EVALUATION OF VARIOUS METHODS USED FOR CULTURING SPUTUM FROM PATIENTS WITH LOWER RESPIRATORY TRACT INFECTIONS

by

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INTRODUCTION

Culture of an appropriately collected sputum specimen is the most useful laboratory test available for establishing the etiology of bacterial infection of the lower respiratory tract. For many years the laboratory diagnosis of bacterial pneumonia and tracheobronchitis had relied upon culture of expectorated sputum. The conventional semiquantitative technique that has been widely used for culturing sputum consists of taking one sample with a single swab from a purulent portion of the specimen and inoculating it onto appropriate media The major drawback of this method is that expectorated sputum is frequently contaminated by organisms from the oropharynx, an area which is known to harbor a variety of bacterial species composing its normal flora (27) (48). The culture therefore not uncommonly shows a number of organisms (oropharyngeal contaminants) in addition to the primary pathogen responsible for the disease in the lower respiratory tract. It has been recognized however, that in patients with lower respiratory tract infection, culture of appropriately selected portions of the expectorated sputum frequently reveals the primary pathogen to be the most predominant organism and on occasion the only organism isolated (47).

In recent years several investigators have reported that culture of transtracheal aspiration is a more reliable method for isolation of the primary pathogen in patients with lower respiratory tract infection. The major advantage of the transtracheal aspirate is that it entirely

by-passes the oropharyngeal area with its normal flora, thus growth in culture represents organisms that exist in lower respiratory tract secretions. In 1959 Pecora (38) proposed this procedure for obtaining material from the lower respiratory tract. In 1964, Lapinski et al. (18) reported the results of culturing transtracheal aspirations from 30 patients with pulmonary infection and 8 patients without known pulmonary infection. Seven of the 8 patients without pulmonary infection had no organisms isolated from specimens collected in this manner. Fourteen of the 30 patients with pulmonary infection had purulent specimens, and 12 of the 14 contained pathogenic organisms while 2 were sterile. Hahn et al. (9) compared the culture results of specimens collected by a transtracheal aspiration procedure with routine expectorated sputa from 61 patients with acute pneumonia. The transtracheal aspirate provided a specimen essentially free of oral contamination and yielded a higher number of pulmonary pathogens than did routine culture of expectorated sputum. Hoeprich (12) analyzed the culture results of 129 patients from whom paired transtracheal aspiration and expectorated specimens were collected. His studies showed that transtracheal aspiration was useful in excluding oropharyngeal flora and providing valid information about lower respiratory tract infection.

In spite of the uniform agreement that the transtracheal aspirate is a superior specimen, it has never been widely used. Most physicians still rely on culture of the expectorated sputum in the management of patients with lower respiratory tract infections. Undoubtedly the ease by which an expectorated specimen is obtained accounts for its wide use at the present. Unlike the transtracheal aspiration, it is

a simple, inexpensive method that requires no instrumentation or the physician's attendance during specimen collection.

In most clinical laboratories, the expectorated sputum still represents the single most common specimen submitted for culture. At the University of Oregon Medical Center, for example, the clinical laboratory processes on the average of 450 sputum specimens each month. Over 75% of these specimens are expectorated and transtracheal aspirations account for less than 5%.

Recognizing the fact that expectorated sputum is the most commonly submitted specimen for culture from patients with pneumonia or tracheobronchitis, several investigators have developed various methods for the treatment of this specimen in an attempt to enhance the recovery of the primary pathogen and eliminate normal oropharyngeal flora. The extent of the problem is emphasized in the recent studies of Barrett-Connor (2). She reported that culture of expectorated sputum by the routine techniques failed to recover Diplococcus pneumoniae in 45% of 51 patients with pneumococcal pneumonia in whom the blood culture was positive for the organism.

The methods that have been developed in an attempt to improve the results of the expectorated sputum culture can be classified into two main groups: a) Washing the sputum prior to culture, and b) Digestion-quantitation.

Washing the expectorated specimen prior to culture was first reported in 1915 by Leutscher (26) who used a serial washing technique for 600 specimens from 459 patients with respiratory tract infections. By examining a Gram smear prepared from the sputum specimens before

and after washing Leutscher stated that washing was successful in eliminating or reducing the normal flora. Mulder (35) stressed the importance of a serial washing technique using serum-broth and more recently (36) (37) he developed a modification of this method using saline. He found that results of culture after washing were as reliable as those obtained from bronchoscopy specimens. In a brief communication, Coster (5) has likewise expressed the opinion that washing sputum before a culture is effective in eliminating practically all adhering throat flora. Lapinski et al. (18) compared the results for 223 expectorated sputum specimens cultured before and after washing. They reported the isolation of Hemophilus influenzae from 51 specimens after washing as compared to 19 isolates from the same specimens before washing. They also found that the culture results of 14 washed expectorated specimens from patients with bronchitis, were identical to the transtracheal aspirate except in 2 specimens. May (29) (31) and Rawlins (40) cultured expectorated sputum from patients with chronic bronchitis after the specimens had been washed. These investigators however, did not do cultures before washing and thus the effectiveness of washing in enhancing the recovery of potential pathogens or eliminating normal oropharyngeal flora could not be evaluated.

A limited number of recent investigations have shown that washing sputum prior to culture did not enhance the recovery of potential pathogens nor eliminate the normal oropharyngeal flora. Dixon and Miller (6) in a study of 250 sputum specimens reported that only 7 (3%) showed clearcut culture results (no growth or pure growth of a potential pathogen) after washing. Kilbourn et al. (17) has also reported that in

patients with cystic fibrosis, washing the sputum prior to culture had no effect on the results.

Digestion with a chemical agent or homogenization of expectorated sputum prior to culture has been investigated by several workers. May was the first to suggest that homogenization of sputum may be useful in enhancing the recovery of pathogens from the specimens. He demonstrated (29) (31) (30) that wide variation occurred in the distribution of organisms within sputum specimens from patients with chronic bronchitis. This variation was not related to the amount of pus present in the specimen. The organisms most frequently demonstrating uneven distribution were Diplococcus pneumoniae and Hemophilus influenzae. In a study of expectorated sputum specimens of 54 patients with chronic bronchitis, May (31) reported the isolation of pneumococcus from 31 specimens and found it to be unevenly distributed in 22 (71%). Hemophilus influenzae was unevenly distributed in 8 of 14 specimens and Staphylococcus aureus in 5 of 7 sputa. He concluded that 5 randomly selected sites within a sputum specimen must be cultured separately in order to obtain a reliable estimate of the proportion of each organism present.

Following May's demonstration of uneven distribution of the primary pathogens in sputum, Elmes et al. (7) homogenized expectorated sputum specimens from 13 patients with chronic bronchitis. The technique involved suspending the specimen in 5 ml of physiologic saline and shaking with glass beads for 1 hour. Two samples from each of 30 specimens treated in this way were cultured. Each was found to contain identical flora present in the same relative proportions.

A variety of chemical agents have been tested for their ability

to liquefy sputum. A detailed description of the mode of action of several mucolytic agents has been provided by Lieberman (22), Levine (20), and Webb (52). A review of the composition of sputum has been discussed by Lieberman (22), White et al. (53), Reas (41), and Sherry et al. (46).

Rowan and Rice (42) reported the use of 2.5% pancreatin to liquefy sputum from 122 patients suspected of having tuberculosis. This agent was found to be an excellent mucolytic agent for use in the culture of mycobacteria. Earlier studies of these workers demonstrated pancreatin to be a superior liquefying agent as compared to pepsin, trypsin, or papain. Rawlins (40) using a modification of their technique found that 1% pancreatin was an effective digestant, which did not effect the viability of organisms. The culture results from digesting 50 expectorated sputum specimens were comparable to the "five culture method" of May. May (32) has subsequently used this procedure in culturing sputa from 85 patients with chronic bronchitis and found that Hemophilus influenzae and Diplococcus pneumoniae were the 2 most frequently occurring primary pathogens.

Sheffner (44) examined the effect of n-acetyl-L-cysteine (NAC) on liquefying sputum. He reported a 77% decrease in the consistency of sputum in 3 minutes using 36.8 mM concentration at pH 7 as measured in Semimicro Ostwald Viscometers. In a subsequent communication (45), he reported that NAC was superior to several chemically related compounds in digesting sputum because of its stability and lack of toxicity.

Mead and Woodham (33) have shown that NAC was an effective sputum liquefying agent for the isolation of mycobacteria and other organisms. These investigators (54) have also compared NAC with pancreatin on

264 sputum specimens submitted for routine examination. The isolation rate of organisms from specimens treated by these 2 methods was identical except for Hemophilus species which showed an increased recovery rate from specimens treated with NAC. It was also found that incubation for up to 90 minutes was required for liquefaction to occur using pancreatin while only 10 minutes were required with NAC. Similar results using NAC have also been reported by Monroe et al. (34), Pirtle et al. (39), and Hurst et al. (13).

Recently dithiothreitol (DTT), a sulfhydryl compound chemically similar to NAC has been tested. Cleland (4) demonstrated its effectiveness in reducing disulfide bonds. Shaw and Dye (43) used it for the isolation of mycobacteria from sputum specimens. In this study DTT was compared to NAC and the culture results were identical. The Ziehl-Neelsen smear results, on the other hand, showed more positive specimens when DTT was used. An in vitro evaluation of a large number of mucolytic agents was carried out by Lieberman (21). All agents were tested on purulent and mucoid sputum specimens from patients with cystic fibrosis and emphysema. DTT was found to be a superior digestant as compared to NAC but not as stable. Hirsch et al. (11) compared the sputum-thinning ability of several agents. They found that the most effective compounds were those containing a free sulfhydryl group. DTT in a 1.5% concentration was more effective in liquefying sputum than a 20% solution of NAC.

Quantitative culture of homogenized (with or without the use of chemical digestants) expectorated sputum has been investigated by many workers as an aid in assessing the significance of the presence of a

given bacterial isolate. Pirtle et al. (39) utilized the quantitative culture for distinguishing between superinfection and colonization (acquisition). This distinction is very important particularly in patients with pneumonia who are receiving antimicrobial drugs. These workers defined superinfection as the appearance of a potential pathogen in the sputum in a concentration of 10^5 organisms/ml of sputum or greater. Colonization, on the other hand, was defined as the appearance in the sputum of at least 10^3 new organisms/ml. Tillotson and Finland (51), on the other hand, defined colonization as the appearance or significant increase in number of any potential pathogen in culture of sputum after antibiotic therapy was begun. The criteria they used for recognizing superinfection (secondary pneumonia) included a recurrence of fever after 1 or more afebrile days associated with increased lower respiratory signs and symptoms, an increase in purulence of sputum, and an increase in number of any potential pathogen.

Louria and Kaminske (23) used quantitative sputum culture techniques for analyzing the frequency of bacterial superinfection in 154 patients treated with antimicrobials following admission to the hospital for acute pulmonary inflammatory disease. The presence of a potential pathogen not recovered previously in a concentration of less than 10^3 organisms/ml of sputum was found to be consistent with colonization (acquisition). The finding, on the other hand, of a new pathogen in a quantity greater than 10^5 organisms/ml, was taken to represent the presence of superinfection. In a later study Louria (24) analyzed the case histories of 7 patients and described the circumstances

under which the routinely employed techniques did not provide the clinician with adequate information for the appropriate selection of antibacterial therapy. He cited examples where unnecessary antimicrobial drugs could have been avoided when simple colonization occurred. In other patients, where appropriate changes in therapy were indicated but not done, a fatal outcome might have been avoided if the diagnosis of superinfection was made.

Monroe et al. (39) in a study of 19 patients with pneumonia and 20 other patients without evidence of lower respiratory tract infection reported that the quantitative culture of homogenized sputum provided information more directly applicable to patient management than did qualitative routine methods. They found that the organism associated with primary pneumonia or superinfection was present in a concentration of 10^7 or more organisms/ml of sputum. Normal mouth flora from patients with lower respiratory tract infection were present in a concentration less than 10^6 organisms/ml of sputum.

Johanson et al. (15), on the other hand, in a study including 26 patients with apparent nosocomial infection of the lower respiratory tract and 31 patients lacking such evidence, reported that quantitative sputum cultures were of no value in distinguishing nosocomial colonization from nosocomial infection. Similar results have been reported by Hahn et al. (9), Bartlett and Melnick (3), and Kalinske (16).

In view of the conflicting reports on the efficacy of washing expectorated sputum prior to culture and the continuing uncertainty of the value of digestion-quantitation methods, it has become evident

that a specific study is needed for comparing all of these various techniques. This is particularly important in view of the fact that these elaborate culture methods are more costly than the simple technique used in the routine diagnostic laboratory.

The present investigation was designed primarily to evaluate the efficacy of the various methods used for processing sputum from patients with bacterial infections of the lower respiratory tract. All of the infections were caused by aerobic or facultative anaerobic microorganisms. Four culture methods were used including; a) The conventional 1 sample-single swab, b) Five samples-single swab, c) Digestion-quantitation using NAC, and d) Digestion-quantitation using DTT. Except for the 5 samples-single swab, all methods were evaluated before and after the sputum specimens were washed. The distribution of pathogens in various portions of sputum was also examined.

MATERIALS AND METHODS

Source of Specimens

The specimens used in this study comprised 94 sputa collected from 84 patients. The sputa included 68 expectorated and 26 non-expectorated specimens. The specimens were selected from sputa submitted for routine culture to the clinical microbiology laboratory of the University of Oregon Medical School. Most specimens selected showed a high degree of purulence both on gross and microscopic examination (presence of numerous polymorphonuclear leukocytes - PML).

A retrospective evaluation of the clinical and radiographic data of the patients who provided the sputum specimens used in this study revealed the following: a) 25 patients with pneumonia provided 25 specimens, b) 6 patients with tracheobronchitis provided 6 specimens, and c) 53 patients without bacterial infection of the lower respiratory tract provided 63 specimens.

Media and Reagents

The media used for sputum culture included 5% sheep blood agar, MacConkey agar, and chocolate agar. Following inoculation, the media were incubated at 35° C in an atmosphere of 5% CO_2. All media were purchased from a local supplier.

Disks for determining the growth requirements (X and V factors) of *Hemophilus* species were obtained from the Difco Company. The 2% desoxycholate used for identification of the pneumococcus was prepared fresh daily.

The buffer used for washing and diluting sputum specimens was Sorenson's phosphate buffered saline (PBS), pH 7.0.

N-acetyl-L-cysteine (NAC) powder (Sigma Chemical Co.) was used for preparing a 2% solution in PBS. The usual procedure was to dissolve 1 gm NAC in about 43 ml PBS and adding about 6.5 ml of 1N NaOH to pH 7.2.

Dithiothreitol (DTT) was obtained from Calbiochem in the form of a 0.1% solution. A 0.01% working solution was made by diluting 1 volume of DTT provided by the manufacturer with 9 volumes of sterile distilled water.

Both NAC and DTT solutions were stored at 4° C after these dilutions had been made and used within 4 days. The NAC powder was kept at 4° C in a desiccator jar. DTT, as supplied by the manufacturer, was kept at room temperature.

Methods Used for Culture

The methods used for culture included the 1 sample-single swab, 5 samples-single swab, digestion-quantitation using NAC, and digestion-quantitation using DTT. Each of these methods was carried out both before and after washing the specimen except the 5 samples-single swab which was done only prior to washing.

The 1 sample-single swab technique involved taking a sterile swab and sampling a purulent portion of the sputum specimen. This small amount of purulent material was then used to inoculate media and to prepare a smear for Gram's stain. An inoculating loop was used to streak the specimen in such a manner to allow for the isolation of single colonies. Five different portions of each specimen were

sampled in this manner.

The 5 samples-single swab method involved touching 5 portions of the sputum with a sterile swab. The swab was used to inoculate appropriate media and to prepare a smear for Gram's stain.

Digestion-quantitation using NAC was carried out in the following manner. A portion of the sputum specimen was placed in a sterile 50 ml conical centrifuge tube. An equal volume of NAC was placed in the tube and mixed for 15-30 seconds on a vortex mixer. The mixture was allowed to stand at room temperature for 15 minutes. PBS was added to give a final dilution of the sputum of 10^{-1}. Ten-fold serial dilutions ranging from 10^{-1} - 10^{-6} were made using PBS as diluent. Each dilution was sampled with a platinum loop calibrated to deliver 0.01 ml and this was used to inoculate appropriate media (blood agar, MacConkey agar, and chocolate agar). The loop was flamed only prior to sampling each dilution; but not between streaking the various quadrants of plated media. Smears for Gram stain were also prepared from each dilution.

Digestion-quantitation using DTT was performed in a manner identical to that described for NAC.

The technique used for washing sputum consisted of placing a portion of the specimen in a Büchner funnel. The funnel was mounted on top of an Erlenmeyer flask equipped with an outlet in the neck which was attached to a negative pressure source on a water line. Fifty ml of sterile PBS was added to the specimen from an automatic filling burette. A small amount of negative pressure was then applied to aid in removing saliva and saline from the specimen. The sputum remaining in the funnel was used for culture.

Outline Used for Processing Specimens

The outline used for processing specimens is illustrated in Chart 1.

The entire sputum specimen was placed in a sterile 155 mm Petri dish. Five purulent (or mucopurulent) portions of the specimen were selected randomly. Each portion was cultured using the 1 sample-single swab method. A sample was also taken from all of the 5 portions using a single swab which was cultured on appropriate media (5 samples-single swab method).

The specimen was then divided roughly into 2 equal portions. One portion (I) was washed with PBS but the second portion (II) was not. Each of these 2 portions was again subdivided into 2 parts. All 4 portions of the specimen were processed by the digestion-quantitation method using either NAC or DTT (See Chart).

Criteria Used for Quantitation of Smear and Culture

Gram smears were examined for the presence of organisms and PMLs. The following criteria were used for quantitation: Many (++++) if greater than 30 organisms or PMLs per oil immersion field were seen, Moderate (+++) if 6 to 30 organisms or PMLs per oil immersion field were seen, Few (++) if 1 to 5 organisms or PMLs per oil immersion field were seen, and Very few (+) if less than 1 organism or PML was seen per oil immersion field.

The organisms isolated in culture using the single swab methods were roughly quantitated utilizing the following criteria: Heavy growth (++++): Growth extending into the 4th segment of the streaks, OR, growth extending with more than 5 colonies into the third segment; Moderate growth (+++): Growth extending with more than 5 colonies into the second, but

not the third segments, OR, growth only in the first segment of streaks with greater than 30 colonies; Light growth (++): Ten to 30 colonies in the first segment only; and Very light growth (+): Less than 10 colonies in the first segment only.

Identification of Isolates

All organisms were identified according to procedures described by Bailey and Scott (1).

Diplococcus pneumoniae was identified by its typical colony morphology on blood agar, solubility in 2% desoxycholate, and sensitivity to optochin.

Hemophilus influenzae was identified by its typical morphology on chocolate agar, its typical appearance on Gram smear, and its specific requirement for both the "X" and "V" factors.

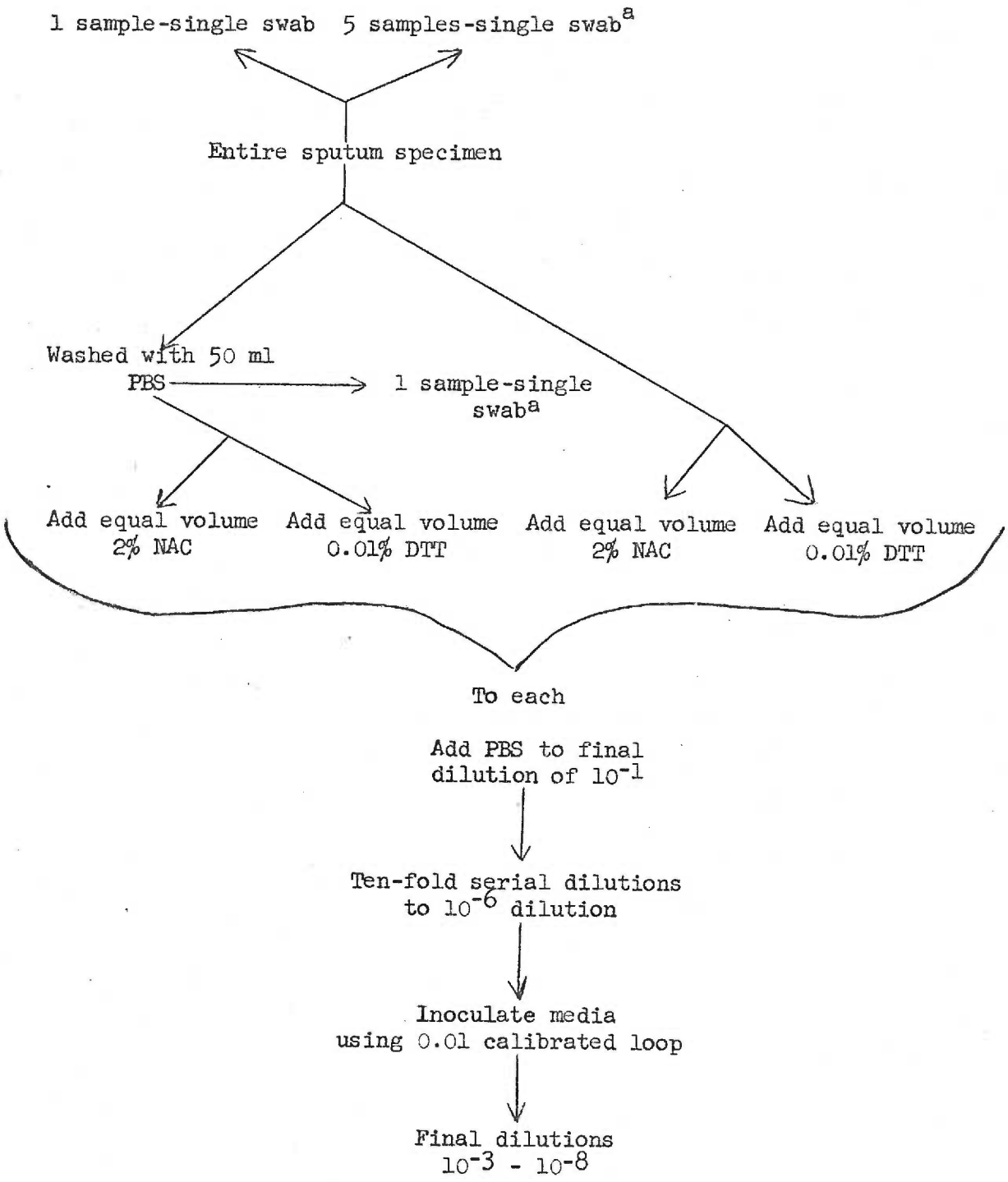
Staphylococcus aureus was identified by its typical colony morphology on blood agar and by a positive slide or tube coagulase test.

Escherichia coli was identified by its typical colony morphology, lactose fermentation, and indol production.

Sources of Methodologic Error in the Quantitative Culture

Loop Error: Statistical analysis of the reproducibility of the calibrated loops used in this study (2 loops quantitated to deliver 0.01 ml were used) was made by sampling a suspension of Escherichia coli 10 times with each loop. The probability of the loops being different was examined. Bacterial counts using both loops were paired randomly and the probability of the difference of the pairs being significant was also examined.

Chart 1



a Five portions were sampled separately

Toxicity Error: The toxic effects of NAC and DTT were tested by subjecting pure cultures of bacteria commonly found in the sputum to treatment with an equal volume of digestant for as long as sputum was exposed to the mucolytic agent before being cultured. The organisms tested were: Hemophilus influenzae, Diplococcus pneumoniae, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Streptococcus viridans, and Neisseria species. The number of organisms surviving in each mucolytic agent was compared to the number surviving in PBS, both immediately after the addition of the agent and 15 minutes later. The probability that the difference was significant was determined.

RESULTS

Clinical Data

Eighty-four patients provided the sputum specimens used in this study and they included 59 males and 25 females. Ages of the patients ranged from 8 - 95 years with the mean being 56.2 and the median 59 years.

The patients were divided into the following 3 groups: Group I comprised 25 patients with clinical, radiographic, and microbiologic evidence for bacterial pneumonia. The primary pathogens responsible for pneumonia in all of these patients were either aerobic or facultative anaerobic bacteria. Group II included 6 patients with clinical and microbiologic evidence for acute tracheobronchitis. Group III consisted of 53 patients lacking definitive evidence for bacterial infection of the lower respiratory tract. Table 1 lists the expectorated and non-expectorated specimens obtained from the patients in each group. The specimens are segregated into those collected from patients receiving and those not receiving antimicrobial drugs. A patient was considered to be on antimicrobial therapy if any antibacterial drug was administered within 48 hours prior to sputum collection. Thirty-three of the 94 specimens were collected from patients receiving antimicrobial drugs. Thirteen of the 25 patients with pneumonia provided expectorated specimens, and in the remaining 12 patients non-expectorated specimens were obtained. The 6 patients with tracheobronchitis provided 3 expectorated and 3 non-expectorated specimens. Sputa collected from 53 patients lacking definitive

Table 1 Classification of 94 sputum specimens obtained from 84 patients into expectorated and non-expectorated groups with reference to antimicrobial therapy at time of collection.

Bacterial Infection Of Lower Respiratory Tract	Number of Specimens / Number of Patients				
	Expectorated		Non-expectorated[a]		
	Antimicrobial Therapy[b]	No Antimicrobial Therapy	Antimicrobial Therapy	No Antimicrobial Therapy	Total
Pneumonia	4/4	9/9	5/5	7/7	25/25
Tracheo-bronchitis	0/0	3/3	0/0	3/3	6/6
Not present	20/14	32/28	4/4	7/7	63/53
Total	24/18	44/40	9/9	17/17	94/84

a Non-expectorated specimens include nasotracheal aspiration, endotracheal suction, transtracheal aspiration, and tracheostomy specimens.

b A patient was considered to be on antimicrobial therapy if any antibacterial drug was administered within 48 hours prior to sputum collection.

evidence of bacterial infection of the lower respiratory tract included 52 expectorated and 11 non-expectorated specimens.

Table 2 lists the methods used for obtaining 26 non-expectorated specimens from 26 patients with reference to antimicrobial therapy at the time of the sputum collection. Eighteen specimens were collected by nasotracheal aspiration, 4 by transtracheal aspiration, 1 by endotracheal suction and 3 via a tracheostomy tube. Five specimens from patients with pneumonia and 4 from patients lacking evidence of lower respiratory tract infection were collected while the patient was receiving antimicrobial drugs.

The clinical and microbiologic data for 13 patients with pneumonia from whom expectorated specimens were collected are listed in Table 3. The age of these patients ranged from 24 - 67 years. Two patients were females and 11 were males. Four of the 13 patients in this group had chronic obstructive pulmonary disease and 4 others suffered from chronic alcoholism. Four patients were receiving antimicrobial drugs at the time of sputum collection, and in two patients No. 30 and 32, the drugs administered were known to be effective against the primary pathogen. Patient No. 30, who had Escherichia coli pneumonia, received ampicillin for 5 days prior to the collection of sputum used in this study. Patient No. 32 was admitted to the hospital with pneumococcal pneumonia. Penicillin was administered for 1 day prior to collection of the sputum specimen used in this study. Penicillin was administered to patient No. 79 for 3 days prior to sputum collection. When culture results revealed Enterobacter aerogenes, penicillin was ceased and kanamycin was administered.

Table 2 Methods used for collection of the non-expectorated sputum specimens

Bacterial Infection of Lower Respiratory Tract	Antimicrobial Therapy	Method of collection[a]				Total Specimens
		NTA	ETS	TR	TTA	
Pneumonia	Yes	2	0	0	3	5
	No	5	1	1	0	7
Tracheo-bronchitis	Yes	0	0	0	0	0
	No	3	0	0	0	3
Not present	Yes	4	0	0	0	4
	No	4	0	2	1	7
Total		18	1	3	4	26

a NTA - Nasotracheal aspiration
ETS - Endotracheal suction
TR - Tracheostomy specimen
TTA - Transtracheal aspiration

Table 3 Clinical and microbiologic data for 13 patients with pneumonia from whom expectorated sputum specimens were obtained.

Patient Number	Age (Years)	Sex	Underlying and Associated Disease(s)	Antimicrobial Therapy Prior to Collection	Degree of Purulence of Sputum[a]	Primary Pathogen Isolated from Sputum	Pathogen Isolated from Blood or Pleural Fluid	Nosocomial Infection
1	64	M	Myocardial infarction	No	+++	*D. pneumoniae*	Not cultured	No
24	49	M	Pancytopenia Liver cirrhosis Chronic alcoholism	No	+++	*D. pneumoniae*	Not cultured	No
31	66	M	Chronic obstructive pulmonary disease	No	+++	*D. pneumoniae*	Blood Culture Negative	No
68	24	M	Inguinal hernia	No	++++	*D. pneumoniae*	Blood Culture Negative	No
78	54	M	Chronic obstructive pulmonary disease Chronic alcoholism	No	++	*D. pneumoniae*	Not cultured	No
83	56	M	None	No	++++	*D. pneumoniae*	Not cultured	No
60	55	M	Parkinson's Syndrome	No	+++	*S. aureus*	*S. aureus* (Pleural Fluid)	No
41	37	F	Hepatic and Renal Failure	No	+++	*S. aureus*	Blood Culture Negative	No
18	67	M	Coma, liver cirrhosis, Chronic alcoholism	No	+++	*E. coli*	*E. coli* (Blood)	Yes
30	57	M	Chronic obstructive pulmonary disease Chronic alcoholism	Ampicillin	++++	*E. coli*	*E. coli* (Pleural fluid)	No
32	63	M	Latent syphillis	Penicillin	+++	*D. pneumoniae*	Not cultured	No
79	59	M	Chronic obstructive pulmonary disease	Penicillin	+++	*E. aerogenes*	Not cultured	No
81	53	F	Asthma	Pennicillin Cephalothin	+++	*P. aeruginosa*	Blood Culture Negative	Yes

a Represents amount of polymorphonuclear leukocytes (PML in Gram smear: ++++ (Many), +++ (Moderate), ++ (Few), + (Very few).

Penicillin and cephalothin were given to patient No. 81 one day before the sputum specimen was obtained. Microscopic examination of the Gram smear revealed many (++++) or moderate (+++) numbers of polymorphonuclear leukocytes (PML) in all but one of the 13 specimens. The primary pathogen was Diplococcus pneumoniae in 7 patients, Staphylococcus aureus in 2, and Gram negative rods in 4. The same pathogenic organism isolated from the sputum was also recovered from the pleural fluid or blood of 3 patients. The pleural fluid was aspirated from 2 patients who developed empyema and the culture was positive for Staphylococcus aureus in one and Escherichia coli in the second. The blood from 5 of the patients was cultured with negative results in 4 and positive results for Escherichia coli in 1 (patient No. 18). Eleven of the 13 patients listed were admitted to the hospital with pneumonia. The remaining two patients developed pneumonia while in the hospital and the disease therefore represents a nosocomial infection. Gram negative rods, Escherichia coli and Pseudomonas aeruginosa, were responsible for the pneumonia in these 2 patients.

Table 4 provides the clinical and microbiologic data for 12 patients with pneumonia from whom non-expectorated specimens were cultured. The age of the patients ranged from 37 - 82 years. Five patients in this group were females and 7 were males. Two patients were in a coma at the time of specimen collection and the other 10 had a variety of underlying and associated diseases. Five patients were receiving antimicrobial drugs at the time of specimen collection. The antimicrobials used in 3 patients (No. 3, 9, and 82) were ineffective against the pathogen causing pneumonia. In the remaining 2 patients

Table 4 Clinical and microbiologic data for 12 patients with pneumonia from whom non-expectorated sputum specimens were obtained.

Patient Number	Age (Years)	Sex	Underlying and Associated Disease(s)	Anti-microbial Therapy Prior to Collection	Method of Collection	Degree of Purulence of Sputum[a]	Primary Pathogen Isolated from Sputum	Pathogen Isolated from Blood or Pleural Fluid	Nosocomial Infection
13	82	F	Coma, Intestinal obstruction, Respiratory failure	No	Nasotracheal aspirate	+++	D. pneumoniae	Not cultured	No
72	70	M	Anemia	No	Nasotracheal aspirate	+++	D. pneumoniae	Not cultured	No
84	62	F	Gastro-intestinal hemorrhage, Chronic laryngitis	No	Nasotracheal aspirate	++	D. pneumoniae	Not cultured	No
25	73	M	Acute renal failure	No	Endotracheal suction	+++	D. pneumoniae	D. pneumoniae (Blood)	No
15	67	M	Aortic stenosis	No	Nasotracheal aspirate	+++	P. mirabilis	Not cultured	Yes
80	70	F	Hypertensive Coronary artery Disease	No	Nasotracheal aspirate	+++	S. aureus	Blood Culture Negative	No
35	52	M	Coma, Cerebral Contusion	No	Tracheostomy	++++	H. influenzae	Not cultured	No
38	37	F	Hepatic failure	Penicillin	Nasotracheal aspirate	++	S. aureus	Not cultured	No
82	82	M	Chronic obstructive pulmonary disease	Cephalexin	Nasotracheal aspirate	+++	H. influenzae	Not cultured	No
9	50	M	Chronic bronchitis, Subdural hematoma	Cephalothin	Transtracheal aspirate	+++	H. influenzae	Blood Culture Negative	No
3	63	M	None	Nafcillin	Transtracheal aspirate	+++	P. maltophilia	P. maltophilia (Pleural fluid)	Yes
62	65	F	Respiratory failure	Lincomycin	Transtracheal aspirate	+++	D. pneumoniae	Not cultured	No

a Represents amount of polymorphonuclear leukocytes (PML) in Gram smear: ++++ (Many), +++ (Moderate), ++ (Few), + (Very few).

the antibiotics used were active against the primary pathogen. Patient 38 had pneumonia caused by Staphylococcus aureus which was susceptible to penicillin. She received penicillin one day before sputum was collected and expired 2 days later. Patient 62 was started on lincomycin for treatment of pneumococcal pneumonia one day before the sputum specimen was obtained. The culture was positive for Diplococcus pneumoniae but subsequent specimens obtained 3 days after initiation of therapy demonstrated that the drug was effective in eliminating the organism. The non-expectorated specimens comprised 7 nasotracheal aspirations, 3 transtracheal aspirations, 1 endotracheal suction, and 1 specimen collected via a tracheostomy. Microscopic examination of the sputum sample revealed many (++++) or moderate (+++) numbers of PMLs in 10 specimens. In the remaining 2 specimens PMLs were few (++). The primary pathogen isolated from sputum was Diplococcus pneumoniae in 5 patients, Hemophilus influenzae in 3, Staphylococcus aureus in 2, Proteus mirabilis in 1, and Pseudomonas maltophilia in 1. Blood was cultured from only 3 patients in this group with positive results for Diplococcus pneumoniae in one (patient No. 25). One patient with pneumonia due to Pseudomonas maltophilia developed empyema and culture of the pleural fluid was positive for that organism.

Data on 6 patients with acute tracheobronchitis are shown in Table 5. Age of the patients ranged from 47 - 95 years and they included 5 males and 1 female. Two patients in this group suffered from congestive heart failure and a third had active pulmonary tuberculosis. None of the patients received antimicrobial drugs prior to sputum collection. Three of the 6 patients provided expectorated specimens and in the remaining

Table 5 Clinical and microbiologic data for 6 patients with acute tracheobronchitis.

Patient Number	Age (Years)	Sex	Underlying and Associated Disease(s)	Anti-microbial Therapy Prior to Collection	Method of Collection	Degree of Purulence of Sputum[a]	Primary Pathogen Isolated from Sputum
42	47	F	Inguinal Hernia	No	Expectorated	+++	*D. pneumoniae*
70	61	M	Fractured rib, Pleural effusion	No	Expectorated	+++	*D. pneumoniae* *H. influenzae*
48	74	M	Congestive Heart failure	No	Expectorated	+++	*H. influenzae*
28	95	M	Congestive Heart failure	No	Nasotracheal aspirate	++	*D. pneumoniae*
39	62	M	Pulmonary tuberculosis, Chronic alcoholism, Left side paresis	No	Nasotracheal aspirate	+++	*D. pneumoniae*
50	62	M	Fractured neck of Humerus, Chronic subdural hematoma	No	Nasotracheal aspirate	+++	*D. pneumoniae* *H. influenzae*

a Represents amount of polymorphonuclear leukocytes (PML) in Gram smear: ++++(Many), +++ (Moderate), ++ (Few), + (Very few).

3 sputa were collected by nasotracheal aspiration. Moderate (+++) numbers of PMLs were seen on microscopic examination of 5 specimens and few (++) were present in the 6th. The primary pathogens included Diplococcus pneumoniae in 3 patients and Hemophilus influenzae in 1. In the remaining 2 patients, tracheobronchitis was caused by both organisms.

Isolation Rates and Distribution of Organisms in Sputum

The quantity of growth of organisms isolated in culture was determined by utilizing the semiquantitative criteria of heavy (++++), moderate (+++), light (++), and very light (+). The presence of uneven distribution of an organism was arbitrarily taken to represent a change in the quantity of growth in different portions of a specimen consistent with any of the following: Variation from 0 to +++ or ++++, + to +++ or ++++, or ++ to ++++.

The frequency of isolation of various organisms and their distribution in different portions was determined prior to washing the specimen in 13 expectorated and 12 non-expectorated sputa from 25 patients with pneumonia. Five portions were cultured from each specimen. The results listed in Table 6 show that the most frequently isolated pathogen in the expectorated group was Diplococcus pneumoniae; 8 isolates were recovered. Staphylococcus aureus and Hemophilus influenzae were the next most common pathogens; 5 isolates of each were recovered. Uneven distribution of the pathogen was detected in 1 out of 8 isolates of Diplococcus pneumoniae. This isolate was the primary pathogen responsible for pneumonia. Uneven distribution was also demonstrated for Hemophilus influenzae in 2 of 5 isolates, Staphylococcus aureus in

Table 6 Frequency of isolation and variation in distribution of micro-organisms in various portions of 13 expectorated and 12 non-expectorated sputum specimens from 25 patients with pneumonia.

Organism isolated	No. of isolates showing uneven distribution[a] / Total No. of isolates	
	Expectorated Group (13 specimens)	Non-expectorated Group (12 specimens)
Respiratory pathogens and potential pathogens		
D. pneumoniae	1/8	1/2
H. influenzae	2/5	1/4
S. aureus	1/5	1/3
E. coli	0/2	0/3
P. aeruginosa	0/1	0/1
K. pneumoniae	0/0	0/1
P. mirabilis	0/0	1/1
P. maltophilia	0/0	0/1
E. cloacae	0/0	1/1
E. aerogenes	0/1	0/0
H. vaginacola	0/1	0/0
C. albicans	0/0	0/3
Beta hemolytic streptococcus not Lancefield group A	1/1	0/0
Total	5/24	5/20
Respiratory non-pathogens (normal flora)		
S. viridans	0/6	1/6
Neisseria species	1/6	1/4
S. epidermidis	1/4	0/3
Corynebacterium species	1/2	1/2
H. parainfluenzae	0/0	0/1
Gamma streptococcus	0/0	0/1
Total	3/18	3/17

a Denotes variation in quantity of organism in different portions of specimen from 0 to +++ or ++++, + to +++ or ++++, or ++ to ++++.

1 of 5 isolates, and beta hemolytic streptococcus not Lancefield group A in 1. Uneven distribution of the respiratory non-pathogens (normal flora) was detected in 3 isolates; Neisseria species, Staphylococcus epidermidis, and Corynebacterium species.

The most frequently isolated pathogen from the non-expectorated specimens was Hemophilus influenzae; 4 isolates were recovered. Staphylococcus aureus and Escherichia coli were next in frequency; 3 isolates of each were recovered. Two isolates of Diplococcus pneumoniae were recovered. Uneven distribution was demonstrated for 5 pathogens. Three of these 5 isolates were the primary pathogens responsible for pneumonia and they included 1 Diplococcus pneumoniae, 1 Staphylococcus aureus, and 1 Proteus mirabilis. The former isolate was recovered from an endotracheal suction and the latter 2 were isolated from nasotracheal aspirations. Three isolates of respiratory non-pathogens demonstrated uneven distribution and they included Streptococcus viridans, Neisseria species, and Corynebacterium species.

Table 7 lists the organisms isolated and the variability of their distribution within 3 expectorated and 3 non-expectorated specimens from 6 patients with tracheobronchitis. In the expectorated specimens, the pathogens isolated included Diplococcus pneumoniae and Hemophilus influenzae. Only 1 isolate showed uneven distribution and the organism, Hemophilus influenzae, was the primary pathogen causing tracheobronchitis. Uneven distribution of 3 non-pathogens, Neisseria species, Staphylococcus epidermidis, and Hemophilus parainfluenzae, was also demonstrated.

In the non-expectorated group, the most commonly isolated pathogen was Diplococcus pneumoniae; 3 isolates were recovered. Uneven distribution was demonstrated for 1 primary pathogen (Hemophilus influenzae), 1 potential pathogen (Klebsiella pneumoniae), and 2 non-pathogens (Streptococcus viridans and Staphylococcus epidermidis).

Table 7 Frequency of isolation and variation in distribution of micro-organisms in various portions of 3 expectorated and 3 non-expectorated specimens from 6 patients with tracheobronchitis.

Organism isolated	No. of isolates showing uneven distribution[a] / Total No. of isolates	
	Expectorated Group (3 specimens)	Non-expectorated Group (3 specimens)
Respiratory pathogens and potential pathogens		
D. pneumoniae	0/2	0/3
H. influenzae	1/2	1/1
P. mirabilis	0/0	0/2
K. pneumoniae	0/0	1/1
E. coli	0/0	0/1
E. aerogenes	0/0	0/1
Beta hemolytic streptococcus not Lancefield group A	0/0	0/1
Total	1/4	2/10
Respiratory non-pathogens (normal flora)		
S. viridans	0/1	1/3
Neisseria species	1/2	0/1
S. epidermidis	1/2	1/2
H. parainfluenzae	1/1	0/1
Total	3/6	2/7

a Denotes variation in quantity of organism in different portions of specimen from 0 to +++ or ++++, + to +++ or ++++, or ++ to ++++.

Table 8 lists the frequency of isolation of organisms and their distribution in 52 expectorated and 11 non-expectorated sputum specimens from 53 patients lacking definitive evidence of bacterial infection of the lower respiratory tract. In the expectorated group, Hemophilus influenzae was the most common potential pathogen; 18 isolates were recovered. Diplococcus pneumoniae was the next most common pathogen; 15 isolates were recovered. Nine isolates of Escherichia coli, 8 Pseudomonas aeruginosa, 7 Klebsiella pneumoniae, and 4 Staphylococcus aureus were also recovered. Uneven distribution was demonstrated by 9 isolates of Diplococcus pneumoniae and 4 isolates of Hemophilus influenzae. Twelve of the respiratory non-pathogens were also found to exhibit uneven distribution in the specimens. In the non-expectorated group of specimens, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae were the most frequently isolated potential pathogens, 3 isolates of each were recovered. Uneven distribution was demonstrated for 1 isolate of Escherichia coli and 1 Klebsiella pneumoniae. Three isolates from the non-expectorated specimens also showed uneven distribution of respiratory non-pathogens and the organisms included Streptococcus viridans, Hemophilus parainfluenzae, and Corynebacterium species.

Table 9 shows the culture results of sputum from 9 patients with lower respiratory tract infection in whom the primary pathogen was found to be unevenly distributed. These patients included 7 of the 25 patients with pneumonia and 2 of the 6 patients with tracheobronchitis. The primary pathogens isolated from the remainder of the patients with lower respiratory tract infection did not exhibit uneven distribution. The

Table 8 Frequency of isolation and variation in distribution of micro-organisms in various portions of 52 expectorated and 11 non-expectorated sputum specimens from 53 patients without evidence for pneumonia or tracheobronchitis.

Organism isolated	No. of isolates showing uneven distribution[a] / Total No. of isolates	
	Expectorated Group (52 specimens)	Non-expectorated Group (11 specimens)
Potential respiratory pathogens		
D. pneumoniae	9/15	0/0
H. influenzae	4/18	0/1
S. aureus	0/4	0/2
E. coli	3/9	1/3
P. aeruginosa	0/8	0/3
K. pneumoniae	0/7	1/3
P. mirabilis	0/3	0/2
E. cloacae	1/3	0/0
Padlewski	0/2	0/0
E. aerogenes	0/1	0/1
Klebsiella species	0/1	0/0
C. albicans	0/5	0/1
H. aphrophilus	0/0	0/1
Beta hemolytic streptococcus not Lancefield group A	0/3	0/2
Total	17/79	2/19
Respiratory non-pathogens (normal flora)		
S. viridans	2/38	1/5
Neisseria species	3/30	0/5
S. epidermidis	4/17	0/2
H. parainfluenzae	2/5	1/2
Corynebacterium species	1/2	1/3
Bacillus species	0/0	0/1
C. guilliermondii	0/0	0/1
C. tropicalis	0/1	0/0
Total	12/93	3/19

a Denotes variation in quantity of organism in different portions of the specimen from 0 to +++ or ++++, + to +++ or ++++, or ++ to ++++.

results of culturing 1 sample (1 sample-single swab), 5 samples separately (5 culture method), and 5 samples combined (5 samples-single swab), are illustrated in Table 9. Specimens from the patients with pneumonia included 1 expectorated sputum, 4 nasotracheal aspirations, 1 endotracheal suction, and 1 transtracheal aspiration. Only one patient (No. 62), from whom the transtracheal aspiration was obtained, was receiving antimicrobial therapy (lincomycin) at the time of specimen collection. The causative agents for pneumonia included Diplococcus pneumoniae in 5 patients, Staphylococcus aureus in 1, and Proteus mirabilis in another. Diplococcus pneumoniae was missed in each of the 5 portions cultured separately from sputum of 3 patients. The 1 sample-single swab and the 5 samples-single swab methods were also not successful in recovering Diplococcus pneumoniae from the sputum of these 3 patients. In 2 of these patients (No. 84 and 62) the organism was isolated by the digestion-quantitation method and in the third patient (No. 72) the organism was isolated only after washing the specimen. Thus in all of the 25 patients with pneumonia studied, the 1 sample-single swab culture method could have missed the primary pathogen (Diplococcus pneumoniae) in 3 instances. Since the disease in 12 of the 25 patients with pneumonia was due to Diplococcus pneumoniae, it is apparent that this organism could have been missed entirely in one-fourth of the patients with pneumococcal pneumonia had the culture been performed by the one sample-single swab method. It is of interest to note that none of the other primary respiratory pathogens was entirely missed in culture in spite of the fact that the quantity may have varied significantly in different portions of the specimen.

Table 9 lists also two patients with tracheobronchitis in whom uneven distribution of the primary pathogen in various portions of sputum was demonstrated. Neither patient was receiving antimicrobial drugs. The pathogen in both patients was Hemophilus influenzae. The organism was present in all portions sampled but the quantity varied greatly (+ to +++) in different parts of the specimen.

Table 10 illustrates the presence of uneven distribution of potential pathogens in 17 specimens obtained from 16 patients without lower respiratory tract infection. Culture of an additional 46 specimens from 37 other patients without pneumonia or tracheobronchitis did not show significant variation in the quantity of the potential pathogens in different portions of the specimen. As shown in Table 10, Diplococcus pneumoniae was the potential pathogen isolated from 8 of the 16 patients who demonstrated uneven distribution of organisms in the specimen. Three Escherichia coli strains and 3 Hemophilus influenzae isolates were also found to be unevenly distributed. The one sample-single swab method missed the potential pathogen in 5 specimens. Culturing 5 samples with a single swab missed the potential pathogen in 4 specimens.

Comparison of Efficacy of Various Culture Methods in Recovering Potential Pathogens from Sputum

The efficacy of 4 culture methods in the recovery of pathogens from expectorated and non-expectorated sputa was tested. The methods used included; 1 sample-single swab, 5 samples-single swab, digestion-quantitation by NAC, and digestion-quantitation by DTT. All cultures were done prior to washing specimens.

Table 11 illustrates the results for 68 expectorated sputum specimens

Table 9 Uneven distribution of the primary pathogen in various portions of sputum from 9 patients with lower respiratory tract infection.

Patient Number	Bacterial Infection of Lower Respiratory Tract	Anti-microbial Therapy Prior to Collection	Method of Collection[a]	Primary Pathogen	1 sample-single swab	5 culture method[b]					5 samples-single swab
						A	B	C	D	E	
31	Pneumonia	No	EXP	D. pneumoniae	+++	-[c]	+++[d]	+++	+++	+++	+++
25	Pneumonia	No	ETS	D. pneumoniae	+++	+++	++	+++	+++	+	+++
15	Pneumonia	No	NTA	P. mirabilis	++	+	++	++	+++	++	+++
80	Pneumonia	No	NTA	S. aureus	+	+	+	+	+++	++	++
72	Pneumonia	No	NTA	D. pneumoniae[e]	-	-	-	-	-	-	-
84	Pneumonia	No	NTA	D. pneumoniae[f]	-	-	-	-	-	-	-
62	Pneumonia	Lincomycin	TTA	D. pneumoniae[f]	-	-	-	-	-	r	-
48	Tracheo-bronchitis	No	EXP	H. influenzae	++	++	+++	++	+	+++	+++
50	Tracheo-bronchitis	No	NTA	H. influenzae	+++	+	+	+++	+++	+++	+++

a EXP - Expectorated, NTA - Nasotracheal aspiration, ETS - Endotracheal suction, TR - Trachostomy specimen, TTA - Transtracheal aspiration

b Five portions of a sputum specimen cultured separately

c Culture negative

d Denote quantity of growth

e Organism was isolated from sputum after washing specimen

f Organism was isolated from sputum using the digestion-quantitation technique

Table 10 Uneven distribution of potential pathogens in various portions of 17 sputum specimens from 16 patients without pneumonia or tracheobronchitis.

Patient Number	Anti-microbial Therapy Prior to Collection	Method of Collection[a]	Potential Pathogen	1 sample-single swab	5 culture method[b]					5 samples-single swab
					A	B	C	D	E	
2	No	EXP	D. pneumoniae	+++	+++	+++	+++	-	-[c]	+++[d]
4	No	EXP	D. pneumoniae	+++	++	+	+++	+++	+	-
20	No	EXP	D. pneumoniae	-	+++	-	-	-	+++	-
21	No	EXP	D. pneumoniae	-	+++	+++	-	-	-	+++
44	No	EXP	D. pneumoniae	-	+++	+++	-	-	+++	+++
75	No	EXP	D. pneumoniae	-	-	+++	-	+++	-	-
22	No	EXP	D. pneumoniae	+++	+++	-	+++	+++	+++	+++
34 - I	No	EXP	D. pneumoniae	+++	-	+++	+++	+++	+++	-
II	Kanamycin	EXP	H. influenzae	++	+	-	++	+++	-	+++
54	Cephalothin	EXP	H. influenzae	++	++	+++	++	+++	+	++
33	No	EXP	H. influenzae	-	++	++	-	+++	++	+++
45	Nafcillin Dicloxicillin Neomycin	EXP	Klebsiella sp.	+++	+	-	+++	+++	+++	+++
57	Penicillin	EXP	E. cloacae	+	+++	++	+	+	+	+++
59	Ampicillin	EXP	E. coli	+++	++	+	+++	+	+++	+
74	Penicillin	EXP	E. coli	+++	+++	+++	+++	+++	-	+++
69	No	TR	E. coli	++	++	++	++	+	+++	++
47	Carbenicillin Gentamycin	NTA	K. pneumoniae	+	+	-	+	+++	++	+++

a EXP - Expectorated, NTA - Nasotracheal aspiration, TR - Trachostomy specimen

b Five portions of a sputum specimen cultured separately

c Culture negative

d Denote quantity of growth

from 64 patients. In 13 patients with pneumonia and 3 patients with tracheobronchitis, the primary pathogen(s) were recovered regardless of the method used for culture. Two potential pathogens (not responsible for pneumonia), 1 Staphylococcus aureus and 1 Hemophilus influenzae, were missed by the 1 sample-single swab and the 5 samples-single swab methods respectively. Fifty-two specimens from 48 patients without evidence of infection in the lower respiratory tract revealed 65 potential pathogens by the one sample-single swab and 68 by the 5 samples-single swab methods. The digestion-quantitation techniques isolated 74 and 77 organisms using NAC and DTT respectively.

The above results show that the 1 sample-single swab method is as effective as the 5 samples-single swab and the digestion-quantitation methods in the recovery of pathogens from expectorated sputa of patients with lower respiratory tract infection. The Student's t test revealed no significant (P less than .05) differences between the methods used.

Table 12 illustrates the results of 4 different methods used for culture of 26 non-expectorated sputum specimens derived from 26 patients. The 1 sample-single swab and the 5 samples-single swab methods missed the primary pathogen, Diplococcus pneumoniae, in 3 of 12 patients with pneumonia. These 3 specimens included 2 nasotracheal aspirates and 1 transtracheal aspirate.

The digestion-quantitation technique using NAC missed the primary pathogen, also a pneumococcus, in 2 specimens. The DTT method missed the primary pathogen (a pneumococcus) in 1. The primary pathogens were recovered from all 3 patients with tracheobronchitis by each of the 4 culture methods used.

Table 11 Value of 4 culture methods in the recovery of pathogens from 68 expectorated specimens derived from 64 patients[a].

Bacterial Infection Of Lower Respiratory Tract	Number of Specimens / Number of Patients	No. of primary pathogens isolated by:				No. of potential pathogens isolated by:			
		1 sample-single swab	5 samples-single swab	NAC[b]	DTT[b]	1 sample-single swab	5 samples-single swab	NAC[b]	DTT[b]
Pneumonia	13/13	13	13	13	13	10	10	11	11
Tracheo-bronchitis	3/3	4	4	4	4	0	0	0	0
Not present	52/48	0	0	0	0	65	68	74	77
Total	68/64	17	17	17	17	75	78	85	88

a All cultures were done prior to washing specimen

b Digestion-quantitation using NAC (n-acetyl-L-cysteine) and DTT (Dithiothreitol)

Table 12 Value of 4 culture methods in the recovery of pathogens from 26 non-expectorated specimens derived from 26 patients[a].

Bacterial Infection Of Lower Respiratory Tract	Number of Specimens / Number of Patients	No. of primary pathogens isolated by:				No. of potential pathogens isolated by:			
		1 sample-single swab	5 samples-single swab	NAC[b]	DTT[b]	1 sample-single swab	5 samples-single swab	NAC[b]	DTT[b]
Pneumonia	12/12	9	9	10	11	10	11	9	9
Tracheo-bronchitis	3/3	4	4	4	4	2	2	3	4
Not present	11/11	0	0	0	0	17	18	18	18
Total	26/26	13	13	14	15	29	31	30	31

a All cultures were done prior to washing specimen

b Digestion-quantitation using NAC (n-acetyl-L-cysteine) and DTT (Dithiothreitol)

Ten potential pathogens (not responsible for pneumonia) were recovered from patients with pneumonia by the 1 sample-single swab method. The 5 samples-single swab technique isolated 11 potential pathogens and the digestion-quantitation procedures using either NAC or DTT yielded 9. Two potential pathogens were recovered from patients with tracheobronchitis by the swab methods. The digestion-quantitation methods using NAC and DTT, revealed 3 and 4 isolates respectively. Almost identical numbers of potential pathogens were recovered from patients lacking evidence for lower respiratory tract infection by each of the culture methods used.

The above results show that the 1 sample-single swab method was as good as the more complex digestion-quantitation techniques and the 5 samples-single swab method in recovering pathogens from non-expectorated sputa of patients with lower respiratory tract infection. The Student's t test revealed no significant (P less than .05) differences between the methods used.

Value of Washing Sputum in Enhancing Recovery of Pathogens and Eliminating Normal Flora

Table 13 illustrates the value of washing expectorated sputum prior to culture in enhancing the recovery of pathogens and eliminating normal oropharyngeal flora. A total of 47 specimens obtained from 37 patients with and without lower respiratory tract infection were cultured after washing. The specimens were first cultured by the 1 sample-single swab method, then subjected to washing and cultured again using the same technique. A portion of each specimen was also subjected to digestion before and after washing. Both NAC and DTT were used as digestants.

The results in Table 13 show that washing the sputa from 8 patients with lower respiratory tract infection had no effect on enhancing the recovery of primary or potential pathogens nor on eliminating normal flora. Similarly, washing 39 specimens from 29 patients without pneumonia or tracheobronchitis did not improve the recovery of potential pathogens and gave inconsistant results in eliminating normal oropharyngeal flora. The Student's t test revealed no significant (P less than .05) differences between the methods used.

Table 14 illustrates the results of washing 12 non-expectorated sputum specimens prior to culture. Specimens obtained by transtracheal aspiration are not included in the Table. The primary pathogen was isolated from each of the 5 patients with pneumonia when the sputum was washed and then processed by the NAC method. Without washing, the NAC method recovered only 4 pathogens. The DTT method also isolated 4 of the 5 primary pathogens whether washing was done or not prior to culture. The 1 sample-single swab culture technique recovered 3 of the primary pathogens when sputum was cultured before washing, as compared to 4 isolates after washing. The Student's t test revealed no significant (P less than .05) differences between the methods used. Washing non-expectorated sputum prior to culture was not successful in enhancing the recovery of potential pathogens nor in eliminating normal flora from patients with or without bacterial infection of the lower respiratory tract.

A variety of other washing techniques were also evaluated on an additional 26 sputum specimens. The methods used included: serial plate washing, washing with centrifugation for various lengths of time

Table 13 Value of washing expectorated sputum prior to culture in recovering pathogens and eliminating normal flora.

Bacterial Infection Of Lower Respiratory Tract	Number of Specimens / Number of Patients	Culture Method[a]	Number of organisms before wash / Number of organisms after wash		
			Primary Pathogens[b]	Potential Pathogens[c]	Non-pathogens[d]
Pneumonia	6/6	A	6/6	4/4	7/7
		B	6/6	5/5	9/9
		C	6/6	5/5	7/9
Tracheo-bronchitis	2/2	A	3/3	0/0	4/4
		B	3/3	0/0	4/4
		C	3/3	0/0	4/4
Not present	39/29	A	0/0	50/46	78/74
		B	0/0	52/49	78/80
		C	0/0	46/45	78/77

a A - 1 sample-single swab
B - Digestion-quantitation using NAC
C - Digestion-quantitation using DTT

b Responsible for disease in lower respiratory tract

c Not responsible for disease in lower respiratory tract

d Normal flora

Table 14 Value of washing non-expectorated[a] sputum prior to culture in recovering pathogens and eliminating normal flora.

Bacterial Infection Of Lower Respiratory Tract	Number of Specimens / Number of Patients	Culture Method[b]	Number of organisms before wash / Number of organisms after wash		
			Primary Pathogens[c]	Potential Pathogens[d]	Non-pathogens[e]
Pneumonia	5/5	A	3/4	6/7	12/11
		B	4/5	6/6	8/10
		C	4/4	7/6	9/9
Tracheo-bronchitis	1/1	A	1/1	0/1	3/2
		B	1/1	1/1	3/3
		C	1/1	1/1	3/3
Not Present	6/6	A	0/0	8/8	10/11
		B	0/0	8/8	10/11
		C	0/0	9/8	11/12

a Not including specimens collected by transtracheal aspiration

b A - 1 sample-single swab
B - Digestion-quantitation using NAC
C - Digestion-quantitation using DTT

c Responsible for disease in lower respiratory tract

d Not responsible for disease in lower respiratory tract

e Normal flora

and at various speeds, and a sieve technique in which the sputum was placed in a wire mesh and rinsed with various quantities of water. None of these techniques was found to be useful in eliminating normal oropharyngeal flora or enhancing the recovery of potential pathogens.

Quantitation of Primary Pathogens in Sputum Specimens of Patients With Lower Respiratory Tract Infection

Quantitation of the primary pathogen isolated by 4 culture methods from sputum of patients with lower respiratory tract infection is illustrated in Tables 15, 16, and 17. The culture methods used included: 1 sample-single swab, 5 samples-single swab, digestion-quantitation using NAC, and digestion-quantitation using DTT. All specimens were processed prior to washing.

Quantitative results of the primary pathogens isolated from 13 expectorated sputum specimens of patients with pneumonia are shown in Table 15. In each specimen, the primary pathogen was recovered in moderate (+++) amounts by the swab techniques. The digestion-quantitation procedure using NAC revealed these organisms to be present in concentrations ranging from 2×10^5 - 4×10^8 organisms/ml of sputum. Nine of the 13 specimens (69%) contained the primary pathogens in quantities of 1×10^7 organisms/ml of sputum or greater. Digestion-quantitation using DTT revealed the organisms to be present in a range from 4×10^5 - 6×10^8 organisms/ml of sputum. Seven of the 13 specimens (54%) contained the primary pathogen in a concentration of 1×10^7 organisms/ml of sputum or greater. The mean value of the two digestion-quantitation techniques shows that the quantity of primary pathogens in expectorated sputum of patients with pneumonia

Table 15 Quantity of primary pathogen isolated from expectorated sputum of 13 patients with pneumonia

Patient Number	Anti-microbial Therapy	Primary Pathogen Isolated	Quantity of primary pathogen in culture before wash				
			1 sample-single swab	5 samples-single swab	Digestion-quantitation		
					NAC	DTT	Mean[a]
1	No	*D. pneumoniae*	+++	+++	4×10^6	4×10^5	9×10^5
24	No	*D. pneumoniae*	+++	+++	3×10^8	4×10^8	3.5×10^8
31	No	*D. pneumoniae*	+++	+++	1.5×10^8	3×10^7	7.3×10^7
68	No	*D. pneumoniae*	+++	+++	1×10^8	1×10^8	1×10^8
78	No	*D. pneumoniae*	+++	+++	2×10^5	4×10^5	3×10^5
83	No	*D. pneumoniae*	+++	+++	6×10^5	1×10^6	8.5×10^5
32	Penicillin	*D. pneumoniae*	+++	+++	1×10^8	5×10^7	7.5×10^7
60	No	*S. aureus*	+++	+++	1×10^7	1×10^6	6×10^6
41	No	*S. aureus*	+++	+++	1×10^6	3×10^6	2×10^6
18	No	*E. coli*	+++	+++	1×10^7	2×10^6	6.5×10^6
30	Ampicillin	*E. coli*	+++	+++	6×10^7	1.2×10^7	3.8×10^7
79	Penicillin	*E. aerogenes*	+++	+++	4×10^8	6×10^8	5×10^8
81	Penicillin Cephalothin	*P. aeroginosa*	+++	+++	2.8×10^7	1×10^7	1.9×10^7

a Mean number of organisms recovered from the two digestion-quantitation procedures (NAC and DTT)

ranges from $3 \times 10^5 - 5 \times 10^8$ organisms/ml of sputum.

Table 16 lists the quantitative results from the culture of non-expectorated sputum of 12 patients with pneumonia. The swab methods failed to isolate the primary pathogen from sputum of 3 patients. The digestion-quantitation technique using NAC missed two of these pathogens, while the DTT method missed only 1. The organism responsible for pneumonia in this latter patient was a pneumococcus and it was recovered from the specimen only after washing. Approximately 63% of the specimens revealed the primary pathogen to be present in moderate (+++) amounts when cultured by the swab methods. The digestion-quantitation techniques using NAC or DTT revealed the primary pathogens to be present in a concentration ranging approximately from less than $1 \times 10^3 - 3 \times 10^8$ organisms/ml of sputum. Five of 12 specimens (42%) revealed the primary pathogen of pneumonia to be present in a concentration of 1×10^7 organisms/ml of sputum or greater.

The quantitation of primary pathogens from 6 patients with tracheobronchitis is illustrated in Table 17. One specimen showed the primary pathogen (Hemophilus influenzae) to be present in light (++) amounts. All of the other isolates were present in moderate (+++) amounts when cultured by the swab methods. The mean of the 2 digestion-quantitation techniques reveals the primary pathogens to be present in a range from $1.5 \times 10^5 - 1.8 \times 10^9$ organisms/ml of sputum. Seven of 8 isolates were present in a concentration of 1×10^7 or greater using DTT, 6 were present in this quantity when the NAC method was used.

The digestion-quantitation techniques were also used for processing 63 sputum specimens from 53 patients lacking definitive evidence of lower

Table 16 Quantity of primary pathogen isolated from non-expectorated sputum of 12 patients with pneumonia.

Patient Number	Anti-microbial Therapy	Primary Pathogen Isolated	Method of Collection[a]	Quantity of primary pathogen in culture before wash				
				1 sample-single swab	5 samples-single swab	Digestion-quantitation		
						NAC	DTT	Mean[b]
13	No	D. pneumoniae	NTA	+++[c]	+++	5×10^7	8×10^7	6.5×10^7
72	No	D. pneumoniae	NTA	-[d]	-	-[e]	-	-
84	No	D. pneumoniae	NTA	-	-	1×10^8	1×10^8	1×10^8
25	No	D. pneumoniae	ETS	+++	+++	1×10^6	5×10^5	8×10^5
62	Lincomycin	D. pneumoniae	TTA	-	-	-	1×10^3	5×10^2
35	No	H. influenzae	TR	+++	+++	2×10^6	2×10^6	2×10^6
82	Cephalexin	H. influenzae	NTA	+++	+++	2×10^7	2×10^8	7×10^7
9	Cephalothin	H. influenzae	TTA	+++	+++	1×10^8	3×10^8	2×10^8
80	No	S. aureus	NTA	+	++	5×10^4	4×10^4	4.5×10^4
38	Penicillin	S. aureus	NTA	+++	+++	4.5×10^6	2×10^6	3.3×10^6
15	No	P. mirabilis	NTA	++	+++	1×10^5	1×10^5	1×10^5
3	Nafcillin	P. maltophilia	TTA	+++	+++	1×10^8	2×10^7	6.5×10^7

a NTA - Nasotracheal aspiration, ETS - Endotracheal suction, TR - Trachostomy specimen, TTA - Trans-tracheal aspiration

b Mean number of organisms recovered from two digestion-quantitation procedures (NAC and DTT).

c Denote quantity of growth

d Culture negative

e Organisms present in a concentration less than 1×10^3 would not be isolated because the lowest dilution used was 10^{-3}

Table 17 Quantity of primary pathogen isolated from sputum of 6 patients with tracheobronchitis.

Patient Number	Primary Pathogen Isolated	Method of Collection[a]	Quantity of primary pathogen in culture before wash				
			1 sample-single swab	5 samples single swab	Digestion-quantitation		
					NAC	DTT	Mean[b]
42	D. pneumoniae	EXP	+++[c]	+++	1×10^8	1×10^8	1×10^8
28	D. pneumoniae	NTA	+++	+++	9×10^6	1×10^7	9.5×10^6
39	D. pneumoniae	NTA	+++	+++	6×10^7	1×10^8	8.5×10^7
48	H. influenzae	EXP	++	+++	1×10^5	2×10^5	1.5×10^5
70	D. pneumoniae	EXP	+++	+++	1.1×10^9	2.5×10^9	1.8×10^9
	H. influenzae	EXP	+++	+++	4×10^8	2×10^8	3×10^8
50	D. pneumoniae	NTA	+++	+++	1×10^7	1×10^7	1×10^7
	H. influenzae	NTA	+++	+++	4×10^7	2×10^8	8×10^7

a EXP - Expectorated, NTA - Nasotracheal aspiration

b Mean number of organisms recovered from two digestion-quantitation procedures (NAC and DTT)

c Denote quantity of growth

respiratory tract infection. The concentration of potential pathogens ranged from less than 1 X 10^3 - 2 X 10^9 organisms/ml of sputum. Twenty-two specimens (35%) contained potential pathogens in a concentration of 1 X 10^7 organisms/ml of sputum or greater.

Sources of Methodologic Error in the Quantitative Culture

The probability of the 2 calibrated loops used in this study being different was examined and the Student's t test revealed no significant (P less than 0.15) difference.

Cultures taken with both loops from the same suspension of Escherichia coli, revealed no significant differences between random pairs of bacterial counts (Student's t - P less than 0.1).

The toxic effects of NAC and DTT were tested. The Student's t test revealed no significant difference between PBS and NAC (P less than 0.5 at 0 minutes and P less than 0.25 at 15 minutes), between PBS and DTT (P less than 0.4 at 0 minutes and P less than 0.25 at 15 minutes), or between NAC and DTT (P less than 0.4 at 0 minutes and P less than 0.2 at 15 minutes).

DISCUSSION

The present study evaluated the efficacy of 4 methods used for sputum culture prior to washing. These comprised the 1 sample-single swab (simple routine laboratory method), 5 samples-single swab, and digestion-quantitation methods using either NAC or DTT. The 1 sample-single swab method was successful in isolating 28 primary pathogens from 31 patients with lower respiratory tract infections. The 5 samples-single swab recovered 28 primary pathogens, the NAC method isolated 29, and the DTT method isolated 30. It is evident from these results that the more elaborate digestion-quantitation method did not significantly increase the isolation rate for primary pathogens from patients with pneumonia or tracheobronchitis.

Similar results were reported by Bartlett and Melnick (3). They compared the 1 sample-single swab method with a digestion-quantitation technique using DTT. Their data showed that the homogenization and quantitative culture failed to decrease the frequency of mixed non-pathogenic flora or increase the recovery of potential pathogens from patients with acute pulmonary infection. Kilbourn et al. (17) in a similar study compared the 1 sample-single swab method with a digestion-quantitation method. They reported that fewer isolates of potential pathogens were recovered using the quantitative method which was probably due to the failure of their technique to recover organisms in a concentration less than 10^4 organisms/ml. Massaro (28), in a study including 75 specimens, reported no increase in the recovery of pathogens using a mechanical homogenization technique.

In view of these findings, one should seriously question the value of the digestion-quantitation method for routine use to culture sputum specimens in the clinical laboratory. Not only are they elaborate and time consuming, but they are also much more expensive tests than the simple 1 sample-single swab method.

The digestion-quantitation technique may, however, prove useful in certain specific situations. Pirtle et al. (39) reported that quantitative cultures made of fresh homogenized sputum were helpful in distinguishing between superinfection and colonization (acquisition) in patients with pneumonia. These workers have arbitrarily defined superinfection as the appearance of a microorganism other than Streptococcus viridans or Neisseria in the sputum at a concentration greater than 10^5 organisms/ml, in addition to the primary pathogen. In their study superinfection was detected in 7 patients and in each instance it was caused by Gram negative organisms. They defined colonization (acquisition) as the appearance of new organisms in the sputum of at least 10^3/ml. All superinfections were associated with the administration of multiple antimicrobial drugs given in high doses intravenously.

Louria (24) has utilized the digestion-quantitation method for separating the harmless carrier state (colonization) from the potentially dangerous bacterial superinfection. He found that the superinfection rate was highest in patients receiving multiple antibiotics. He has also found that the quantitative technique was useful in evaluating the effectiveness of antibiotic treatment. Successful therapy was associated with a gradual decrease in the numbers of pathogenic organisms isolated from sputum specimens.

Our results also show that the digestion-quantitation methods using either NAC or DTT were equally effective in recovering the primary pathogens from patients with lower respiratory tract infection. Both methods recovered the pathogen in roughly the same concentration.

Several investigators have examined the value of liquefying sputum in enhancing culture results. A variety of agents were used in these studies. Woodham and Mead (54) compared pancreatin and NAC as sputum liquefying agents for the culture of organisms other than mycobacteria. They found that NAC liquefied sputum more rapidly than pancreatin (5-10 minutes compared to 60-90 minutes) and had a slight advantage in the isolation of Hemophilus species. With organisms other than Hemophilus species, the isolation rates were similar.

Hurst et al. (13) compared the sputum liquefying activity of NAC with trypsin and pancreatic dornase. They found that NAC was the most active agent in decreasing sputum consistency. These workers also reported that NAC was useful in the clinical management of patients with tenacious sputum. Similar results have also been reported by Sheffner et al. (45). Shaw and Dye (43) compared NAC and DTT in processing sputum from patients with pulmonary tuberculosis. Both agents were equally effective in isolating mycobacteria in culture.

In a study designed to compare the sputum liquefying ability (not culture results) of NAC and DTT, Lieberman (21) reported that NAC caused liquefaction of both purulent and mucoid specimens when used in concentration between 0.14 and 1.4 M. DTT, on the other hand, was found to have a greater mucolytic action than NAC at lower concentrations (0.1 to 0.001 M). Hirsch et al. (10) in a similar study evaluated the

sputum liquefying efficacy of 11 agents. The most effective group of agents were those containing a reactive sulfhydryl group such as NAC or DTT.

In a study of 19 patients with pneumonia prior to the administration of antimicrobial therapy, Monroe et al. (34) utilized a quantitative method for culture of liquefied sputum using 2% NAC. They found that the primary pathogens (organisms responsible for pneumonia) were present in populations of 10^7 organisms/ml or greater. Normal oropharyngeal flora, on the other hand, were present in a concentration less than 10^6 organisms/ml of sputum.

The results reported in this study show that the primary pathogen isolated from patients with bacterial infections of the lower respiratory tract was not always present in a concentration of 1×10^7 or greater. In 16 patients with pneumonia studied prior to the administration of antimicrobial therapy, the NAC method recovered the primary pathogen in a concentration less than 10^7 from 8 patients. The DTT method isolated the primary pathogen in a concentration less than 10^7 from 6 patients. In the patients with pneumonia studied while on antimicrobial drugs, both the NAC and DTT methods recovered the primary pathogen in a concentration less than 10^7 from 2 of 9 patients. Our data on 6 patients with acute tracheobronchitis, however, show that the primary pathogens were present in a concentration of 10^7 organisms/ml or greater in all but 1. None of these patients were receiving antimicrobial drugs prior to specimen collection. The normal flora in the sputum of patients with pneumonia or tracheobronchitis were present in concentrations ranging from 1×10^3 - 2.6×10^9

organisms/ml of sputum. Our study also showed that potential pathogens were recovered in concentrations of 10^7 organisms/ml or greater from 35% of the sputum specimens collected from 53 patients lacking definitive evidence for lower respiratory tract infection. It appears, therefore, that the use of a single breakpoint of 10^7 organisms/ml or greater to signify the presence of bacterial infection of the lower respiratory tract is not always accurate. The present study shows that not only the primary pathogens can be recovered from patients with pneumonia in a quantity less 10^7 organisms/ml, but the potential pathogens can also be present in the sputum of patients without lower respiratory tract infection in quantities of 10^7 organisms/ml or greater.

The results reported in this study show that washing sputum of patients with or without lower respiratory tract infection was not an effective procedure for enhancing the recovery of pathogens nor eliminating the normal flora. The primary pathogen responsible for the disease in patients with pneumonia or tracheobronchitis was as readily recovered from the sputum specimens before washing as after washing. A number of investigators have examined the value of washing sputum prior to culture. All of these reports were done on specimens submitted to routine microbiology laboratories for culture. In these studies, no attempts were made to evaluate the washing technique specifically for sputum from patients with bacterial pneumonia. Dixon and Miller (6) compared methods for culturing 250 sputum specimens submitted to the routine laboratory. One portion of the sputum was cultured after washing; the remainder of the specimen was liquefied by pancreatin and serial dilutions were used for culture. The method used for washing consisted of taking up a purulent portion of sputum on a wire loop,

immersing and agitating it in 3 ml of peptone water for 20 seconds, and inoculating appropriate media. Culture plates inoculated with a 10^{-4} dilution of homogenized sputum gave 70% clearcut results (either negative or positive for 1 single bacterial species); whereas direct culture of washed sputum generally gave mixtures of organisms (3% clearcut results). Kilbourn et al. (17) have also examined the value of washing sputum from patients with cystic fibrosis in enhancing the recovery of pathogenic organisms. These workers utilized a liquefaction-quantitation technique. Their results showed that washing the sputum did not significantly alter the isolation of pathogens.

Lapinski et al. (18) evaluated washing of sputum from patients with bronchitis. A large loopful of sputum, selected from the purulent portion, was dispersed in 10-15 ml of sterile physiologic saline and rinsed for 5-10 seconds. This procedure was repeated twice. One of the purulent flecks was removed with a loop and cultured. The results obtained from 120 purulent specimens showed that washing increased the recovery of potential pathogens from 85 isolates before to 108 isolates after washing.

Results from the present study show that the pneumococcus was the most frequent pathogen responsible for the pneumonia in the 25 patients studied. This organism accounted for the disease in 12 of these patients. This finding is consistent with other reports. Sullivan et al. (50) reported Diplococcus pneumoniae to be the etiologic agent in 62% of patients with pneumonia. Hoeprich (11) reported the pneumococcus to be responsible for 90% of all bacterial pneumonias.

Our data showed that the primary pathogen responsible for pneumonia

was isolated from the blood of 2 patients out of a total of 8 patients from whom blood cultures were performed. Tillotson and Finland (51) reported that blood cultures were positive in 12% of the 149 patients with pneumonia. Bailey and Scott (1) reported that one-third of patients with untreated pneumococcal pneumonia have an associated bacteremia. Hoeprich (11) stressed the importance of collecting blood cultures from patients suspected of having pneumonia.

Our results show that the infection in 3 of the patients with pneumonia was hospital associated. These patients were in the hospital for a varying period of time prior to the development of pneumonia. Gram negative rods were the primary pathogens responsible for these cases. A number of investigators have recognized the importance of the Gram negative rods in hospital acquired infections. Johanson et al. (14) examined the oropharyngeal flora and found that hospitalized patients frequently become colonized with Gram negative bacilli. The colonization correlated best with the clinical severity of illness. These workers stressed the importance of this finding in the pathogenesis of bacterial pneumonias in the hospitalized patients which almost always begins with the aspiration into the lung of bacteria present in the upper respiratory tract. In a later study (15) these investigators reported that 45% of patients admitted to a medical intensive care unit became colonized in the oropharynx with Gram negative rods, and 23% of these colonized patients developed nosocomial respiratory infections. Tillotson and Finland (51) also reported that Gram negative bacilli are the most frequent pathogens cultured from the sputum of patients receiving antimicrobial therapy for pneumonia. Lorian and Topf (25) reported

that three-fourths of all organisms from nosocomial infections of the lower respiratory tract were caused by Gram negative bacilli. Similar results have also been reported by Glover and Jolly (8).

The results reported in this study demonstrated that primary and potential pathogens can be unevenly distributed in sputum. The culture of expectorated specimens from 16 patients with lower respiratory tract infection showed uneven distribution for 6 of 28 potential pathogens isolated. In the non-expectorated specimens obtained from 15 patients, 7 out of 30 potential pathogens isolated were found to be unevenly distributed. Sputum specimens from patients lacking definitive evidence for lower respiratory tract infection have also demonstrated uneven distribution of potential pathogens.

The irregular distribution of pathogens in the sputum of patients with lower respiratory tract infection may lead to entirely negative results in culture if the portion sampled happened to lack the organism in question. In our study when the 1 sample-single swab was used for culture, the primary pathogen responsible for pneumonia was missed entirely in 3 of 25 patients. The pathogen in all of the 3 patients was Diplococcus pneumoniae. Only 1 of these 3 patients was on antimicrobial therapy prior to sputum collection.

It is of interest to note that pathogens other than pneumococcus were found to be unevenly distributed in sputum specimens of patients with pneumonia but none were missed when 1 sample was cultured.

May (31) compared the results of culturing 5 portions from the sputa of 54 expectorated specimens collected from patients with chronic bronchitis. Uneven distribution was demonstrated for Diplococcus pneumoniae

in 71% of specimens, Staphylococcus aureus in 71%, and Hemophilus influenzae in 57%. Similar results were reported by Rawlins (40) who compared culture results from various portions of sputa from 50 patients with chronic bronchitis.

It is not clear why the pneumococcus, unlike other pathogens, was absent from certain portions of the sputum specimens in patients who had pneumococcal pneumonia. Barrett-Connor (2) reported recently that routine sputum culture (1 sample-single swab method) of patients with pneumococcal pneumonia associated with bacteremia, missed the pneumococcus in nearly 50% of the cases examined. The failure to recover pneumococcus in culture can be due to an inadequate specimen, in other words not good sputum, or to the uneven distribution of Diplococcus pneumoniae in sputum. All of the specimens used in our study represented good sputum samples as evidenced by purulence both grossly and microscopically.

Lepow et al. (19) have similarly found that pneumococcus could not be identified on culture from nearly 50% of sputum specimens showing many Gram positive Diplococci on the smear. It is obvious that some of the organisms on the smear may have been other streptococci. It is evident, however, from our studies and the study of Barrett-Connor that the pneumococcus can be missed when 1 sample is used for culture by the routine method. Furthermore, our studies show that neither the 5 samples-single swab method nor the digestion-quantitation methods used for culture of sputum prior to washing were 100% successful in recovering the pneumococcus. One possible explanation for this phenomenon may be due to an antagonistic effect of products produced

by other bacterial populations found in the specimen upon the pneumococcus. Sprunt and Redman (49) in a study of 53 adults and 84 children reported in vitro tests indicating that strains of Streptococcus viridans inhibited the growth of some other organisms. The mechanism of the interbacterial inhibition is not clearly understood but in their study the addition of catalase reversed the effect in some instances, thus hydrogen peroxide would seem to play a role. They have also demonstrated that media depletion and decreasing pH were not important factors in the mechanism of inhibition. The almost invariable contamination of expectorated sputum by pharyngeal organisms plus the undesirable but usual delay in transport of the specimen to the laboratory permits overgrowth of other bacteria. This was evidenced in Barrett-Connor's study which showed that another potential pathogen was recovered from sputum culture from 20% of specimens from patients with a pneumococcal isolate. Our results also show similar findings.

It is tempting to speculate that prior antibiotic usage may contribute to uneven distribution of the pathogens in various portions of the sputum specimen. Our results, however, clearly show that this is not an important factor. Of the 9 patients who demonstrated uneven distribution of the primary pathogens in the sputum, only 1 was on antibiotic therapy at the time of specimen collection. This patient was on lincomycin for 1 day before sputum was obtained by transtracheal aspiration. In this case, the organism, a pneumococcus, was recovered by the digestion-quantitation technique using DTT. Another mechanism that should be entertained particularly for the pneumococcus is that this organism may undergo autolysis in certain parts of the sputum or

its viability may be seriously affected by the action of antibody or other non-specific inhibitors found in bronchial secretions. It is known that old cultures of pneumococci frequently undergo autolysis (1). It would be of interest to examine the distribution of pneumococci in different parts of sputum specimens of patients with pneumococcal pneumonia not only by the conventional culture method but also by immunologic tests utilizing the Quellung reaction. The latter may identify pneumococcus in certain portions of the specimen which may yield negative culture results. This would provide support for the hypothesis that the organism is actually present but is inhibited by some other factor. Lapinski et al. (18) found a much higher yield of Hemophilus influenzae from saline washed sputa than from unwashed specimens. They reported that a combination of Neisseria catarrhalis and mucin was responsible for the inhibition of Hemophilus influenzae growth in culture.

SUMMARY

Some reports have recently advocated the culture of washed sputum or the use of a digestion-quantitation procedure instead of the routine culture method currently employed in the clinical laboratory. The use of such elaborate methods for the routine culture of sputum undoubtedly will result in increased cost to the patient. The present study evaluated 4 culture methods: 1 sample-single swab, 5 samples-single swab, digestion-quantitation using NAC, and digestion-quantitation using DTT. A total of 94 sputum specimens obtained from 25 patients with pneumonia, 6 patients with tracheobronchitis, and 53 patients without lower respiratory tract infection were processed by these methods. All of the respiratory tract infections in this group of patients were caused by aerobic or facultative anaerobic organisms. The 1 sample-single swab method was found to be as effective as the 5 samples-single swab and the digestion-quantitation techniques in recovering the primary pathogens from patients with pneumonia or tracheobronchitis. The Student's t test revealed that the differences between the methods was not statistically significant ($P < .05$).

The pneumococcus was the most frequently isolated pathogen from the pneumonia group (48%). Hemophilus influenzae and Diplococcus pneumoniae were the 2 main pathogens isolated from patients with acute tracheobronchitis.

Uneven distribution of the primary pathogen was detected in various portions of the sputum from 7 patients with pneumonia and 2 patients with tracheobronchitis. The pneumococcus was found to be unevenly distributed in the specimens from 5 of the patients with pneumonia. The swab methods

missed it in three instances. The digestion-quantitation methods also missed it in 2 patients when NAC was used and in 1 when DTT was used. Antibiotic therapy was not a factor responsible for the uneven distribution of pathogens in the specimens. Only 1 out of the 9 patients that exhibited irregular distribution of the primary pathogen was on antimicrobial therapy. Uneven distribution was also demonstrated for potential pathogens in 17 sputum specimens from 16 patients without lower respiratory tract infection.

The value of washing prior to culture was evaluated. Washing had no effect on enhancing the recovery of pathogens nor eliminating normal oral flora.

The quantity of the primary pathogen in patients with pneumonia varied in the expectorated specimens from 3×10^5 - 5×10^8 organisms/ml of sputum, and in non-expectorated sputa from less than 1×10^3 - 2×10^8 organisms/ml. The primary pathogens isolated from patients with tracheobronchitis ranged in concentration from 1.5×10^5 - 1.8×10^9 organisms/ml of sputum. In 13 of 25 patients with pneumonia and 2 of 6 patients with tracheobronchitis the primary pathogen was isolated in quantities less than 10^7 organisms/ml of sputum. In patients without lower respiratory tract infection, potential pathogens were isolated from sputa in concentration of 1×10^7 organisms/ml or greater from 22 of 63 specimens.

These data do not justify the claims that culture of washed sputum or the use of the digestion-quantitation techniques are superior to the routine method (culture of 1 sample of unwashed sputum by a single swab) currently employed in many clinical laboratories. Results of quantitative culture also indicate that the presence of a pathogen in the sputum in a concentration of 10^7 organisms/ml or greater is not always indicative of lower respiratory tract infection.

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