clinical microbiology proficiency testing

CMPT Clinical Microbiology Program

Innovation, Education, Quality Assessment, Continual Improvement

Challenge M091-3

May 2009

Diabetic Foot Wound Swab: *Pseudomonas aeruginosa*

The sample has a gram smear companion (G091)

HISTORY

The sample was sent as a wound swab from a 60 year old diabetic patient with an inflamed foot ulcer. The sample was sent only to category A and B laboratories.

CMPT QA

The culture yielded 4+ pure growth of *P.aeruginosa* that was viable for 17 days.

SURVEY RESULTS

Identification results (Table 2):

The sample was processed by 94% of the participating laboratories (103/109). Of the participating laboratories, 98% correctly identified *Pseudomonas aeruginosa* in the sample and received an acceptable grade of 4. All 15 of the reference labs reported *P.aeruginosa*.

The current results are consistent with previous PT challenge results (M074-3: 96%, M064-2: 97%, M054-2: 97%).

One laboratory reported *Pseudomonas* species, refer and received a grade of 4.

One laboratory reported "Wound" and received a grade of zero.

Susceptibility Testing results (Table 3):

Susceptibility testing was performed by 94% of the laboratories. The remainder of the labs referred the sample for testing. Consensus was achieved among the 15 reference labs regarding susceptibility results and was considered suitable for grading. See Table 1.

Antimicrobial agents were divided into four groups for grading:

a. Piperacillin and or Piperacillin-tazobactam and or Ticarcillin, Ticarcillin-clavulanic acid

b. Ceftazidime

c. Gentamicin and or Tobramycin

d. Ciprofloxacin

86% of the laboratories (83% of A labs and 85% of B labs) tested and reported susceptibility for group a, b, c and d agents.

3% (3% of A labs and 4% of B labs) tested and reported groups a, b and c agents.

6% (6% of A labs and 4% of B labs) tested and reported groups b, c and d agents.

4% (3% of A labs and 8% of B labs) tested and reported groups a, c and d agents.

1% (1A lab) tested and reported groups c and d agents.

2% (8% of B labs) tested and reported groups a and c agents.

1% (1A lab) tested and reported only group c (Ciprofloxacin) agent.

All antibiotics tested were reported as susceptible by all laboratories.

Laboratories were awarded 4 points for each antibiotic tested.

Table -1 Susceptibility results obtained by CMPT and reference laboratories.

Antibiotic(s)	CMPT	Reference labs
Piperacillin/Piperacillin-Tazobactam/Ticarcillin/Ticarcillin-clavulanic acid	S	15/15 labs reported S
Cetazidime	S	15/15 labs reported S
Gentamicin/Tobramycin	S	15/15 labs reported S
Ciprofloxacin	S	13/15 labs reported S, 2 labs did not report.

Grading

Maximum grade = 20

Grading was based on identification of the organism (maximum 4), and antimicrobial susceptibility testing (maximum 16, 4 per each antibiotic tested for the groups in table 1).

98% of the laboratories correctly identified *P. aeruginosa* and received a grade of 4.

All laboratories reported the applicable antibiotics as Susceptible and received the maximum points.

83% of the labs reported the 4 antibiotics recommended, receiving a grade of 16.

13% of the labs reported 3 out of 4 antibiotics and received a grade of 12.

2% of the labs reported two antibiotics and received a grade of 8.

One laboratory only reported Ciprofloxacin and received a grade of 4

Table-2: Identification Results for M091-*3 Pseudomonas aeruginosa* challenge

	Laboratory category			
Reported	**A**	**B**	**%**	**grade**
Pseudomonas aeruginosa	75	25	97	4
Pseudomonas aeruginosa 2 strains	0	1	1	4
Pseudomonas species, refer	0	1	1	4
wound	1	0	1	0
snnp, refer	2	4	n/a	ungraded
Total	**78**	**31**	**100**	

snnp: sample not normally processed

The Committee recommends that all Proficiency Testing samples should be processed as routine samples even when there is a staff shortage or high workload.

SAMPLE PROCESSING

Specimen acquisition techniques for the diagnosis of diabetic foot infections include the wound swab, curettage, tissue biopsy and fine-needle aspiration.

Punch biopsy is usually recognized as the reference test[1].

Soft tissue infections, such as diabetic foot infections, are usually defined as the presence of $>10^5$ colony forming units (CFUs) per gram of tissue[1].

Swabs should be taken after the debridement and the cleaning of the wound. If the swab method is the only method available, quantitative and semi-quantitative techniques should be performed to help differentiate between colonization and infection.

Quantitative techniques for analyzing specimens obtained from wound swabs involve identifying the type and counting the numbers of microorganisms present.

Semi-quantitative techniques entail classifying a level of bacterial growth by observing growth on four quadrants of an agar plate where each quadrant has been streaked in sequence using a sterile loop for each quadrant, thus making dilutions of the original streak onto each sequential quadrant. The greater the quantity of bacteria on the original swab, the more quadrants will display bacterial growth [1].

A study by Ratliff et al.[3] showed that bacterial growth in 3 or more quadrants resulted in a sensitivity of 79% and specificity of 90% .

Pure or predominant bacteria seen on Gram smear and isolated from culture are likely significant pathogens[2].

The processing of swab samples for anaerobes is controversial. Anaerobic culture is not recommended if the sample was obtained with a swab. The recommended specimens for anaerobic culture are tissue biopsy and needle aspirated samples.

ISOLATION AND IDENTIFICATION

Culture isolation

Pseudomonas species have very simple nutritional requirements. Most *P. aeruginosa* iso-

CLSI guidelines:

"P. aeruginosa may develop resistance during prolonged therapy with all antibiotics. Therefore, isolates that are susceptible may become resistant within 3-4 days after initiation of therapy. Testing of repeat isolates may be warranted."

Penicillins and β-lactam/β-lactam combination: "The susceptible category of these drugs implies the need of high-dose therapy for serious infections caused by *P. aeruginosa*. For these infections, monotherapy has been associated with clinical failure. The addition of a second antimicrobial agent (eg. Fluorouinolone, aminoglycoside) with in vitro activity against *P. aeruginosa* should be considered[9]."

Many patients with diabetic foot infections are treated on an outpatient basis. Treatment with an oral agent is helpful – ciprofloxacn is one of the few oral antimicrobials effetive against *P. aeruginosa*.

Table-2: Susceptibility Results for M091-*3 Pseudomonas aeruginosa* challenge

Pip/Pip-Ta	CAZ	Gen/Tob	Cip	No of labs	% Total	% A labs	% B labs	Grade
S	S	S	S	86	83	83	85	**16**
S	S	S	NR	3	3	4	0	**12**
NR	S	S	S	6	6	6	4	**12**
S	NR	S	S	4	4	3	8	**12**
NR	NR	S	S	1	1	1	0	**8**
S	NR	S	NR	2	2	0	8	**8**
NR	NR	NR	S	1	1	0	4	**4**
snnp	snnp	snnp	snnp	6	n/a	n/a	n/a	

Pip/Pip-Ta: Piperacillin and or Piperacillin-Tazobactam or Ticarcillin and or Ticarcillin-Clavulanic acid; **CAZ**: ceftazidime; **Gen/Tob**: Gentamicin and or Tobramycin; **Cip**: ciprofloxacin **NR**: not reported **S**: susceptible **R**: resistant

lates are easily recognizable on primary isolation media. *P. aeruginosa* produce a diffusible pigment and a grape-like odor.

Colonies are usually flat with a metallic sheen. Other morphologies exist, including mucoid colonies, which are typically isolated from cystic fibrosis patients.

Identification methods

Pseudomonas spp. are aerobic, non-spore-forming gram negative rods which are straight or slightly curved. They are strictly aerobic and usually motile. Most *Pseudomonas* species are oxidase positive (except *Pseudomonas luteola* and *Pseudomonas oryzihabitants*). *Pseudomonas* spp are catalase positive[8].

P. aeruginosa is the only clinically relevant fluorescent pseudomonads (pyoverdin positive) able to grow at 42°C. In addition, *P.aeruginosa* is arginine positive[8].

ANTIMICROBIAL SUSCEPTIBILITY

According to the CLSI Standards, the antibiotics that should be tested and reported routinely (Group A) for *P. aeruginosa* isolates are: ceftazidime, gentamicin/tobramycin and piperacillin[9].

Group B agents (amikacin, aztreonam, cefepime, ciprofloxacin/levofloxacin, imipenem/meropenem, piperacillin-tazobactam/ticarcillin) may be used for primary testing. However, CLSI group B agents are reported only selectively depending on: the results of the agents tested in group A, specimen source, allergy to the primary drugs, failure to respond to an agent in group A or for infection control purposes[9].

In a multicenter study by Citron et al.,[5] *P. aeruginosa* strains isolated from diabetic foot infections were largely susceptible to imipenem, piperacillin-tazobactam, ceftazidime, aminoglycosides, and ciprofloxacin.

CLINICAL RELEVANCE

Foot infections occur in up to 15% of diabetic patients and account for 20% of all hospitalizations of diabetic patients [7]. Predisposing factors include peripheral neuropathy, vascular disease local trauma and pressure.

Staphylococcus aureus is the most frequently isolated and virulent pathogen in diabetic foot infections. Streptococci (various groups of β-hemolytic, and others) are also important pathogens. Gram-negative bacilli (mainly *Enterobacteriaceae*, occasionally P*seudomonas aeruginosa*) are usually isolated from patients with chronic or previously treated infections; they are often, but not always, true pathogens[5].

Mixtures of anaerobic bacteria, including gram-negative anaerobic bacilli may be present in up to 40% of infections [4]

P. aeruginosa is hydrophilic and can be recovered from moist environments even antiseptic solutions.

P. aeruginosa rarely colonize healthy humans. However, individuals receiving frequent courses of antibiotic therapy are at risk of gastrointestinal colonization.

Normal skin does not support *P. aeruginosa* colonization, but burned skin or wounds are an attractive site for this bacterium[8] since the physical barrier of the intact dermis has been broken.

TREATMENT

According to the IDSA GUIDELINES for the diagnosis and treatment of diabetic foot infections,[10] an empirical antibiotic regimen should be selected on the basis of the severity of the infection and the likely etiologic agent(s). It is important to remember that anaerobes may be involved in these infections.

Broad-spectrum empirical therapy is not routinely required but is indicated for severe infections, pending culture results and antibiotic susceptibility data.

Definitive therapy should be based on the culture results, the susceptibility data and the clinical response to the empirical regimen.

Mesaros et al., [11] recommend that any suspicion of pseudomonal infection should be confirmed bacteriologically because reliance on empirical treatment entirely is no longer acceptable.

Therapy should be initiated as soon as possible because early therapy is associated with a better outcome. Therapy usually includes an anti-pseudomonal β-lactam associated with either an aminoglycoside or a fluoroquinolone (preferably ciprofloxacin).

Treatment should be fine tuned once laboratory data are available to avoid developing resistant strains.

INTERESTING:

In a 2008 study of chronic wounds, Bjarnsholt et al[6] investigated the similarities between *P. aeruginosa* infections in cystic fibrosis and chronic wound patients. They proposed that the lack of proper wound healing is, in part, caused by the inefficient eradication of infectious and opportunistic pathogens.

They hypothesize that the infections are kept chronic by the bacterial burden, mainly *P. aeruginosa*.

The authors propose that bacterial persistence including the extreme tolerance to antibiotics are caused by the ability of these microorganisms to form biofilms and excrete damaging virulence factors that contribute to form an efficient PMN shield.

If broad spectrum antibiotics are required to include an anaerobic component, addition of a beta-lactam/beta-lactamase inhibitor combination, such as piperacillin-tazobactam, may be useful[4]

REFERENCES

1. O'Meara S, Nelson EA, Golder S, Dalton JE, Craig D, Iglesias C. 2006 Systematic review of methods to diagnose infection in foot ulcers in diabetes. Diabet Med Apr;23 (4):341-7.
2. Lipsky BA. A report from the international consensus on diagnosing and treating the infected diabetic foot. Diabetes Metab Res Rev 2004; 20(Suppl. 1): S68-S77
3. Ratliff C, Rodeheaver G. 2002 Correlation of semi-quantitative swab cultures to quantitative swab cultures from chronic wounds. Wounds. December 2002 Link
4. Citron DM, Goldstein EJC, Merriam CV, Lipsky BA, Abramson MA. Bacteriology of moderate to severe diabetic foot infections and in vitro activity of antimicrobial agents. *J Clin Microbiol* 2007; 45: 2051-2056.
5. Lipsky BA. New developments in diagnosing and treating diabetic foot infections. Diabetes Metab Res Rev. 2008 May-Jun;24 Suppl 1:S66-71
6. Bjarnsholt T, Kirketerp-Møller K, Jensen PØ, Madsen KG, Phipps R, Krogfelt K, Høiby N, Givskov M. Why chronic wounds will not heal: a novel hypothesis. Wound Repair Regen. 2008 Jan-Feb;16(1):2-10.
7. L.A. Lavery, D.G. Armstrong, R.P. Wunderlich, M.J. Mohler, C.S. Wendel and B.A. Lipsky, Risk factors for foot infections in individuals with diabetes, Diabetes Care 29 (2006), pp. 1288–1293
8. Murray, P.R., Baron, E. J., Jorgensen, J.J.,Landry ML,. and Pfaller, M.A., . Manual of Clinical Microbiology, 9th ed. ASM Press: Washington, DC, 2007.
9. CLSI Performance Standards for Antimicrobial Susceptibility Testing; 19th Informational Supplement. 2009. M100-S19 Vol. 29 No.3
10. Lipsky BA, Berendt AR, Gunner Deery H, Embil JM, Joseph WS, Karchmer AW, LeFrock JL, Lew DP, Mader JT, Norden C, Tan JS. IDSA guidelines. Diagnosis and Treatment of Diabetic Foot Infections Clinical Infectious Diseases 2004;39:885–910
11. Mesaros N, Nordmann P, Plésiat P, Roussel-Delvallez M, Van Eldere J, Glupczynski Y, Van Laethem Y, Jacobs F, Lebecque P, Malfroot A, Tulkens PM, Van Bambeke F. Pseudomonas aeruginosa: resistance and therapeutic options at the turn of the new millennium. Clin Microbiol Infect. 2007 Jun;13(6):560-78
12. CMPT Critique M054-2 Ear (malignant otitis externa): *Pseudomonas aeruginosa*
13. CMPT Critique M064-2 Ear (otitis externa): Pseudomonas aeruginosa
14. CMPT Critique M074-3 Foot (gangrenous tissue): Pseudomonas aeruginosa, Clostridium perfringens