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CMPT Clinical Bacteriology Program

Innovation, Education, Quality Assessment, Continual Improvement

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Challenge M243-3

November 2024

Sputum: *Pasteurella multocida*

HISTORY

A simulated sputum sample collected from a 65 year old ICU patient with pneumonia was sent to category A laboratories.

Participants were expected to isolate and report *Pasteurella multocida* and perform susceptibility testing.

CMPT QA/QC/STATISTICS

All simulated sputum samples are produced at CMPT according to CMPT internal protocols. The sample contained a pure culture of *Pasteurella multocida*

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 14 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

Identification: 13/13 (100%) labs reported *Pasteurella multocida*

Susceptibility: 8/13 labs reported Ampicillin susceptible (S), 1 lab reported a comment, 4 labs did not report; 7/13 labs reported AmoC susceptible (S), 2 labs reported a comment, 4 labs did not report; 7/13 labs reported Ceftriaxone susceptible (S), 1 lab reported a comment, 5 labs did not report; 5/13 labs reported Levofloxacin or Moxifloxacin susceptible(S), 1 lab reported a comment, 7 labs did not report; 7/13 labs reported SXT susceptible (S), 1 lab reported SXT resistant, 1 lab reported a comment, 4 labs did not report

No consensus was reached for any of the antibiotics evaluated.

Participants

Identification: 43/51 (84%) of labs reported *Pasteurella multocida*. 1 lab reported *Pasteurella* species, refer; 1 lab reported gram negative bacillus refer; 1 reported *Haemophilus* species, refer (Table 1)

Susceptibility: 18/51 labs reported ampicillin S, 2 labs reported ampicillin R , 2 labs reported a comment, 20 labs did not report, 1 lab n/a no ID to report, 8 labs referred, not normally processed; 18/51 labs reported AmoC S, 2 labs reported AmoC R, 3 labs reported a comment, 17 labs did not report, 1 lab n/a no ID to report, 10 labs referred, not normally processed;

MAIN EDUCATIONAL POINTS from M243-3

1. Human isolates are mostly found in wound or soft tissue infections and are associated with bite wound infections from dogs and cats
2. Pasteurella multocida can cause osteomyelitis, bacteremia, endocarditis, meningitis, brain abscesses, ophthalmic infections, peritonitis, pneumonia, lung abscess, UTI, empyema and septicemia (cirrhosis of liver particular risk factor). Less frequent are colonization or infection of the respiratory tract
3. In the respiratory tract, colonization may eventually lead to sinusitis or bronchitis, as well as pneumonia and empyema

Grading

Maximum grade: 4

Reporting *Pasteurella multocida* or *Pasteurella* species, refer was graded 4.

Susceptibility results were ungraded.

Table 1. Identification results

Reported	Total	Grade
Pasteurella multocida	43	4
Pasteurella species, refer ± presumptive	2	4
gram negative bacillus, refer	1	3
Haemophilus species, refer	1	0
no report	1	0
sample not normally processed	2	ungraded
Total	**51**	

19/51 labs reported Cro S, 3 labs reported a comment, 19 labs did not report, 1 lab n/a no ID to report, 9 labs referred, not normally processed; 9/51 labs reported levofloxacin S, 6 labs reported moxifloxacin S, 1 lab reported moxifloxacin with MIC=35, 2 labs reported a comment, 22 labs did not report, 1 lab n/a, no ID to report, 10 labs referred, not normally processed; 24/51 labs reported SXT S, 1 lab reported SXT R, 2 labs reported a comment, 14 labs did not report, 1 lab n/a, no ID to report, 9 labs referred, not normally processed

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; no antimicrobial agent reached consensus therefore, susceptibility testing results could not be graded.

COMMENTS ON RESULTS

Identification

Overall, participants had no difficulty identifying this organism to the genus level, and most participants correctly identified the organism to the species level.

Susceptibility

No antimicrobial agent reached consensus therefore, susceptibility testing results could not be graded.

ISOLATION AND IDENTIFICATION

Pasteurella grows well on various commercial media, including chocolate and sheep-blood agar, however, it does not grow on MacConkey agar.

Colonies of *Pasteurella* species are usually grey and viscous, with a strong mucinous odour resembling *Haemophilus influenzae*. There is no hemolysis on blood agar.[3]

Pasteurella species are spherical, ovoid, or rod-shaped cells 0.3-1.0µm in diameter and 1.0-2.0µm in length. Cells are gram negative and can be seen in pairs or short chains. All species are non-motile, are facultatively anaerobic, and most strains are positive for catalase, oxidase, indole, acid production from sucrose, and ornithine decarboxylase; nitrates are reduced to nitrites by almost all species.[3-5] Biochemically, *P. multocida* and *P. canis* are very similar, with acid production from mannitol being the main difference between them (*P. multocida* positive, *P. canis* negative).[1]

MALDI-TOF MS systems have been shown to be able to correctly identify *Pasteurella* isolates to the species level in 89% of cases.[1] In a recent study comparing the performance of two MALDI - TOF MS systems, the Vitek2 (with a companion mannitol set up

Table 2A - E. Susceptibility testing results

2A Ampicillin	**Total**	**Grade**
S	18	ungraded
Comment*	2	ungraded
R	2	ungraded
no report	20	ungraded
n/a, no ID report	1	ungraded
refer/sample not normally processed	8	ungraded
Total	**51**	
2B Amoxacillin/Clavulanic Acid	**Total**	**Grade**
S	18	ungraded
Comment*	3	ungraded
R	2	ungraded
no report	17	ungraded
n/a, no ID report	1	ungraded
refer/sample not normally processed	10	ungraded
Total	**51**	
2C Ceftriaxone	**Total**	**Grade**
S	19	ungraded
Comment*	3	ungraded
n/a, no ID report	1	ungraded
no report	19	ungraded
refer/sample not normally processed	9	ungraded
Total	**51**	
2D Levofloxacin/Moxifloxacin	**Total**	**Grade**
Lev S	9	ungraded
Mox S	6	ungraded
Comment*	2	ungraded
Mox MIC = 35mm	1	ungraded
n/a, no ID report	1	ungraded
no report	22	ungraded
refer/sample not normally processed	10	ungraded
Total	**51**	
2E SXT	**Total**	**Grade**
S	24	ungraded
Comment*	2	ungraded
R	1	ungraded
no report	14	ungraded
n/a, no ID report	1	ungraded
refer/sample not normally processed	9	ungraded
Total	**51**	

*Comment - Our lab does not perform susceptibility testing on this source if β-lactamase negative; instead of routine susceptibility testing, a predictability comment would be added: "Pasteurella species are usually susceptible to penicillin, ampicillin, amoxicillin/clavulanate, TMP-SMX , quinolones and tetracyclines. They are resistant to first generation cephalosporins (cephalexin)."

manually), and traditional methods against the sodA-based gene sequencing used as the gold standard, both MALDI-TOF MS systems performed equally well when identifying *P. multocida* and *P. canis*, and had the advantage of speed, producing results within 10 minutes. In the same study, the Vitek 2 was able to correctly identify 50% of all isolates tested, and only 47.6% of the *P. multocida* isolates tested.

Traditional biochemical methods tested butcation were found to perform very well, correctly identifying 95% of all isolates tested, but required greater turnaround time due to the need for overnight (or longer) incubation times.[6]

ANTIMICROBIAL SUSCEPTIBILITY

According to the CLSI guidelines, there is no need for routine testing of isolates from bite wounds as empiric therapy is generally effective for *P. multocida*.

Isolates from normally sterile sources (blood cultures, deep tissue, implanted prosthetic devices) and respiratory specimens may warrant testing, including β-lactamase using a chromo-genic cephalosporin test, especially in immuno-deficient patients.[7]

Although *Pasteurella* species are normally sensitive to penicillin, some β-lactamase-producing strains have been recovered. The organism also is sensitive to ampicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, tetracyclines, second- and third-generation cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole (SXT).[5]

CLINICAL RELEVANCE

Pasteurella species, commonly found as commensals in the oral flora of dogs, cats, other mammals, and birds, can become significant pathogens, particularly following animal bites. These bacteria are associated with a variety of infections, including bite wound infections, osteomyelitis, bacteremia, endocarditis, meningitis, brain abscesses, ophthalmic infections, peritonitis, pneumonia, lung abscesses, urinary tract infections, empyema, and septicemia. Individuals with liver cirrhosis are particularly at risk for severe infections.

While *Pasteurella multocida* is not a frequent cause of pneumonia, it can contribute to respiratory infections, especially in individuals with underlying health conditions or compromised immune systems. In the respiratory tract, colonization by this organism may lead to complications such as sinusitis, bronchitis, pneumonia, and empyema, underscoring the importance of recognizing its potential clinical implications.

REFERENCES

1. Zbinden R, von Graevenitz A. *Aggregatibacter, Capnocytophaga, Eikenella, Kingella, Pasteurella*, and Other Fastidious or Rarely Encountered Gram-Negative Rods. In: Carrol K. C. et. al., ed. Manual of Clinical Microbiology. Vol 1. 12th ed. ASM; 2019:656.

2. Rasmussen D, Landon A, Powell J, Brown GR. Evaluating and treating mammalian bites. JAAPA Off J Am Acad Physician Assist. 2017;30(3):32-36.

3. Standards Unit Microbiology Services Public Health England. Identification of Pasteurella species and Morphologically Similar Organisms. UK Stand Microbiol Investig. 2015; ID13 Issue 3 (Journal Article).

4. Oehler RL, Velez AP, Mizrachi M, Lamarche J, Gompf S. Bite-related and septic syndromes caused by cats and dogs. Lancet Infect Dis. 2009;9(7):439-447. doi:10.1016/S1473-3099(09)70110-0

5. Kristinsson G. Pasteurella multocida infections. Pediatr Rev Am Acad Pediatr. 2007;28(12):472-473. doi:10.1542/pir.28-12-472

6. Zangenah S, Güleryüz G, Boräng S, Ullberg M, Bergman P, Özenci V. Identification of clinical Pasteurella isolates by MALDI-TOF — a comparison with VITEK 2 and conventional microbiological methods. Diagn Microbiol Infect Dis. 2013;77(2):96-98. doi:10.1016/j.diagmicrobio.2013.06.024

7. Clinical Laboratory Standards Institute. Methods for Antimicrobial Dilution and Disk susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline- Third Edition. 2016;(Generic):M45-A3 CLSI Wayne, PA.

8. Dendle C, Looke D. Review article: Animal bites: an update for management with a focus on infections. Emerg Med Australas EMA. 2008;20(6):458-467.

9. Christenson ES, Ahmed HM, Durand CM. Pasteurella multocida infection in solid organ transplantation. Lancet Infect Dis. 2015;15(2):235-240. doi:10.1016/S1473-3099(14)70895-3

10. Ellis R, Ellis C. Dog and cat bites. Am Fam Physician. 2014;90(4):239-243.