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There's *What* In My Brain?

What We Can Learn from NGS Testing of Bacterial Meningitis Cases

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NORTHEAST BRANCH-AMERICAN SOCIETY FOR MICROBIOLOGY
54TH ANNUAL REGION I MEETING
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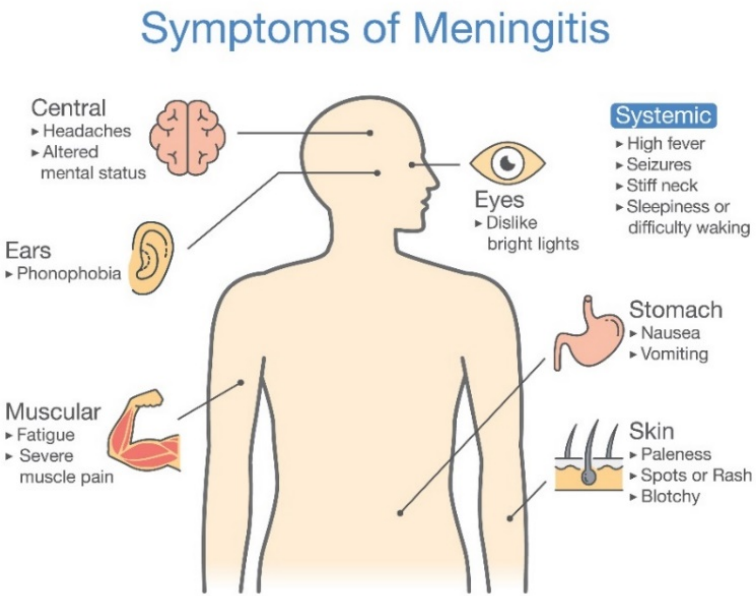
Bacterial meningitis is a serious and potentially deadly infection of the CNS

- Inflammation of the meninges
- Immediate diagnosis critical for patient care
- Sudden onset of fever, headache, stiff neck, altered mental status, nausea/vomiting
- Symptoms usually appear 3-7 days after exposure
- Children and older adults are the highest risk groups



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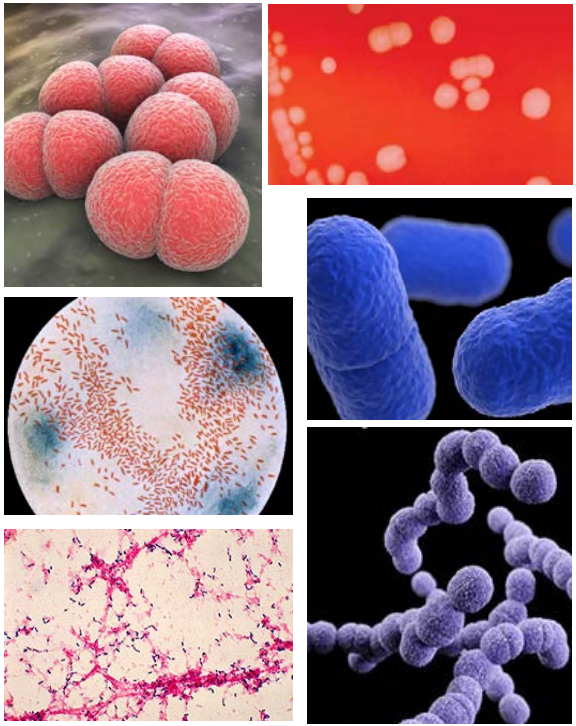
Bacterial meningitis is a serious and potentially deadly infection of the CNS

- Most common causes of bacterial meningitis is US are:
  - *Neisseria meningitidis*
  - *Streptococcus pneumoniae*
  - *Haemophilus influenzae*
  - *Group B Streptococcus*
  - *Listeria monocytogenes*
- Vaccines available for protection against *N. meningitidis, S. pneumoniae, and Haemophilus influenzae*
  - Serotyping performed to determine if it's a vaccine preventable strain
- Identification of contacts important for prophylaxis and vaccination clinics in certain settings



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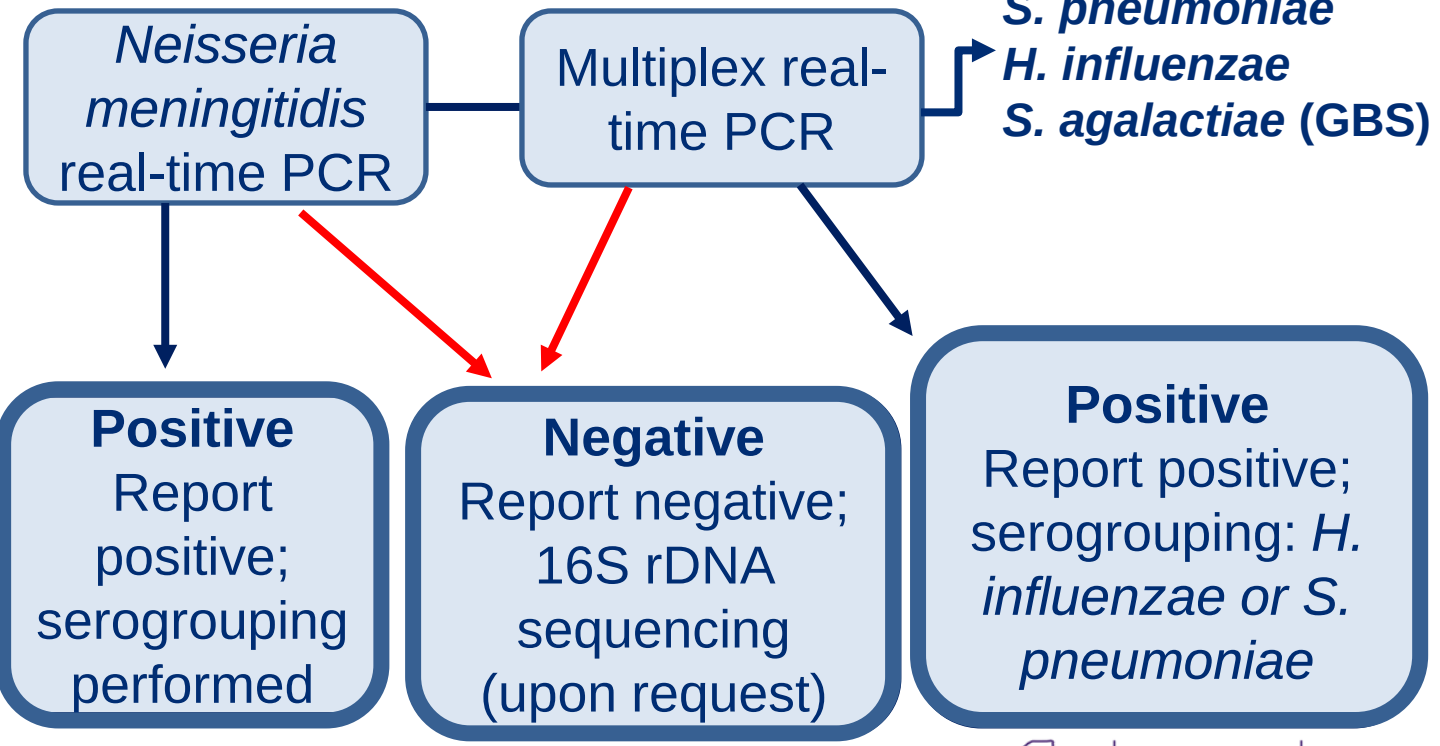
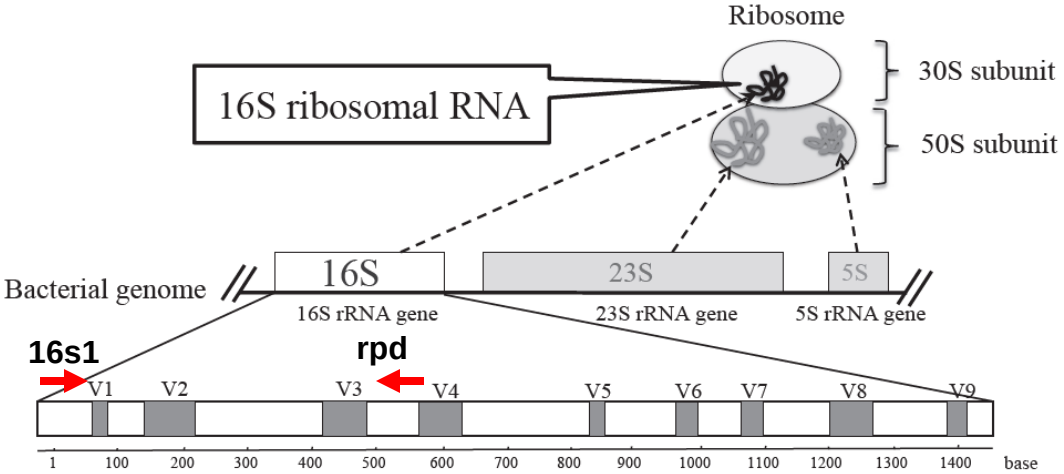
Current testing algorithm for bacterial meningitis cases



16S rDNA sequencing is commonly used for bacterial identification



- Universally found in all bacteria; highly conserved
- Allows for identification of fastidious organisms and culture-negative specimens
- Alternative testing method when unsure of the pathogenic bacteria

Meningitis testing in the Bacteriology Laboratory from 2015-2017

- Specimens tested:
  - Young children (ages 0-10): ~25%
  - Teenage/college-aged (ages 15-25): ~19%

- ~19% of specimens tested by real-time PCR were positive for targeted bacteria

- When 16S rDNA sequencing was requested: other bacteria identified in ~20% of specimens
  - Many specimens remain unidentified

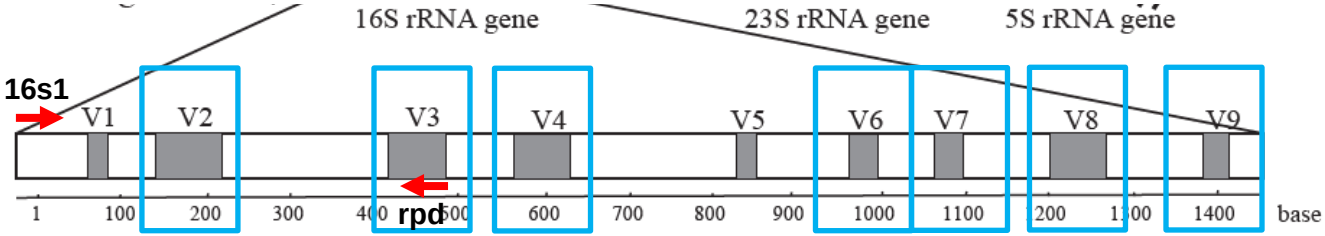
- Can NGS can help resolve when organisms are not detected/identified?

In this study, we aimed to evaluate the performance of the Ion 16S™ Metagenomics Kit to identify bacteria in CSF in comparison to the current 16S Sanger sequencing method.

Increased identification = Better patient outcomes

Improve laboratory testing methods

Bacterial 16S rRNA gene: Primer targets of Ion 16S™ Metagenomics Kit

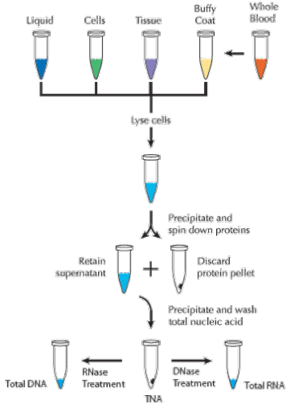


Fukuda et al. Molecular Approaches to Studying Microbial Communities: Targeting the 16S Ribosomal RNA Gene. *J UOEH*. 2016

Workflow for sequencing using the Ion 16S™ Metagenomics Kit

Prepare samples, Epicentre DNA extraction → PCR setup → NGS

- Library Prep (manual)
- Templating
- Sequencing, Analysis



Overview of the MasterPure™ Complete Kit protocol. © Epicentre

Ion S5

ION REPORTER™ SOFTWARE

MicroSEQ™

GREENGENES
The 16S rRNA Gene Database and Tools

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Setting an Analysis Threshold

Appendix A: List of organisms found in negative aCSF controls across retrospective study runs 1-5; organisms recorded if *Genus species* was identified

Genus	Species	% of Total Reads
Aciditerrimonas	*sp.*	0.11
Agrococcus	*jejuensis*	0.02
Blastococcus	*aggregatus*	0.11
Blastococcus	*saxobsidens*	0.06
Corynebacterium	*afermentans*	0.12
Corynebacterium	*tuberculostearicum*	0.02*
Corynebacterium	*sp.*	0
Yimella	*lutea*	0.01
Dietzia	*cercidiphylli*	0
Dietzia	*maris*	0.01
Dietzia	*natronolimnaea*	0.05
Microbacterium	*ginsengisoli*	0.05
Kocuria	*marina*	0.04
Nocardioides	*oleivorans*	0.4
Prevotella	*maculosa*	0.07

- "Background bacteria" can be challenging
- Organisms in negative artificial CSF (aCSF) controls were identified and used to measure background/ contamination
- Threshold to identify a bacterial species: ≥1.0% of total reads in the sample

Retrospective study

Tested archived CSF specimens that had been tested previously

68 total specimens tested

- 15 known positives
- 53 “unknowns”
  - No prior 16S sequencing performed
  - Not positive for any other real-time PCR targets
  - Not tested by another laboratory at Wadsworth

Retrospective study results

Targeted 16S NGS Results:	16S Sanger Sequencing Results:
• 15/15: Identification of meningitis positive samples correlated	• 10/15: Identification of meningitis positive samples correlated
• 15/53: Samples initially negative by PCR were found to be positive for at least one bacterial organism (28%)	• 3/53: Samples initially negative by PCR were found to be positive for at least one bacterial organism (6%)
• 38/53: Samples previously determined negative by PCR were negative by NGS	• 50/53: Samples previously determined negative by PCR were negative by 16S sanger sequencing

Retrospective study results: breakdown of Ion Torrent NGS Positives

	Results		
Sample	Ion Torrent NGS	16S Sanger Sequencing	Other Real-time PCR
17	*Streptococcus anginosus, Streptococcus intermedius, Fusobacterium necrophorum*	NBD*	*Streptococcus anginosus*
18	*Streptococcus salivarius*	NBD*	*Streptococcus pyogenes*
23	*Clostridium septicum, Klebsiella pneumoniae, Klebsiella variicola*	*Klebsiella*	*Klebsiella* sp.
26	*Staphylococcus auricularis*	NBD*	—
41	*Prevotella maculosa, Prevotella oris*	NBD*	*Streptococcus constellatus*
43	*Streptococcus pasteurianus*	NBD*	—
47	*Streptococcus salivarius*	NBD*	—
48	*Diaphorobacter oryzae*	NBD*	—
51	*Klebsiella pneumoniae*	NBD*	*Klebsiella* sp.
55	*Bacteroides caccae, Bacteroides dorei, Prevotella, Prevotella sp., Lactobacillus gasseri, Ruminococcus gnavus*	NBD*	—
61	*Streptococcus salivarius*	*Streptococcus salivarius*	—
65	*Corynebacterium sp., Cloacibacterium normanense, Enterococcus cecorum*	NBD*	—
67	*Nocardioides sp., Propionibacterium acnes*	NBD*	—

***NBD: no bacterial DNA detected**

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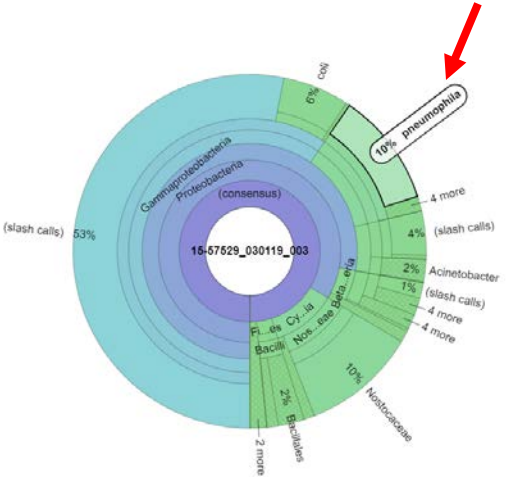
Sample #30

- 59 year-old female
- Suspected meningitis – culture negative at hospital laboratory
- CSF sent to Wadsworth for meningitis testing
- With NGS *Legionella pneumophila* was identified
- Confirmed result with lab developed real-time PCR

Data View: Consensus

Primer	Phylum	Class	Order	Family	Genus	Species	% ID	Count	DB counters	F:R %	% of total reads	% of valid reads	% of mapped reads	% of mapped reads per prime
(consensus)								594642	495178 : 99464		39.01	81.56	100	100
			Legionellales					70117	68531 : 1586		4.6	9.62	11.79	11.79
				Legionellaceae				70117	68531 : 1586		4.6	9.62	11.79	11.79
					(family level ID only)			77	0 : 77		0.01	0.01	0.01	0.01
					Legionella			70040	68531 : 1509		4.6	9.61	11.78	11.78
						(genus level ID only)		1509	0 : 1509		0.1	0.21	0.25	0.25
						(slash calls)		6879	6879 : 0		0.45	0.94	1.16	1.16
						bellardensis	100 - 100	67	67 : 0	0 : 100	0	0.01	0.01	0.01
						pneumophila	99.09 - 100	61510	61510 : 0	37.98 : 62.02	4.04	8.44	10.34	10.34
						quinlivanii	99.30 - 100	75	75 : 0	0 : 100	0	0.01	0.01	0.01
			Pseudomonadales					38819	36878 : 1941		2.55	5.32	6.53	6.53



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	Results		
Sample	Ion Torrent NGS	16S Sanger Sequencing	Other Real-time PCR
18	***Streptococcus salivarius***	NBD*	*Streptococcus pyogenes*
47	***Streptococcus salivarius***	NBD*	—
61	***Streptococcus salivarius***	*Streptococcus salivarius*	—

- *Streptococcus salivarius* identified in 3 samples
- Normally found in the oral cavity, and is an uncommon cause of invasive infections.

	Results		
Sample	Ion Torrent NGS	16S Sanger Sequencing	Other Real-time PCR
18	***Streptococcus salivarius***	NBD*	*Streptococcus pyogenes*
47	***Streptococcus salivarius***	NBD*	—
61	***Streptococcus salivarius***	*Streptococcus salivarius*	—

- *Streptococcus salivarius* identified in 3 samples
- Normally found in the oral cavity, and is an uncommon cause of invasive infections.
- Has been associated with meningitis in past cases

Bacterial Meningitis After Intrapartum Spinal Anesthesia --- New York and Ohio, 2008--2009

Weekly
January 29, 2010 / 59(03);65-69

In June 2007, the Healthcare Infection Control Practices Advisory Committee (HICPAC) recommended for the first time that su
procedure operators to prevent infections associated with these procedures (*1*). HICPAC made the recommendation in respons
following myelography procedures. In September 2008, three bacterial meningitis cases in postpartum women were reported t
Department of Health (NYSDOH); in May 2009, two similar cases were reported to the Ohio Department of Health. All five won
spinal anesthesia. Four were confirmed to have *Streptococcus salivarius* meningitis, and one woman subsequently died. This re
investigations of these five cases, which determined that the New York cases were associated with one anesthesiologist and th
with a second anesthesiologist. In Ohio, the anesthesiologist did not wear a mask; wearing a mask might have prevented the i
underscore the need to follow established infection-control recommendations during spinal procedures, including the use of a n
technique.

Case Reports

New York. In September 2008, a healthy woman aged 24 years (patient A) was admitted in active labor to a New York City h
spinal-epidural anesthesia from anesthesiologist A, and delivered a healthy baby. Approximately 22 hours after receiving anest
headache, back pain, rigors, nausea, vomiting, and disorientation.

CDC MMWR Weekly Report

Eur J Neurol. 1997 Jan;4(1):90-3. doi: 10.1111/j.1468-1331.1997.tb00305.x.

Streptococcus salivarius meningitis: a case report and literature review.

Berrouschot J[1], Sterker M, Schneider D.

⊕ **Author information**

Abstract

Twelve hours after spinal anaesthesia, a 61-year-old patient developed meningitis with fever, somnolence, h
neck. The cerebrospinal fluid was found to contain 5.279/mm(3) cells (95 granulocytes), 12.800 mg/l protein
Streptococcus salivarius was detected in the culture. The patient was treated with antibiotics (initially cefota
later piperacillin and sulbactam). Restitutio ad integrum took place after 7 days. Thirteen cases of Streptocc

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	Results		
Sample	**Ion Torrent NGS**	**16S Sanger Sequencing**	**Other Real-time PCR**
17	*Streptococcus anginosus, Streptococcus intermedius, Fusobacterium necrophorum*	NBD*	*Streptococcus anginosus*
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23	*Clostridium septicum, Klebsiella pneumoniae, Klebsiella variicola*	*Klebsiella*	*Klebsiella* sp.
41	*Prevotella maculosa, Prevotella oris*	NBD*	** *Streptococcus constellatus*
51	*Klebsiella pneumoniae*	NBD*	*Klebsiella* sp.

- 5 Samples had organisms identified that we currently have real-time PCR assays developed for
  - *Streptococcus anginosus group*
  - *Klebsiella pneumoniae*
  - *Steptococcus pyogenes*
  - *** in two cases we identified the pathogen and confirmed with real-time prior to setting our threshold cutoffs.*
- Other organisms were identified in these samples that could were not confirmed by real-time PCR – no current assays
  - *Fusobacterium necrophorum*
  - *Prevotella* sp.

	Results		
Sample	**Ion Torrent NGS**	**16S Sanger Sequencing**	**Other Real-time PCR**
17	*Streptococcus anginosus, Streptococcus intermedius, Fusobacterium necrophorum*	NBD*	*Streptococcus anginosus*
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  - *** in two cases we identified a pathogen and confirmed with real-time prior to setting our threshold cutoffs.*
- Other organisms were identified in these samples that could were not confirmed by real-time PCR – no curr

Eur J Pediatr. 2000 Jul;159(7):527-9.

Streptococcus pyogenes meningitis: report of a case and review of the literature.

Berner R[1], Herdeg S, Gordjani N, Brandis M.

Author information

Abstract

Streptococcus pyogenes is a very uncommon cause of bacterial meningitis beyond the neonatal period. A case report and recent literature is presented. We report on a previously healthy 7-year-old boy who developed S. pyogenes meningitis foll

Germs. 2018 Jun; 8(2): 92–95.
Published online 2018 Jun 4. doi: 10.11603/germs.2018.1136

PMCID: PMC6019955
PMID: 29951302

Community acquired *Klebsiella pneumoniae* meningitis: a case report

Bianca Lee,[1,*] Kevin Yeroushalmi,[2] Hay Me Me,[3] Paresh Sojitra,[4] Usman Jilani,[5] Syed Iqbal,[6] Shadab Ahmed,[7] Janice Verley,[8] and Jagadish Akella[9]

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	Results		
Sample	**Ion Torrent NGS**	**16S Sanger Sequencing**	**Other Real-time PCR**
26	*Staphylococcus auricularis*	NBD*	—
43	*Streptococcus pasteurianus*	NBD*	—

- Additional samples:
  Staphylococcus auricularis
  Streptococcus pasteurianus

- Can cause opportunistic infections
  - Rarely associated with infection or meningitis

Perinatal/Neonatal Case Presentation | Published: 26 July 2007

Perinatal/Neonatal Case Presentation

Early-onset sepsis with *Staphylococcus auricularis* in an extremely low-birth weight infant – an uncommon pathogen

D J Hoffman ✉, G D Brown & F A Lombardo

Journal of Perinatology **27**, 519–520 (2007) | Download Citation ↓

J Clin Microbiol. 2010 Jun; 48(6): 2247–2249. PMCID: PMC2884481
Published online 2010 Mar 31. doi: 10.1128/JCM.00081-10 PMID: 20357211

Streptococcus gallolyticus Subspecies *pasteurianus* (Biotype II/2), a Newly Reported Cause of Adult Meningitis▽

Amy S. Sturt,[1,2,*] Liying Yang,[2] Kuldip Sandhu,[3] Zhiheng Pei,[2,3] Nicholas Cassai,[3] and Martin J. Blaser[2,3]

	Results		
Sample	Ion Torrent NGS	16S Sanger Sequencing	Other Real-time PCR
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65	*Corynebacterium sp., Cloacibacterium normanense, Enterococcus cecorum*	NBD*	—

- Numerous samples (17, 23, 55, and 65) had multiple organisms identified, unlikely mixed infections
  - Could represent contamination from specimen collection or the laboratory
- For many of these bacteria, meningitis reported in rare cases
- Important to consider whole clinical picture!

Summary of major findings

- Targeted 16S rDNA NGS shows increased sensitivity for detection of gram-positive and gram-negative bacteria

- Targeted 16S rDNA NGS identified other bacteria in previously negative clinical CSF specimens
  - 28% of specimens vs 6% by 16S Sanger sequencing

- Public health impact:
  - Results could lead to implementation of new assays (ex: real time PCR for *S. salivarius*)
  - Integration of existing assays into current testing algorithm (ex: S. pyogenes)
  - Improved testing methods = better patient and community health outcomes

Challenges of NGS for Bacterial ID

- NGS most cost-effective when sequencing volume is high
- Increased sensitivity can lead to issues with result interpretation
  - Background
  - Contamination
- Lack of standardization
  - NGS platforms
  - Bioinformatics
- Limitations of 16S rDNA sequencing

Lab personnel

Instrumentation

Reagents and plastics

Conclusions and future directions

- NGS can be a valuable tool for sensitive identification of bacteria in clinical CSF specimens

- Future studies
  - Illumina MiSeq
  - Oxford Nanopore Technologies' MinION
  - Continued retrospective testing of clinical specimens
  - Expand to other specimen sources, including whole blood
  - Clinical Validation of Targeted 16S NGS assay

Acknowledgements

Dr. Kim Musser
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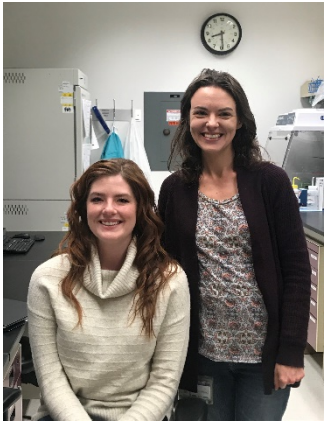

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Applied Genomic Technologies Core

Bacteriology Laboratory

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Calculated costs of Ion Torrent NGS vs 16S Sanger sequencing and Real-time PCR

	Ion Torrent NGS[a]	16S Sanger Sequencing[b]	Real-time PCR
Cost per sample	$412.71	$26.65	$15
TAT (days)	4	Standard: 7-10 Priority: 2	<1

- Ion Torrent NGS: High reagent costs, high cost/sample, labor intensive (manual library prep)
- Bacterial identification using Ion 16S™ Metagenomics Kit not feasible for routine use in the Bacteriology Lab

DISTINCTIONS BETWEEN MEDICINE & PUBLIC HEALTH

	Public Health	Medicine
Primary Focus	Population/Entire Community	Individual
Emphasis	Disease prevention and health promotion for the whole community	Disease diagnosis, treatment, and care for the individual patient
Paradigm	Interventions aimed at the environment, human behavior and lifestyle, and medical care	Places predominant emphasis on medical care
Specializations	Analytical method (epidemiology, toxicology) Setting and Population (occupational health, international health) Substantive health problem (environmental health, nutrition)	Organ system (cardiology, neurology) Patient group (obstetrics, pediatrics) Etiology and pathophysiology (infectious disease, oncology) technical skill (radiology, surgery)

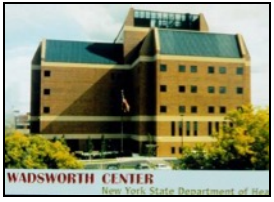
Levels of Specimen Testing

Patient specimen

Clinical Microbiology Laboratory
Hospital (Initial Testing/Diagnosis)

Patient management
Infection control

Short TAT is critical



State Public Health Laboratory
(Confirmation/ additional testing)

Surveillance
Characterization
Epidemiology
Investigation

Federal Public Health Laboratory
(Confirmation/ additional testing)

Monitoring
National trends

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Wadsworth Center

Laboratories in four scientific divisions:

- Environmental Health
- Infectious Disease
- Genetics
- Translational Medicine

Division of Infectious Diseases

Viral Diseases	Bacterial Diseases	Bloodborne Diseases	Mycotic & Parasitic Diseases	Viral Replication and Vector Biology
Virology	Bacteriology	Bloodborne Virus	Parasitology	Arbovirology
Viral Encephalitis	Mycobacteriology	Diagnostic Immunology	Mycology	Rabies
Enteric Virus	Biodefense			

Research Laboratories
PI- grant funded programs

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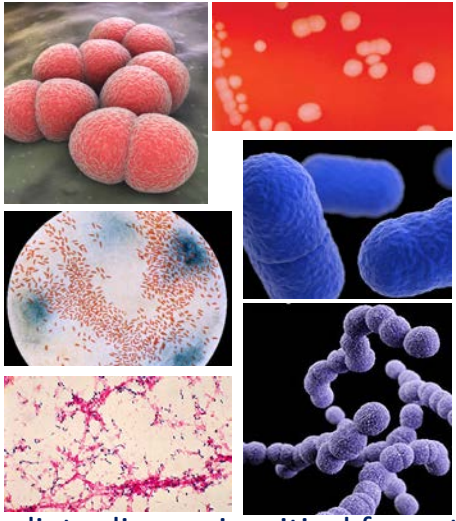
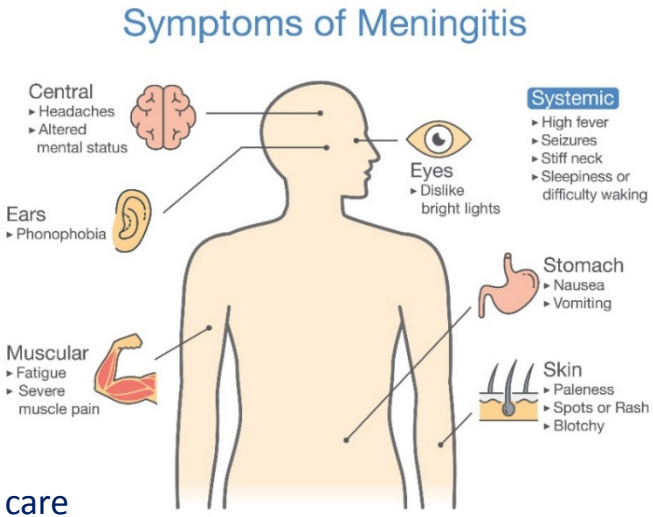
Bacterial meningitis is a serious and potentially deadly infection of the CNS





- Immediate diagnosis critical for patient care
- Most common causes of bacterial meningitis is US are: *Neisseria meningitidis, Streptococcus pneumoniae, Haemophilus influenzae, Group B Streptococcus, , Listeria monocytogenes*
- Children are the highest risk group
- Vaccines for protection against *N. meningitidis, S. pneumoniae, and Haemophilus influenzae*