Bacterial Respiratory Infections: A Review of the Problems of Etiology and Antimicrobial Susceptibility Testing

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ABSTRACT

The etiologic relationships of bacteria to infections of the respiratory tract are reviewed, as well as the antimicrobial susceptibility tests that can be used for each microorganism or groups of microorganisms. The respiratory infections which are discussed are those associated with the pleura and lungs, bronchi, trachea, larynx, paranasal sinuses and middle ear.

Introduction

Statistics on respiratory infections are probably more incomplete than for any other class of infection; however, a close look at the figures that are available, such as the Morbidity and Mortality Weekly Report compiled at the Center for Disease Control, reveals the degree to which these infections occur in this country. For example, in the compilation of optionally reported acute diseases for 1971, almost 500,000 cases of respiratory infections, streptococcal sore throats, and scarlet fever were reported. Since this report was not from all of the states in the country, and since most cases of respiratory disease are never reported, the number of cases per year in this country alone can be assumed to be in the millions.

The etiologic agents involved with respiratory disease include viruses, mycoplasma, rickettsia, protozoa, and bacteria. Viruses and mycoplasma are probably the major causes of these respiratory infections, although it is often very difficult to define the etiologic agent in a specific episode of this type of infection. In this review, only bacteria will be considered. Their etiologic relationships to infections of the respiratory tract will be reviewed first, and the antimicrobial susceptibility tests that can be used for each microorganism or groups of microorganisms will be discussed second. Respiratory infections will be considered as those associated with the middle ear, paranasal sinuses, throat, larynx, trachea, bronchi, pleura and lungs.
and the microbiologist must make.\textsuperscript{5,12,15,19,20} Although the efficacy of antimicrobial agents used for certain respiratory infections, such as penicillin to treat pneumococcal pneumonia, is dramatic, it is often very difficult to identify the responsible organism. In discussing respiratory disease as the clinician usually sees it, Lambert\textsuperscript{19} said “... tidy outlines disappear and the picture is full of confusion and contradiction.” Based upon careful history and examination, the clinician can usually classify a respiratory illness into one of several syndromes. These syndromes usually have an anatomical-pathological label such as otitis media, sinusitis, nasopharyngitis, and tonsillopharyngitis.\textsuperscript{19} Whether or not antimicrobial treatment should be started before results of bacterial cultures are known depends upon the individual patient and his disease, but this decision should be based upon sound clinical judgment. In any case, specimens for bacteriological analysis should be obtained first, and treatment should be adjusted according to subsequent bacteriology reports. Of course, these bacteriology reports should be interpreted with caution. Many of the bacteria that must be considered as potential pathogens also occur as part of the flora of subjects who do not have respiratory disease. Although certain bacteria have been associated with respiratory disease since the beginning of the study of bacteriology, it can be said today that almost any species can be part of the normal respiratory flora or under certain conditions can be the etiologic agent of a respiratory infection.

Therefore, it is obvious that care should be taken in the collection of specimens for microbiological study. For example, when swabs are taken from persons with suspected nasopharyngitis or tonsillitis, it is essential that the swab not be contaminated with organisms from the mouth. Although sputum samples will always be contaminated to some degree with oral microorganisms as they pass through the mouth, the specimens should be from the lower respiratory tract and should not contain any of the patient's saliva.

Some laboratories have attempted to retrieve more useful information from the study of sputum samples by quantitation of the microbiological agents present.\textsuperscript{1,7,17,22,23,24,28,31,32,35,45} In these studies, the sputum specimen is usually liquefied with a mucolytic agent and then completely homogenized. The mucolytic agents used have been pancreatin,\textsuperscript{7,28} sodium ethylhexylsulfate plus potassium iodide,\textsuperscript{17} alpha amylase\textsuperscript{1} and N-acetyl-cysteine.\textsuperscript{11,28,32,45} The latter has been used most often. After liquefaction, the specimen is homogenized and dilutions of the homogenate are inoculated onto appropriate media.

This procedure permits not only a qualitative report of the kinds of microorganisms present but also the approximate numbers of each per unit volume of specimens. According to the aforementioned authors, this information is valuable in establishing the identity of the etiologic agent, monitoring the efficacy of antimicrobial therapy, determining when therapy should be modified or stopped and recognizing dual infections and superinfections. Some of these studies have also shown that there is little correlation between the results obtained by the quantitation technique and those obtained by the conventional techniques. Kilbourn et al\textsuperscript{17} also compared quantitative results for the study of sputum and saliva, and, as expected, found large differences. Balows et al\textsuperscript{4} Munroe et al\textsuperscript{28} and Gerlach\textsuperscript{11} point out the necessity of using fresh specimens. Balows et al\textsuperscript{1} were unable to recover pneumococci from sputum after it was refrigerated overnight, but they readily isolated them when fresh specimens were cultured. Gerlach\textsuperscript{11} has also studied the effects of overnight refrigeration on sputum,
and reports the loss of pneumococci and *Haemophilus influenzae* and a sharp diminution in the numbers of group A streptococci.

Some of these groups have used different interpretations of the data obtained by the quantitative technique. Louria has reported that in staphylococcal pneumonia the patient starts out with about 10^8 staphylococci per ml of sputum, and at the end of 7 to 14 days of therapy, the patient will be afebrile and clinically well but will still have about 10^3 staphylococci per ml of sputum. If therapy is stopped at this point, the organisms will persist in low numbers or eventually disappear. In their studies, Munroe et al reported that the numbers of a particular pathogen prior to therapy was 10^7 or greater per ml of sputum; concentrations of less than 10^5 organisms per ml of sputum were considered low. Gerlach made his interpretations on the comparison of numbers of potential pathogens and the number of saprophytes; a particular potential pathogen is considered to be present in significant numbers if these numbers are equal to or greater than the number of saprophytes. Gerlach has reported that with this technique *H. influenzae*, *S. pneumoniae* and group A streptococci are often predominant, while they are in the minority by conventional methods.

Other methods have also been used to arrive at a more conclusive diagnosis. Bronchoscopy is, of course, a routine technique. Bacteriological studies of bronchial specimens obtained by this technique generally show that patients with pulmonary disease have more organisms present in the lower respiratory tract than those that do not have pulmonary disease, and that the latter may not have any pathogens present at all. However, there are those that feel techniques which by-pass the upper respiratory tract, such as transtracheal aspiration or lung puncture, give much more reliable bacteriological information. Bartlett and Finegold report that transtracheal aspirations or empyema fluid are particularly useful in the diagnosis of pleuro-pulmonary infections due to anaerobes.

Other criteria have also been used to establish a diagnosis and the etiologic agent. Tillotson and Finland emphasize the advantages of using a gram stain before culture and sensitivity testing. In their studies of pneumonia due to gram negative bacilli, Tillotson and Lerner used as their criteria for diagnosis the isolation of the same species two or more times, or isolation of the same species from the sputum and blood, or the isolation of bacteria from pleural fluid. Pneumonias due to particular organisms can also often be correlated with a particular anatomic pathology. Many times the type of pneumonia depends upon the kinds of patients, their underlying disease and the therapy in use. For example, pneumonias due to *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacteroides* sp. generally involve the lower lobe and, frequently, there is empyema; pneumonias due to *H. influenzae* occur chiefly in elderly men with chronic obstructive lung disease.

Respiratory infections are also a significant part of nosocomial disease. The microbial flora involved in hospital infections will vary from hospital to hospital and from time to time, but it is markedly dependent upon the kinds of patients. Important factors affecting the colonization of hospitalized patients are age, pre-existing condition, duration of hospitalization and types of therapy, particularly antimicrobial, in use. Two epidemiologic patterns of nosocomial disease can be seen: epidemic, where there is one, or at most only a few
sources, or endemic, where the reservoir is diffuse and often includes endogenous flora of the patient population.

The microbial flora of hospitals, in general, are often more resistant to a variety of antimicrobics than the so-called street strains. In this situation, nosocomial disease becomes more serious. The development of resistant strains has been correlated with the intensive use of antimicrobics in the hospital.\(^\text{10}\)

Kantor and Shaw\(^\text{16}\) report that the use of one or more antimicrobials in a patient alters the normal pattern of microorganisms in the upper respiratory tract and makes the patient more susceptible to infections. In this regard, they define colonization as a significant increase in the number of any potential pathogen after antibiotics are given. Supra-infection is the appearance of both bacteriological and clinical evidence of a new infection developing during the course of antibiotic therapy for a previous infection. To Kantor and Shaw, superinfection is the development of a new infection unrelated to the antibiotic therapy.

**Potential Pathogens for Areas of the Respiratory Tract**

Even though identification of the specific etiologic agent of respiratory infections is often difficult, lists of potential pathogens can be made for each anatomic area of the respiratory tract. Most any organism can be a pathogen under certain conditions, but these are the species that are likely to occur in these areas if they are involved in an infection. The potential pathogens from the middle ear are *Streptococcus pneumoniae*, group A streptococci, *Haemophilus influenzae*, *Staphylococcus aureus*, *Enterobacteriaceae* and *Pseudomonas* sp. The pathogens for the paranasal sinuses include pneumococci, group A streptococci, *S. aureus*, *H. influenzae*, the *Enterobacteriaceae* and *Pseudomonas* sp. Potential pathogens for the throat include group A streptococci, *H. influenzae*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Corynebacterium diphtheriae* and *Bordetella pertussis*. There are reports\(^\text{4,34}\) that meningococci may, on occasion, cause pharyngitis. However, the gonococci may not belong on this list because there appears to be some doubt that they cause pharyngitis, even though they have been isolated from the throat.\(^\text{8}\) The pathogens of the larynx, trachea, and bronchi include pneumococci, group A streptococci, *H. influenzae*, *C. diphtheriae*, *S. aureus*, the *Enterobacteriaceae* and *Pseudomonas* sp. The potential pathogens for the pleura are *S. aureus*, *S. pneumoniae*, group A streptococci, *Peptostreptococcus* and *Peptococcus* (the anaerobic cocci), *H. influenzae*, the *Enterobacteriaceae*, *Pseudomonas* sp. and *Mycobacterium* sp. The potential lung pathogens causing pneumonia are pneumococci, group A streptococci, *H. influenzae*, *S. aureus*, the *Enterobacteriaceae*, *Pseudomonas* sp., *Mycobacterium* sp. and *Pasteurella*. Abscesses of the lungs are usually caused by one or more of the following organisms: anaerobic cocci, *Bacteroides* sp., *S. aureus*, *Enterobacteriaceae*, *Pseudomonas* sp. and pneumococci. Some other organisms found in various respiratory infections are *Bordetella bronchiseptica*, *Moraxella* sp., *Corynebacterium haemolyticum*, *Corynebacterium ulcerans*, group B streptococci, *Staphylococcus salivarius*, *Actinomyces* sp., *Fusobacterium* sp., *Eubacterium* sp., *Propionibacterium* sp. and *Clostridium* sp. The last five genera in the list are anaerobes.

It should be emphasized again that almost any microorganism can be the etio-
logic agent of a respiratory infection if the conditions are favorable for the organism. These conditions are particularly favorable in the compromised host where the innate resistance is impaired and the balance of power is altered to favor the microorganism. In some instances, the microorganism gains the upper hand by acquiring resistance to antimicrobial agents, as is the case with the microbic flora of most hospitals.

Antimicrobial Susceptibility Techniques

Once the identity of the etiologic agent has been determined, the microbiologist must decide if antimicrobial susceptibility tests are needed. If they are needed, he must decide what test to use based on his knowledge of the cultural and physiological characteristics of the organism.

There are basically four kinds of susceptibility tests that the microbiologist can use. These include the agar diffusion test, the broth dilution test (both micro and macro), the agar dilution test for organisms other than mycobacteria and the agar dilution test used for mycobacteria. Each of these tests has its advantages and disadvantages. To use each one properly, the microbiologist must be familiar with them all. The proper selection of a procedure depends upon knowledge of the organism and knowledge of the test.

The agar diffusion test is the easiest of these tests to run and it is also the most widely used. This procedure can be applied to most of the organisms isolated in a clinical laboratory; if used properly, useful, reliable, reproducible results can be obtained. The agar diffusion test used by most laboratories in this country was developed at the University of Washington, Seattle, and is commonly called the Kirby-Bauer procedure. This procedure is the most standardized of all the tests and principally for this reason, it has recently been recommended by the FDA and by the National Committee for Clinical Laboratory Standards Subcommittee on Antimicrobial Susceptibility Testing.

In the Kirby-Bauer procedure, single high-content discs are placed on a Mueller-Hinton agar plate that has been inoculated in a precise manner with a culture that has been adjusted to a standard inoculum. After the plates are incubated for 18 to 24 hrs at 35°C, the diameters of the zones of inhibition are measured. The culture is judged to be sensitive, resistant, or intermediate to an antimicrobial based on the size of the zone of inhibition around the disc containing that drug. A table of interpretive zone sizes has been developed for use with this test.

The interpretive zone size values were derived in the following manner. Recent clinical isolates of various bacteria were tested with each antimicrobial by agar diffusion and by a dilution method to obtain diameters of the zones of inhibition and minimal inhibitory concentrations (MIC). The zone sizes and MIC were compared to determine the zone size associated with an MIC for an organism considered to be susceptible. This MIC value was compared to concentrations of the antimicrobial that could be obtained in a patient's serum with the usual dosage. Finally, the judgment based on the in vitro data was confirmed by clinical efficacy studies. For these reasons, this test is not a qualitative one but a quantitative one.

The Kirby-Bauer agar diffusion test does have limitations; however, it can be used only with rapidly growing bacteria, principally staphylococci, the Enterobacteriaceae and Pseudomonas aeruginosa. The interpretive standards cannot be used for the more fastidious, slower-growing bacteria or for those requiring other than an aerobic environment for growth.

In the dilution procedures, varying concentrations of antimicrobial agents are incorporated into the culture media. These
media are inoculated with a culture of the organism to be tested and incubated for 24 to 48 hrs for the organisms, other than mycobacteria, which obviously require a much longer incubation. For these organisms, other than mycobacteria, the concentration of an antimicrobial that can be seen by the unaided eye to completely inhibit growth of the organism is the minimum inhibitory concentration (MIC). With the broth dilution test, a minimum bactericidal concentration (MBC) can be determined by subculturing the broth from the tubes showing no growth. In the agar dilution sensitivity test for mycobacteria, the interpretation is somewhat different in that the percentage of organisms resistant to a particular concentration of a drug is calculated.4,42

Dilution susceptibility tests are more difficult to perform than the agar diffusion test, but they offer some advantages. In addition to providing determinations of inhibitory and bactericidal end-points (the latter in broth tests), dilution tests can be used to test more fastidious or slow-growing organisms not amenable to the agar diffusion tests and can be used to test combinations of drugs. They are also adaptable to automation procedures.

In addition to their use with fastidious organisms, dilution tests should be used to test organisms from persons with bacterial endocarditis and serious systemic infection, those that have failed to respond to therapy and patients deficient in immunological responses. The tests should also be used in the evaluation of new chemotherapeutic agents.

Susceptibility tests are influenced by the kind of culture medium, pH of the medium, size of inoculum, and incubation time and temperature. Therefore, standardized methods must be used, and an active quality control program is essential if meaningful results are to be obtained. Standard multiply-sensitive control cultures should be tested daily, or whenever tests are performed.

**Susceptibility Tests to be Used with Each Potential Pathogen**

As stated previously, the decision to perform a susceptibility test or not to perform it, and the choice of which test to use depends upon the identity of the organism, a knowledge of the tests themselves, and the information desired.

Susceptibility tests are necessary on some organisms because they vary in their susceptibility patterns. Other organisms should not be tested because they are universally susceptible to a drug of choice.

Organisms which should be invariably tested are *S. aureus*, *E. coli*, *Klebsiella* sp., *Enterobacter* sp., *Proteus* sp., *Serratia* and *Pseudomonas aeruginosa*. These commonly isolated organisms are rapid growers that can be tested by the Kirby-Bauer technique. They can also be tested by a dilution technique if an MIC is desired.

*Haemophilus influenzae* can also be tested by the Kirby-Bauer technique, even though chocolate agar is used and incubation is in a candle jar or an atmosphere of carbon dioxide. There are some, however, who feel that susceptibility tests need not be done because the sensitivity of these organisms to antimicrobics has changed little over the years.50

*Streptococcus pneumoniae* and group A streptococci are commonly isolated organisms that should not be submitted to susceptibility tests. Group A streptococci are always susceptible to penicillin, and pneumococci should be considered in the same category even though penicillin-resistant strains have been reported.14 Group A streptococci resistant to erythromycin and lincomycin have been reported, but these strains are extremely rare. A significant number of each of these genera are resistant to tetracycline.
Neisseria species should not be routinely tested for susceptibility. Both *N. meningitidis* and *N. gonorrhoeae* are susceptible to penicillin, even though there has been a steady increase in the resistance of gonococci to penicillin. There is an agar diffusion method for testing the sensitivity of meningococci to sulfonamides, but the Kirby-Bauer interpretive zone sizes cannot be used. Any other susceptibility testing for *Neisseria* species should be done by an agar dilution method. Gonococci will require the use of supplemented chocolate agar.

There appears to be little need for routine testing of *C. diphtheriae*, since this species appears to be uniformly susceptible to penicillin or erythromycin. Group B streptococci probably fall into this category also, but there is only a limited amount of information on the antimicrobial susceptibility of this group. The *Moraxella* species are also susceptible to penicillin and need not be tested. Even though our experience with *Staphylococcus salivarius* is very limited, *in vitro* tests have shown this species to be very susceptible to penicillin. It is recommended that it not be tested.

If antimicrobial therapy is to be used effectively for *B. pertussis* infections, it must be initiated before the cough develops to the paroxysmal stage. In addition, oral drugs may be vomited. Although the organism is reportedly sensitive in vitro to ampicillin, this drug has apparently failed to be effective clinically. However, erythromycin has been shown to eradicate the organism. Susceptibility testing of these bacteria probably will not be useful; however, if it is performed, it should be done by a dilution technique.

Mycobacteria should be tested for susceptibility to the antituberculosis drugs by the special agar dilution tests that are used for mycobacteria.

The remaining microorganisms in the lists have had only a limited number of antimicrobial susceptibility tests performed on them. Therefore, they cannot be tested by the Kirby-Bauer agar diffusion test because the interpretive values may not be applicable to these organisms. However, if susceptibility tests on *Pasteurella, Bordetella* or *Corynebacterium* isolates are needed, a dilution test should be used. The same rule applied to other microorganisms that may be isolated on occasion but still fit into the category of having not been studied enough to permit them to be tested by agar diffusion. Certainly, the Kirby-Bauer interpretive table cannot be used for these organisms.

Furthermore, Kirby-Bauer tests should not be used for anaerobes. Although some recent progress has been made in the development of agar diffusion tests for anaerobes, the methods and interpretive values of these authors must be followed exactly if these tests are used. Otherwise, the anaerobes should be tested by either agar or broth dilution tests. There are clear indications for further studies of the *in vitro* testing of anaerobes, but there is probably a greater need for data on the clinical efficacy of chemotherapeutic agents in cases of bacteriologically proven anaerobic infections. Only with these data can a correlation of *in vitro* and *in vivo* data be made.

To reiterate, almost any microorganism can be a respiratory pathogen under certain conditions, i.e., when a compromised host is involved. If organisms other than those mentioned here are judged to be etiologic agents, susceptibility testing should be done by a dilution procedure until enough data is accumulated to show that they can be tested by an agar diffusion procedure.

**Summary**

The number of bacterial species that are potential respiratory pathogens is quite large. However, to prove that one of them
is the etiologic agent of a respiratory infection is usually quite difficult. If a species is designated as the etiologic agent, the decision to do an antimicrobial susceptibility test and the choice of the test to use depends upon the identification of the organism. Only rapidly growing aerobic or facultative bacteria should be tested by the Kirby-Bauer agar diffusion technique. Other organisms should be tested by the broth or agar dilution methods. Mycobacteria should be tested by the special agar dilution technique used only for those organisms.

References


"It has been my experience that folks who have no vices have very few virtues."

Abraham Lincoln