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| **SOP Number:** | M.3.20 | **Effective Date:** | 03/2013 |
| **Department:** | Microbiology | **Revision Date:** | 02/21/13 |
| **Policy (P), Procedure (PR)or Both (P/P):** | P/P | **Version:** | 1 |

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| Applicable Standards |  | Version History |
| Standard | Organization  |  | Version | Effective Date | Retired Date |
| MIC.13250 | CAP |  | 1 | 02/2013 |  |
| MIC.15000 | CAP |  |  |  |  |
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| Related Documents |  |  |  |  |
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| Review History (Up to the Last 15 Occurrences) |
| Date | Version | Revision Type | Review By/Initials & Date |
| 02/2013 | 1 | New Policy/Procedure | J. Lewis 02/21/2013 |
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**PRINCIPLE:** *Otitis media* is an infection of the middle ear and accounts for more than 30 million office visits per year. More than 80% of children have one or more episodes of otitis media by age 6 years, with the highest number of cases between 2 and 6 years of age. Hearing loss and deficits in learning are a few of the complications. The most common agents of otitis media are *Streptococcus pneumoniae,Haemophilus influenzae, Moraxella catarrhalis,* and *Alloiococcus otitis* although *Streptococcus pyogenes* is found on a seasonal basis. (*Note:* Although the name *Alloiococcus otitidis* is in common usage, that name has not been officially adopted; *Alloiococcus otitis* is the official name. Amoxicillin and amoxicillin-clavulanate are the drugs of choice for initial treatment . Although *S. pneumoniae* organisms are becoming increasingly more resistant, the concentrations of amoxicillin that can be achieved in the middle ear fluid are sufficient to eliminate all but the most resistant strains. Treatment generally resolves the infection, but treatment failures occur and surgical intervention can be necessary.

*Tympanocentesis and culture of the middle ear fluid* constitute a valuable tool for definitive diagnosis, to guide therapy, to evaluate treatment failures, and for research studies to determine the efficacy of antimicrobials against the most common agents. However, the diagnosis is usually made on clinical grounds, because of the invasive nature of tympanocentesis. Culture is usually reserved for persistent infections. Historically pediatricians cultured the nasopharynx to predict the pathogens in the middle ear fluid. This practice is no longer recommended, since the presence of pathogens in the nasopharynx does not predict the pathogens present in the middle ear. However, lack of isolation of any pathogen has a 96% negative predictive value for lack of a pathogen in the middle ear fluid.

*Otitis externa* is an infection of the external auditory canal. Unique problems occur with this infection because of the narrow and tortuous nature of the canal and its tendency to trap foreign objects, wax, and water. Infections are classified as acute and chronic. Acute infections are often referred to as “swimmer’s ear.” *Pseudomonas aeruginosa* is a frequent cause of freshwater otitis, and *Vibrio alginolyticus* is a cause in oceanic swimmers, although other aerobic bacteria can be involved. Localized infections with *Staphylococcus aureus* or *S. pyogenes* can also occur. Contaminating skin bacterial microbiota (corynebacteria and staphylococci) can be present, which are not significant. More invasive infections are caused by extension of bacteria into the adjacent soft tissues and bone, with the formation of a cholesteatoma. Chronic otitis is usually caused by bacterial infection, and although *P. aeruginosa* may be predominant, a variety of anaerobes may also be present. Tissue and bone samples from the inner ear should also be cultured for fungi, *Nocardia,* and mycobacteria, which may also be etiologic agents of chronic infection. Underlying diseases such as tertiary syphilis may also cause chronic otitis, but this presentation is rare in the developed world

**Specimen collection**

1. *External ear*
	1. Insert sterile swab into ear canal until resistance is met.
	2. Rotate swab and allow fluid to collect on swab.
2. *Tympanocentesis fluid*

NOTE: Because of the invasive nature of the collection process, these specimens are usually submitted primarily to diagnose middle ear infections only if previous therapy has failed.

1. Clean the external canal with mild detergent.
2. Using a syringe aspiration technique, the physician will obtain the fluid from the ear drum.
3. Send the specimen in a sterile container or in the syringe capped with a Luer-Lok and with the needle removed.
4. If the eardrum is ruptured, collect exudate by inserting a sterile swab through an auditory speculum.

**Specimen transport**

1. Submit swabs in tube of transport medium.
2. Submit aspirates in a sterile container or in the original syringe capped with a Luer-Lok to prevent leakage.
3. Label specimens with demographic information, date and time of collection, and site of collection.
4. List the diagnosis of otitis media, chronic otitis, or otitis externa.

**PROCEDURE:**

**Specimen Inoculation**

1. Inoculate specimen to BAP, CHOC, and MAC.
2. Firmly roll swab over one-sixth (no more) of the agar surface, or aspirate 3or 4 drops of fluid onto agar. Streak carefully for isolation in four quadrants to minimize overgrowth by other microorganisms.
3. For cultures of invasively collected ear fluid samples, incubation may be extended to 4 days, if results are negative. Anaerobic cultures may be indicated.
4. Perform a Gram stain from the swab or fluid if requested.

**Culture examination**

***External otitis***

1. Observe plates at 24, 48 and 72h for growth of enteric gram negative rods, pseudomonads, vibrios, streptococci, coryneforms, and *S. aureus.* Since only one pathogen is generally responsible for otitis externa, mixed cultures of gram-negative rods should be minimally identified.
2. For gram-negative rods, perform spot oxidase and indole tests to avoid misidentifications.
	1. *V. alginolyticus* is an oxidase- and indole-positive, gram-negative rod that grows well on MAC. It will grow on MH with 4% salt. As applicable, place a colistin disk on MH. It is further identified by most kit systems, although the reactions are improved if the inoculums are made in saline rather than water.
	2. *P. aeruginosa* is oxidase positive and indole negative and often has a characteristic odor or blue-green or brown pigment, for definitive identification.
	3. Other gram-negative rods are less common and are usually identified by commercial kits.
	4. Perform antimicrobial susceptibility testing (AST) on the predominant microorganism. Evaluate swarming *Proteus* organisms on MAC, where they generally do not swarm, to be sure they are the predominant microorganism before performing AST; they can be an important pathogen from a patient with diabetes mellitus.
3. Examine cultures for predominant gram-positive cocci. Generally *S. aureus* and *S. pyogenes* are the most common gram-positive cocci involved in otitis externa.
4. Identify *S. pyogenes* and other beta-hemolytic streptococci.
5. Identify and perform AST on *S. aureus*
6. Identify yeasts and molds, if present. *Aspergillus* and *Candida albicans* have been implicated in chronic infections.
7. Since resident cutaneous microbiota (coagulase-negative staphylococci and coryneforms) are normal in the external ear canal, they should not be further evaluated.

***Otitis media***

1. Observe plates at 24, 48 and 72h for growth. Pursue all organisms present, since the specimen is collected by an invasive procedure and any microorganism can be considered the agent of disease.
2. Fastidious gram-negative rods and diplococcic
	1. Identify *H. influenzae* and *M. catarrhalis* and perform beta-lactamase test on *H. influenzae.* Since more than 90% of *M. catarrhalis* organisms are beta-lactamase positive, testing is not helpful to treatment.
	2. *Bordetella trematum* is an oxidase-negative, catalase-positive, gram negative rod that has been implicated in ear infections. It is motile, frequently reduces nitrate, and may or may not grow on MAC.
3. Observe for growth of gram-positive cocci. Perform rapid identification tests to identify the following.
4. *S. pyogenes* and other beta-hemolytic streptococci. No susceptibilility is needed as “Drug of Choice is Penicillin”
5. *S. pneumoniae - Perform AST using standardized methods (reference 5 and section per laboratory protocol and physician policy.*
6. Normal skin microbiota (coagulase-negative staphylococci and corynebacteria) are not generally identified to the species level unless they are the only predominant species in the culture and are present in large numbers.
	1. Identify *Turicella otitidis,* a long coryneform rod implicated in otitis media. It is catalase positive, asaccharolytic, and CAMP test positive.
7. Examine for *Nocardia* in chronic infections.
8. Examine, on request, for *A. otitis* from middle ear fluid. Prepare ***BHI agar with 5% rabbit blood*** and inoculate with specimen. Incubate plates for 5 days; increased CO2 is not required. A few strains have been reported to grow on sheep blood agar, but it is not optimal.
	1. *A. otitis* is slow growing and produces pinpoint colonies that are moist and slightly yellow. No hemolysis is observed. Eventually the colonies adhere to the agar.
	2. No growth is seen on CHOC.
	3. On Gram stain, *A. otitis* organisms are gram positive cocci in clusters and tetrads without chains and cannot be distinguished from staphylococci.
	4. Perform the following tests to confirm the identification. Note the expected reactions.
* Catalase negative or weak
* Pyrrolidonyl-b-naphthylamide (PYR) positive
* Vancomycin susceptible

**REPORTING CULTURE RESULTS**

1. Negative results
2. Report preliminary and final results as “No growth.”
3. Indicate the number of days the culture was incubated.
4. If bacteria were seen on smear but did not grow on culture, extend the incubation and make a notation on the report to indicate the discrepancy.
5. Positive reporting
6. Indicate the presence of skin microbiota, without identification.
7. If the culture is mixed but with no predominating pathogen, indicate the genera and do not report further: e.g., “Mixed microbiota present, consisting of (#) morphologies of (description). Please contact laboratory if further testing is clinically indicated.”
8. Report all pathogens and susceptibility tests performed, using preliminary reports as indicated.
9. For *Vibrio,* report that the organism is resistant to colistin, if there was no zone around the disk, since ear drops often contain this antimicrobial if colistin disk testing was performed.

**PROCEDURE INTERPRETATION**

1. A positive external ear culture with a predominant gram-negative rod, betahemolytic streptococci, or *S. aureus* generally indicates infection with that agent.
2. A positive middle ear culture with *S. pneumoniae, H. influenzae, M. catarrhalis,* and *A. otitis* generally indicates infection with that organism.
3. A negative culture cannot rule out otitis media. In fact, in chronic infections, a pathogen is often not isolated.
4. Controversy regarding the need for treatment of otitis media has been found in recent literature, but most physicians agree on its benefits. *A. otitis* is resistant to sulfamethoxazole-trimethoprim and often to erythromycin, but it does not have a beta-lactamase.

**LIMITATIONS**

1. Methods that employ PCR for the detection of pathogens responsible for otitis media are more sensitive than culture techniques and increase the rate of detection of a pathogen to 75%.
2. False-negative cultures can result from overgrowth of the culture with normal cutaneous microbiota.
3. False-positive results can be caused by overinterpretation of the culture results.
4. *T. otitidis,* a coryneform rod, has been infrequently isolated from ear fluid and may be a cause of otitis media.
5. *A. otitis* is difficult to culture and may not be detected.

**REFERENCE:**

* + - 1. Clinical Microbiology Procedures Handbook. 3.11.5 Otitis Cultures. *March 2007.*