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THE BACTERIAL FLORA OF THE RESPIRATORY TRACT. SOME KNOWNS AND UNKNOWNS

The upper respiratory tract of man and terrestrial mammals, composed in part of the nose, nasopharynx, mouth and oropharynx, harbors an extraordinarily varied bacterial flora. Rosebury, in his book entitled *Microorganisms Indigenous to Man*[1] lists no less than 21 genera of bacteria (Table 1)

TABLE 1. GENERA OF BACTERIA INHABITING THE UPPER RESPIRATORY TRACT*

Staphylococcus	Klebsiella
Streptococcus	Proteus
Peptostreptococcus	Pseudomonas
Diplococcus	Moraxella
Neisseria	Mima
Veillonella	Haemophilus
Lactobacillus	Bacteroides
Corynebacterium	Fusobacterium
Leptotrichia	Spirillum
Escherichia	Vibrio
Paracolobactrum	

* Modified from Reference 1, pp. 318-321.

that may be isolated from the mucous membranes lining these structures. Although the lower respiratory tract is in direct continuity with the secretions draining the upper respiratory tract, it is, by contrast, normally sterile.[2-4] This remarkable difference is brought about by a series of coordinated mechanisms of the mammalian host which act to protect the tracheobronchial tree and pulmonary parenchyma from infection. Only when injury, acute or chronic, has occurred does bacterial infection of the lower respiratory tract usually arise. There are some exceptions to this generalization such as diptheria and perhaps pertussis, pneumonic plague and tularemia, and tuberculosis; but in general, this view seems valid.

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There is little if any evidence that bacterial infection of the lower respiratory tract plays a significant primary role in the development of chronic obstructive bronchitis or of pulmonary emphysema. It would appear rather that, once structural or physiological changes or both have occurred in the structures of the lower respiratory tract, both saprophytic and pathogenic bacteria may obtain a foothold that would have been denied them in normal subjects. Having done so, they may bring about further impairment of ventilatory and respiratory functions and threaten both the well-being and life of the patient. It would not be possible in the time allotted to consider the entire bacterial flora of the respiratory tract. Instead, an attempt will be made to highlight some of the areas of our ignorance, which are legion, and to indicate certain lines of approach that may be useful in future studies. By so doing, others may be moved to explore further some of the bacteriologic problems of respiratory infection which are sorely in need of solution. Among these are the means whereby pathogenic bacteria injure the respiratory tract, the classification and potentially pathogenic role of those bacterial species often looked upon as saprophytes or as opportunist pathogens, and the discovery of more suitable experimental hosts in which to study the behavior of these organisms.

Of the bacteria isolated from the sputum and from tracheobronchial secretions of patients with chronic obstructive airway disease, most attention has been focused upon *Diplococcus pneumoniae, Haemophilus influenzae,* coagulase-positive *Staphylococcus aureus, Streptococcus pyogenes* and the enterobacteriaceae. Although alpha and nonhemolytic streptococci. neisseriae and coagulase-negative staphylocci have often been found associated with the aforementioned organisms in specimens obtained directly from the lower respiratory tract,[3,4] little emphasis has been placed upon their potential role as infectious agents because of their recognized limited capacity to invade the tissues of normal individuals. Whether or not such an attitude is justifiable is perhaps open to question. In considering the flora of the respiratory tract, I should like to comment briefly upon our observations on some of the species indigenous to it and to indicate additional areas in which further studies are needed.

Pneumococcal infection

Although pneumococcal infection is no longer regarded by many to be as prevalent or as serious as it was two decades ago, available evidence suggests that it occurs as frequently as it did in the era antedating the availability of antimicrobial drugs. Investigations by the author of the incidence of primary pneumococcal pneumonia in several American cities in the past 15 years indicate that 40 to 50 bacteremic infections with this or-

ganism will be observed per annum for every 200 to 250 ward beds on a city hospital medical service. Many of those so afflicted are individuals with underlying systemic illness, including disease of the respiratory tract. In addition to giving rise to pneumonia, pneumococcus appears to be an important cause of intercurrent purulent bronchitis in patients with chronic obstructive airway disease.[5] In this latter role, it shares an importance comparable to that of *Haemophilus influenzae.* When sought with the aid of well-established bacteriologic techniques, it is commonly present in the respiratory secretions of patients with respiratory disease. From 2,485 cultures of the respiratory tract examined between July 1, 1966 and June 30, 1967, 371 strains of pneumococcus were isolated. This figure is a crude one, not having been adjusted for patients receiving antimicrobial drugs inhibitory to pneumococcus before or after admission to the hospital. The distribution of pneumococcal types isolated is similar, with few exceptions, to that observed prior to the advent of antibiotics. The virtual absence of pneumococcus Type II from the Eastern United States for the past two decades has been noteworthy.

Even though it is still an important respiratory pathogen, almost nothing is known about the means whereby pneumococcus brings about tissue injury and death. Solution of this problem may be of considerable importance in reducing both the morbidity and mortality resulting from pneumococcal infection. Of potential value, in the meantime, to persons of high risk of injury from pneumococcal infections is polyvalent pneumococcal vaccine.[6] It is hoped that such vaccine may become available again in the not too distant future.

Streptococci in the respiratory tract

The alpha and nonhemolytic streptococci are almost universal inhabitants of the upper respiratory tract. Though they share a number of properties in common with pneumococcus, they are regarded by many as playing a negligible role in chronic bronchial disease. This view is based in part upon the observations that elimination of these organisms from respiratory secretions may not be followed by diminution of the purulence of the sputum of bronchitics with infection and that relapse following antimicrobial therapy may not occur promptly after the reappearance of such streptococci in the sputum.[7] Although these arguments may have some validity, the alpha and nonhemolytic respiratory streptococci are so commonly present in infected respiratory secretions and resemble pneumococci in such a variety of attributes that we have thought it worthwhile to attempt to learn more about this heterogeneous and poorly classified group of organisms.

Like pneumococcus, most of the alpha and nonhemolytic streptococci of the respiratory tract are capsulated, a fact that has been overlooked frequently in attempts to classify these organisms serologically in the past. Awareness of this fact has been heightened by the availability of a serologic reagent designated "omni-serum" which reacts with all 82 of the capsular serotypes of pneumococcus.[8] In the examination of sputum spreads for the presence of pneumococci with this reagent, it has been observed not infrequently that organisms in chains resembling streptococci give a small but definitely positive quellung or capsular precipitin reaction. As early as 1925, the production of capsular antigens by streptococci was noted by Lancefield[9] and, in 1941, Mørch[10] described the occurrence of a capsular cross-reaction between a streptococcus and pneumococcus. These facts have led to a systematic study of the capsular antigens of alpha and nonhemolytic streptococci with the following results.

From 196 cultures of the respiratory tract containing colonies of streptococci, 98 or 50 per cent included capsulated organisms cross-reacting with pneumococcus (Table 2). Twenty cultures yielded two strains of streptococcus each cross-reacting with a different pneumococcal serotype, and three cultures contained three serologically distinct cross-reacting strains of streptococci. To date, cross-reactions between alpha and nonhemolytic streptococci and 22 capsular serotypes of pneumococci have been identified (Table 3). Among these cross-reactions are several between streptococci classified in Lancefield's groups F and K. Group F streptococcus Type II cross-reacts with pneumococcus Type 21 and Type 7 or 51, Type III (*Streptococcus MG*) with pneumococcus Type 13, and Type V with pneumococcus Type 17. Cross-reactions of streptococci in Group K include strains giving capsular precipitin reactions with antisera to pneumococci of Types 36 and 47. It is noteworthy that antisera for the grouping of streptococci in Lancefield's groups F and K are prepared both in this country and in Great Britain with streptococci endowed with sufficient capsular poly-

TABLE 2. STREPTOCOCCI CROSS-REACTING WITH PNEUMOCOCCI IN CULTURES OF THE HUMAN RESPIRATORY TRACT

Source	*Total cultures*	*Total positive*	*Number of cross-reacting types per culture*		
			1	*2*	*3*
Sputum	172	80 (46%)	61	16	3
N. P.	24	18	14	4	0
	—	—	—	—	—
Total	196	98	75	20	3

TABLE 3. ALPHA AND NONHEMOLYTIC STREPTOCOCCI FROM THE HUMAN RESPIRATORY TRACT CROSS-REACTING WITH PNEUMOCOCCI

Pneumococcal type	*Streptococci (No. of strains)*	*Pneumococcal type*	*Streptococci (No. of strains)*
5	1	36	10
9	7	39	3
13	11	43	1
	8(MG)	47	1
15	6	52-75	3
20	3	59	98
21	10	69	1
24	1	72	18
27	1	75	12
32	1	80	1
33	5	82	1
34	1		

saccharide to stimulate formation of anticapsular antibodies. These antisera are not specific for the cell wall polysaccharides of their respective streptococcal groups and yield positive precipitin and quellung reactions with organisms possessing related capsular polysaccharides, a situation that may lead to errors in streptococcal classification.

Because of the evidence that many alpha and nonhemolytic respiratory streptococci produce capsular polysaccharide, antisera have been prepared with a number of strains of filamentous streptococci that do not cross-react with pneumococcus and that have been isolated from the respiratory tract. To date, 14 serotypes have been identified and given provisional type designations 83 through 96 to avoid ambiguity vis à vis pneumococcal capsular serotypes. The frequency of these strains is shown in Table 4.

Serologic cross-reactions between pneumococcus and alpha and nonhemolytic streptococci are not limited to those between capsular antigens. Cross-reactions between the cell wall polysaccharides of these two groups of organisms have been noted also. Highly potent antisera to the C polysaccharide of pneumococcus can be prepared with mutants of this organism producing a capsule of material very similar to, though not identical with the cell wall polysaccharide of this organism. Hot acid extracts of a variety of alpha and nonhemolytic streptococci give positive precipitin reactions with these antisera but fail to do so when the antiserum has been absorbed with pneumoccocal C polysaccharide (Table 5). Among the organisms showing this reaction are all five serotypes of streptococci in Lancefield's

TABLE 4. PROVISIONAL SEROTYPES OF ALPHA AND NONHEMOLYTIC STREPTOCOCCI FROM THE HUMAN RESPIRATORY TRACT

Provisional type	*Number of strains*
83	62
84	1
85	2
86	5
87	1
88	1
89	1
90	3
91	1
92	1
93	11
94	1
95	1
96	2
Untypable	97
	189

Group F, but some organisms possessing the same or closely related capsular antigens but lacking Group F cell wall polysachharide do not react with antiserum to pneumococcal C polysaccharide.

In addition to these similarities of alpha and nonhemolytic streptococci to pneumococcus, others exist. Streptococci are known which, like pneumococcus, are sensitive to optochin and ferment inulin. The most striking evidence of similarity of some streptococci to pneumococcus, however, lies in the realm of genetics. Studies of the base ratios of the deoxyribonucleates of several strains of streptococci have shown them to be similar to those of pneumococcus.[11] More significant, however, is the fact that heritable characters, such as drug resistance, can be transferred from streptococci to pneumococcus or from pneumococcus to streptococci by means of transformation reactions.[12] With the use of the transforming system, it has been possible to transform pneumococci to a variety of streptococcal capsular serotypes (Table 6), reactions involving transfer of multigenic pathways. Such transformation has been possible not only with DNA from streptococcal serotypes cross-reacting with pneumococcal capsular and somatic polysaccharides but also with DNA from strains having no demonstrable serologic relation to pneumococcus. The latter transformed pneumococci

TABLE 5. CROSS-REACTIONS OF LANCEFIELD EXTRACTS OF ALPHA AND NONHEMOLYTIC STREPTOCOCCI WITH ANTISERUM TO PNEUMOCOCCAL C POLYSACCHARIDE

Streptococcus cross-reacting with pneumococcus type:	*Positive/No. tested*
21	5/9
33	1/1
36	2/15
59	11/36
72	8/20
75	7/9
Provisionally classified streptococcus type:	
83	6/32
84	1/1
85	1/2
86	2/5
87	0/1
88	1/1
89	1/1
90	1/1
91	1/1
92	1/1
93	4/4
94	1/1
95	1/1
96	1/1

produce capsular polysaccharides unrelated to any of the known pneumococcal capsular serotypes.

These many similarities of pneumococci and alpha and nonhemolytic streptococci raise the question of whether or not the latter should be regarded as being of little importance in certain types of respiratory disease. It should be borne in mind that many capsular types of pneumococcus have a very low invasive potential both for man and for experimental animals. Only 3 per cent of all the pneumococcal bacteremias of man are caused by capsular types 34 through 82[18] inclusive and mice will survive intraperitoneal infections with 10^7 organisms of a number of types.[14] In this respect, some strains of pneumococcus are comparable in virulence to a number of alpha and nonhemolytic streptococci. Under natural and experimental conditions, both the composition and quantity of capsular polysaccharide produced by an organism appear to determine in part its invasive properties, and the latter can be changed through genetic manipulation of the capsule without

TABLE 6. CAPSULAR TRANSFORMATIONS OF PNEUMOCOCCUS WITH STREPTOCOCCAL DNA

Source of DNA: Str. cross-reacting with pneum. type	*Capsular type of transformed pneum.*
9	9 and III-9*
13	13
20	20
47	47
59	59
72	72
75	75
Str. provisional type	
85	85
86	86

* Binary capsular phenotype producing Type III pneumococcal capsular polysaccharide and streptococcal capsular polysaccharide cross-reacting with pneumococcus Type IX.

obvious alteration of the soma. In addition, it has been shown by Rich and McKee[15] that noncapsulated pneumococci produce lesions in agranulocytic rabbits closely akin to those produced by capsulated strains in normal and agranulocytic rabbits. The means whereby pneumococci bring about the injury and death of an infected subject is still unknown. It is not outside the realm of probability that, given an equal foothold, some strains of alpha and nonhemolytic streptococci may produce injury in a fashion similar to pneumococcus. The question cannot be resolved until more is learned about the pathogenic properties of both groups of organisms. Similarities already demonstrated are such, however, as to make additional studies of the alpha and nonhemolytic streptococci of the respiratory tract worthwhile. Of great interest would be examination of those strains isolated during properly executed bronchoscopy or from secretions obtained by sublaryngeal tracheal puncture and aspiration. There is a need also for a suitable animal model in which to study the behavior of these organisms.

The beta hemolytic streptococci appear to play a numerically less important role in the infection of patients with chronic bronchial disease than do other recognized bacterial pathogens of the respiratory tract. The beta hemolytic streptococci of Lancefield's Group A have been described so extensively elsewhere that they will not be considered here.

Staphylococci comprise another group of organisms which are apparently significant in causing infection of the tracheobronchial tree. Hemolytic,

coagulase-producing, pigmented strains are recognized to be virulent whereas their nonpigmented, noncoagulase-producing counterparts are thought to be of lesser importance. Much remains to be learned about both groups of organisms with regard to the mechanisms whereby they produce disease and especially with regard to the role of the immunologic responses of the host as a means of limiting infection by them.

Haemophilus influenzae

Of the organisms associated with infection of patients with chronic obstructive bronchial disease, none has been stressed more strongly in the past decade, especially in Great Britain, than *Haemophilus influenzae.* This bacillus is a frequent inhabitant of the upper respiratory tract, though one that is easy to overlook unless appropriate cultural techniques are employed. A recent compilation of surveys for its presence in normal subjects indicates carrier rates between 25 per cent and 84 per cent in groups of individuals examined on one occasion. These surveys include both capsulated and noncapsulated variants of which the former constitute only 5 to 6 per cent.[16] Relatively few data are available on the isolation of the six capsulated types of *Haemophilus influenzae,* though the more recent availability of capsular typing sera from commercial sources has resulted in augmentation of earlier information. Table 7 represents a compilation of the type distribution of capsulated *H. influenzae* recovered from the respiratory tract, taken from several sources,[16-18] and includes strains from both normal subjects and those with respiratory disease. Types b, e, and f appear to be those most commonly isolated. Longitudinal study of a group of chronic bronchitics has resulted in the isolation of four different capsular types of *H. influenzae* from a single patient though more than one capsular type was never re-

TABLE 7. DISTRIBUTION OF CAPSULAR TYPES OF HAEMOPHILUS INFLUENZAE ISOLATED FROM THE RESPIRATORY TRACT*

Type	*Number*	*Per cent*
a	38	8.4
b	234	51.5
c	13	2.9
d	16	3.5
e	78	17.2
f	75	16.5
Total	454	100

* Combined data. See rfs. no. 16, 17 and 18.

covered from a single culture.[18] Of the 599 cultures typed by slide agglutination in this study, 26 per cent were capsulated, a considerably higher proportion than that noted by most investigators.

Insofar as classification is concerned, noncapsulated strains of *H. influenzae* have posed a much more difficult problem than their capsulated counterparts. Agglutination of such strains by appropriately prepared antisera suggests a wide degree of antigenic heterogeneity. More recent studies of extracts of organisms solubilized by a variety of techniques have demonstrated the presence of several species-specific antigens, some possibly polysaccharide in nature.[19,20] There is currently a need, however, for a more thorough biochemical investigation of the structural components of *H. influenzae* with modern techniques to shed light on the antigenic elements of this organism. Wider use of the quellung or capsular precipitin reaction in the study of *H. influenzae* might also be worthwhile, for organisms failing to produce irridescent colonies on suitable media are usually regarded as lacking capsular antigens by this somewhat insensitive technique.

Another area of investigation that has not been exploited and that might prove fruitful is genetic study of the noncapsulated strains of *H. influenzae* isolated from the respiratory tract. Like pneumococcus, *H. influenzae* is readily susceptible to genetic transformation.[21] Transfer of the genes controlling capsulation and drug resistance from suitable donor strains to noncapsulated strains isolated from the respiratory tract might shed additional light on their relatedness to capsulated variants.

From studies of sputum and of respiratory secretions obtained directly from the lower respiratory tract, it is evident that both capsulated and noncapsulated organisms may be present in patients with chronic obstructive airway disease during clinical episodes of infection. The potentially hurtful role of noncapsulated variants of *H. influenzae* has been inferred both from their demonstrated presence in the bronchial tissues[22] of patients with chronic bronchial disease and from the improvement of patients concomitant with the elimination of the organism from the respiratory tract following antimicrobial therapy. More will need to be learned about the biology of this fastidious organism, however, before its pathogenic role can be understood fully. Like pneumococcus, it may produce neuraminidases,[23] although the role of these bacterial enzymes in respiratory disease remains to be elucidated. In addition, it may be necessary to explore further its behavior in subhuman primates to obtain a suitable animal host in which more satisfactory, controlled pathologic study of its behavior in the respiratory tract can be accomplished.

The enterobacteriaceae comprise another group of organisms with the capacity to injure the lower respiratory tract. Unlike the bacteria just

considered, these organisms reside more commonly in the gastrointestinal tract and are a frequent cause of abdominal and genitourinary tract infection. They are found at times, however, in the normal mouth and pharynx[1] and are not infrequent inhabitants of the upper respiratory tract when the normal flora of gram-positive bacteria has been suppressed by selective antimicrobial drugs. They have been isolated from the bronchial secretions of patients with chronic bronchial disease under conditions which exclude contamination by organisms in the upper respiratory tract and are the cause of perhaps 5 per cent of the bacterial pneumonias seen in hospitals. A recent study of 82 pneumonias caused by enterobacteria suggests that more than half of such infections are attributable to organisms in the Klebsiella-Enterobacter group and to *E. coli.*[24]

Klebsiella pneumoniae

Klebsiella pneumoniae has long been associated with infection of the respiratory tract, as its name implies. Like pneumococcus and a number of other inhabitants of the respiratory tract, it is found usually to be capsulated when isolated from man. More than 70 different capsular serotypes of *Klebsiella pneumoniae* are now known; but as has been the case with *H. influenzae,* because of the unavailability of typing sera, very few data have been collected regarding the role of different serotypes of *K. pneumoniae* in disease of the human respiratory tract. In the earlier studies of Edwards and Fife[25,26] and of Ørskov,[27] a total of 292 strains from respiratory secretions were typed. To add to these data, the collaboration of Dr. William H. Ewing, Chief, Enteric Bacteriology Unit, Bacteriology Section of the Communicable Disease Center, Atlanta, Georgia was obtained. Through his cooperation, an additional 186 strains of *K. pneumoniae* isolated from human respiratory secretions have been typed. These strains were all recovered from patients hospitalized on the medical service of the Philadelphia General Hospital with suspected respiratory disease between February, 1966 and July, 1967. The data have not yet been analyzed to determine the role of klebsiellas in the illnesses of the patients from whom they were isolated. The relative frequency of the isolation of klebsiellas with respect to pneumococci, however, is not without interest. Between July 1, 1966 and June 30, 1967, 2,485 cultures of respiratory secretions were examined. From these cultures, 371 strains of pneumococcus and 198 strains of *K. pneumoniae* were isolated. These figures have not been analyzed for the selective effect of antimicrobial therapy prior to or during hospitalization. They are indicative, however, that *K. pneumoniae* is a common inhabitant of the respiratory tract of hospitalized patients. Fifty-three capsular serotypes were represented among the strains isolated, 10 of which

TABLE 8. TYPE DISTRIBUTION OF KLEBSIELLA PNEUMONIAE ISOLATED FROM THE HUMAN RESPIRATORY TRACT*

Type	*No. of strains*	*Type*	*No. of strains*
2	53	25	12
1	47	19	11
4	39	5	10
3	16	17	10
8	14	7	9
35	14	27	9
9	13	41	9
18	12	Other	198
	Total — 476		

* Combined data. See rfs. no. 25, 26, 27.

had not been included in the earlier studies of Edwards and Fife and of Ørskov. The limited data on the distribution of klebsiella types are shown in Table 8. Fourteen types account for 62 per cent of the isolates from respiratory secretions, and Types 1, 2 and 4 for 34 per cent. There is reason to believe that typing sera for klebsiellas may be available commercially in the not too distant future. If so, it will be possible to accumulate considerably more information about this respiratory pathogen than has been possible in the past. With regard to *E. coli,* there is a similar lack of information about serotypes responsible for respiratory infection. Here again, information is needed.

The foregoing fragmentary data have been presented to illustrate something of what is known about some of the bacterial inhabitants of the respiratory tract that play a role in chronic bronchial infection and to indicate certain of the approaches that can be employed to learn more about them. No mention has been made of many of the genera listed in Table 1, some of which, including the destructive anaerobes, may play an important role in bronchial disease and require further study. At our present point in time it is obvious that we need to know a great deal more about the biology of the bacteria that inhabit the human respiratory tract and the means whereby they produce tissue injury. To solve these important problems, much additional study of bacteria isolated directly from the lower respiratory tract with the aid of biochemical, immunologic, genetic and epidemiologic techniques will be required. Of comparable importance, if an understanding of pathogenetic mechanisms is to be achieved, will be the development of suitable animal models in which the behavior of bacteria

in the respiratory tract can be studied. To gain this latter end, it will probably be necessary to turn to primate hosts. The increasing availability of such hosts in the National Primate Centers holds promise that some of today's difficult problems can be subjected to more meaningful study in the not too distant future.

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