Challenge M233-2

November 2023

Wound: *Neisseria weaveri*

HISTORY

A simulated wound sample collected from a 9-year-old male with a dog bite on his arm was sent to category A laboratories.

Participants were expected to isolate and report *Neisseria weaveri* or *Neisseria* species.

CMPT QA/QC/STATISTICS

All simulated wound samples are produced at CMPT according to CMPT internal protocols. The sample contained a pure culture of *Neisseria weaveri*.

The samples are assessed for homogeneity and stability using in-house quality control methods and random selection of samples before and during production, and post sample delivery. The number of random samples selected is 15% of the total production batch.

The challenge sample lot was confirmed to be homogeneous and stable for 22 days. Organism identification was confirmed by a reference laboratory.

All challenge components have in-house assigned values based on the most clinically appropriate result; the most clinically appropriate result is determined by expert committee evaluation. No further statistical analysis is performed on the results beyond that described under "Suitability for grading."

SURVEY RESULTS

Reference laboratories: 11/12 (92%) labs reported *Neisseria weaveri*, 1 lab reported gram-negative bacilli, refer, and one lab indicated it does not normally perform testing on this type of sample.

Participants: 44/49 (90%) participants provided an acceptable response (33 reported *Neisseria weaveri;* 5 reported *Neisseria* sp.; 11 reported gram negative bacilli). 4 labs reported *Pseudomonas* (Table 1), which was incorrect.

MAIN EDUCATIONAL POINTS from M233-2

1. *Neisseria weaveri* is infrequently recovered from dog/cat bite wounds.
2. Like *Neisseria elongata*, *Neisseria bacilliformis*, and a few other species of *Neisseria*, *Neisseria weaveri* is a gram negative bacilli
3. Like several other organisms associated with dog bite wounds, *N. weaveri* strains are susceptible to penicillin, fluoroquinolones, and tetracycline

Suitability for Grading

A challenge is considered suitable for grading if agreement is reached by 80 percent of selected reference group and at least 50 percent of the participants.

Organism identification was correctly performed by at least 80 percent of reference laboratories and greater than 50 percent of all laboratories and was thus, determined to be suitable for grading.

Grading

Maximum grade: 4

Reporting *Neisseria weaveri/* species was graded 4.

Reporting other organism was graded 0.

Table 1. Identification results

Reported	Total	Grade
Neisseria weaveri	28	4
Neisseria species, presumptive, refer	1	4
gram-negative bacilli/bacillus/rods, refer ± fastidious	11	4
Bâtonnets gram négatif, Faible possibilité de *Neisseria* sp (*elongata*, *weaveri*, *animaloris*, *zoodegmatis*), refer	1	4
Neisseria species	1	4
Neisseria species, not *gonorrhoeae* or *meningitidis*	2	4
Neisseria animaloris, presumptive, refer	1	4
Pseudomonas species, refer	1	0
Pseudomonas alcaligenes	3	0
no report	1	0
shipping delay, no report	1	ungraded
refer/sample not normally processed	3	ungraded
Total	**54**	

COMMENTS ON RESULTS

Participants performed well on this challenge. The majority of participants provided an acceptable response of N. weaveri, *Neisseria* sp., or gram negative bacilli with referral. Because this organism was from a superficial wound infection and the exact speciation of *Neisseria* was not considered critical from a management perspective, laboratories received a grade of 4 for reporting *Neisseria* to the genus level.

Several laboratories reported *Pseudomonas alcaligenes* or *Pseudomonas* sp., which was incorrect.

Differences were noted with respect to the identification methods used at the different laboratories (Table 2).

From these results, it becomes clear that laboratories using MALDI-TOF, no matter the platform, performed well in identification of this organism. Vitek 2, when used with the NH card, similarly performed well, though the number was much smaller. The Vitek 2 when used with the GNI card, however, did not perform well, and the other methods struggled to obtain an identification, though the API NH was able to identify the *Neisseria* genus.

ISOLATION AND IDENTIFICATION

Neisseria weaveri, formerly CDC M-5 group, was first named in 1993 in honor of Robert E. Weaver, for his substantial contributions to the characterization, identification, and classification of this species.[1]

Unlike the rest of the species of the *Neisseria* genus that are true cocci, *Neisseria elongata* and *N. weaveri* are medium to large, plump rods that appear in Gram-stained smears as pairs or short chains.[1–4] It has a tendency to grow in chains or longer rods in broth culture.

N. weaveri is aerobic, and grows well between 25 and 35°C; most strains grow at 42°C. Colonies are grey-white with an entire border, flat, slightly glistening, and smooth and variable in size. They are 1 to 2 mm in diameter after 24 hours of incubation at 35°C and 2 to 4 mm after 48 hours of incubation on sheep blood agar plate (SBAP). A zone of alpha-hemolysis is produced on SBAP in areas of heavy growth.[1]

The species is nonmotile, strongly oxidase and catalase positive, indole negative, and does not ferment carbohydrates. It reduces nitrite but not nitrate and has a weakly positive phenylalanine deaminase reaction from culture grown on SBAP. [1–3,5,6]

For further details on the biochemical characteristics of *N. weaveri* and differential diagnosis please see Andersen 1993 [1], Forsblom 2002 [2] and Cools 2007 [4].

Differential diagnosis is difficult when working with samples from dog bite wounds since many species in the normal canine oral flora have similar biochemical characteristics.

As observed in this challenge, commercial identification systems commonly used in the clinical laboratory frequently fail to identify *N. weaveri.* The organism is not listed in the database of MicroScan, Phoenix, VITEK 2 or API 20NE. Other identification systems including, RapID NH and RapID NF, may give unacceptable profiles.[2]

Following classic phenotypic tests reference laboratories may perform cellular fatty acid profiling or do 16S rRNA sequencing to confirm organism identification.

Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) is the preferred method of identification for all bacteria in the clinical laboratory and may be particularly useful for the identification of fastidious gram negative bacilli and other bacteria that are difficult to culture. [7]

ANTIMICROBIAL SUSCEPTIBILITY

Like several other organisms associated with dog bite wounds, *N. weaveri* strains are susceptible to penicillin, fluoroquinolones, and tetracycline.[8,9]

Antibiotic susceptibility testing has been performed by some groups using the BSAC (British Society for Antimicrobial Chemotherapy) method [10] using broth dilution and E-test. These tests showed the organism to be sensitive to penicillin, ciprofloxacin and gentamicin, but resistant to trimethoprim.[6] The chromogenic cephalosporin test can be used for detection of β-lactamase production. [6]

Currently, there is no a standardized method for susceptibility testing recommended by CLSI for this microorganism.

Table 2. Identification methods used by laboratories

ID Method	# of labs	# Correct Genus & Species	Correct Genus		Wrong Genus	No ID
			No sp.	Wrong sp.		
MALDI (Vitek MS or Bruker Biotyper)	28	28	0	0	0	0
Vitek 2 (GNI Card)	14	2	1	1	7	3
Vitek 2 (NH Card)	2	2	0	0	0	0
API 20 NE	3	0	0	0	1	2
API NH	4	0	0	3	0	1
BD Phoenix	3	0	0	2	0	1
MicroScan WalkAway	1	0	0	0	0	1

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N. weaveri is typically susceptible to penicillin. However, in the setting of a dog bite wound, empiric coverage for other common isolates including *Pasteurella* species, *Staphylococcus aureus* and anaerobes requires a broad-spectrum agent such as amoxicillin-clavulanate. Because of the resistance of certain bacteria first generation cephalosporins, cloxacillin, clindamycin and erythromycin are not considered adequate therapy for bite wounds.[9]

CLINICAL RELEVANCE

N. weaveri is a part of the normal canine oral flora (in 10 to 12% of oral flora samples). [1]

Clinical sources of *N. weaveri* are most often dog bite wounds,[5,8] although it has also been isolated from cat, or in one case tiger bite wounds.[11]

In a study involving 50 dog bite wounds, *Neisseria* species were isolated in 32% of the wounds; the most frequently isolated species was *N. weaveri* (14%), followed by *N. zoodegmatis* (10%), *N. animaloris* (6%), and *N. subflava* (2%).[12]

N. weaveri has been isolated from sites other than the wound site. A case of *N. weaveri* septicemia was reported in a 69-year-old man after a dog bite [8] and the organism was isolated from sputum of a 60-year-old man admitted to the hospital because of an acute exacerbation of his bronchiectasis.[6]

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