asks for clarification on an isolate reported as *Shigella*, but the patient is not experiencing continued diarrhea. The recognition of a mistaken identification may not be made until the major *Shigella* serogroups A, B, C, and D are tested and found to be negative. Confirmatory identification can be made with the API system. Should an isolate suspected to be inactive *E. coli* be found, NPHL will perform additional testing when requested for confirmation.