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BACTERIAL ETIOLOGY OF LOWER RESPIRATORY TRACT INFECTIONS IN PARTS OF EASTERN NIGERIA

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ABSTRACT

Quantitative bacteriology of 200 sputa from patients with chronic bronchopulmonary disorders was carried out. *Mycobacterium tuberculosis* was the most frequently isolated pathogen (19%), followed by *Streptococcus pneumoniae* (13.6%), *Haemophilus influennzae* (9.0%), *Staphylococcus aureus* (7%), *Klebsiella pneumonia* (4%), *Pseudomonas aeruginosa* (4%), *Escherichia coli* (4%), *Nocardia asteroides* in association with others (6.5%), Neisseria catarrhalis (1%), and *Peptostrptococcus* sp. (1%). 52% of the infections were caused by single aetiologic agents while 32% were polymicrobial.

INTRODUCTION

Pulmonary diseases and their diagnosis have always constituted complex problems in public health care. The literature is full of reports on the microbiology of acute and chronic lower respiratory tract infections, but most of these reports are of investigations conducted in the temperate or monsoon regions of the world and very few in the tropics. In the opinion of Warrel 1975 respiratory infections are a major cause of morbidity and mortality in tropical countries, yet they are generally ignored in textbooks of tropical medicine.' Warrel's report 1975 appeared to be a brief review of a few documented cases in the tropics; those of Noah et al. 1976 Fayinka 1977 Aderele 1977 and Diallo et al. 1979 were noteworthy but covered a limited scope. Certain diseases or infections have been assumed, not proved, to be rare in Africa, because no proper investigations are carried out due to lack of adequate facilities, or because such infections, though common are not reported. The need for fuller investigations of causes of microbial diseases in the tropics and particularly in Africa is therefore compelling. This investigation was therefore undertaken in order to cover a wider spectrum of the subject in Nigeria as far as bacterial are concerned. The roles of viruses and fungi are recognized but not covered in this report. An attempt has been made to show the probable incidence of some of the major potential aetiologic agents and their combinations where the infections present mixed bacterial flora.

MATERIALS AND METHODS

The study was carried out on 200 samples of mucopurulent or purulent sputum obtained from patients clinically diagnosed as suffering from pulmonary disorders which included chronic bronchitis, pulmonary tuberculosis, pneumonia, multiple lung abscesses, and undefined chest

infections. The average age was 34 years with a range of 5-75. The sampling zones covered parts of Nsukka and Enugu in Anambra State and Umuahia and Owerri in Imo State. All specimens were sampled randomly at the clinic or laboratory into sterile wide-mouth disposable containers obtained from Messrs. "Sterillin Ltd.'. (Teddington, Middlessex, England). These specimens were processed within two hours of expectoration or where this was not possible (as in a few cases) they were refrigerated at 4-5°C immediately they were received in the laboratory and processed within 24 hours of expectoration. Where transportation over a long distance was involved, specimens were carried in an ice chamber in a large Thermos flask and transferred to the refrigerator in the laboratory.

Early steps in processing the specimen involved macroscopic examination for blood and colour, microscopic examinations of a wet mount for cells and ova of *Paragonimus* species; Gram's and Ziehl-Neelsen's stained slides for various organisms, and to exclude acid-fast bacilli. Direct cultures were put up on blood agar, chocolate or Levinthal's agar and MacConkey agar plates, and incubated in carbon-dioxide atmosphere for 24-48 hours. Mucopurulent specimens were washed by Osoagbaka's modification of the method of Bartlett and Finegold 1974. All specimens were liquefied with 2% N-acetyl-L-cysteine as used by Percival and Roberts 1971 and Wilson and Martin 1972 liquefaction being complete in less than 10 minutes with light shaking on Gallenkamp's flask-shaker. The liquefied specimens were then carefully diluted to 10^{-2} and 10^{-4} with sterile physiological saline as used by Pirtle et al. (1969).

Blood agar and Levinthal's (or Chocolate) agar plates were inoculated in duplicate by streaking from the 10^{-2} dilution from which also 2 cm^3 was pipetted into McClung's carbon-free paraffin-bait broth as used by Mishra and Randhawa 1969. On each of the blood agar and Levinthal's plates was placed a bacitracin disc containing 10 units of the drug (Baber, 1969) while the blood plates got in addition, a disc containing 30 ug of kanamycin to select *Bacteroides* species. The bacitracin plates were incubated in Co_2 at 37°C for 24-48 hours while the kanamycin-containing plates were incubated anaerobically at 37°C for 48-72 hours. 0.2 cm^3 of the 10^{-2} dilution was pipetted into each of two tubes of mycoplasma diplasic medium (Bailey and Scott, 1974) which were then incubated at 37°C for up to 4 weeks before being discarded as negative. Blood and Levinthal's plates were inoculated in duplicate with 0.025 cm^3 of the 10^{-4} dilution of the specimen using a 40-dropper. This was then spread evenly with a sterile glass spreader. One set was incubated in Co_2 at 37°C for 24-48 hours while the second set was incubated anaerobically for 48-72 hours. For the anaerobic cultures the Gaspak system from Messrs. BBL (Division of Baction Dickinson and Company, Cockeysville, U.S.A.) was used. Growth from all five sets of cultures were compared and identified by accepted conventional methods but counts were made from the 10^{-4} dilution cultures. (Cowan and Steel 1974; Cruickshank et al. 1975; Duorden et al. 1976; Slitter and Finegold 1975).

RESULTS

In more than 90% of all cases, the results of the cultures agreed very closely with the expected organisms as indicated in the gram-stained smears of the sputum. 28 specimens had AFB in the direct Ziehl-Neelsen's slides but 36 grew *Mycobacterium tuberculosis* in the L-J Culture medium. From the microscopic examination of direct wet preparations some ova morphologically resembling ova of *Paragonimus wastermanii* were seen in three specimens. More than 600 strains of 20 different species of micro-organisms were encountered in the study. Of these, 15 bacterial species were isolated in numbers greater than 1 x 10^7 colony-forming units (CFU)/cm^3 and were recorded as of aetiologic significance. Some fungi particularly *Candida albicans* and *Aspergillus* species were also encountered in appreciable quantities. The months of November and February which marked the end of the rainy season, the beginning

of the dry season, and the end of the harmattan season appeared to be the most favourable periods for these infections, with more cases of *H. influenzae*, *Str. pneumoniae* and *Nocardia asteroides* being isolated in February.

Out of the 200 cases investigated, quantitatively, 170 had positive cultures yielding up to or more than 1 x 10^7 CFU/CM^3 105 (52.5%) of these 200 cases grew single organisms at the test dilution while 65 (32.5%) were polymicrobial, most of them growing two organisms of aetiologic note (Tables 1 & 2) and 30 (15%) were either sterile or had no significant growth.

DISCUSSION

Laboratory investigations of bronchopulmonary infections constitute problems in public health care mainly because of the large numbers of potential pathogens normally inhabiting the oropharyngeal region but which could cause pulmonary disorders in any diseased lung (laurenzi et al. 1961, Lees and McNaught 1959, Mulder 1964). This could therefore prejudice the findings of bacteriological investigations particularly in lower respiratory tract infections carried out on expectorated sputum since it gets contaminated with these normal oropharyngeal flora.

TABLE 1

FREQUENCY OF MICRO-ORGANISMS IN DIFFERENT CLINICAL PULMONARY DISORDERS (200)

Chest Disorders	No. Tested	No. Positive	Micro-organisms	Frequency
Bronchitis	50	42	H. influenzae: Pure	14
			" + Mycobacterium sp.	8
			" + Nocardia asteroides	3
			" + Pneumococci	5
			" + Bacteroides sp.	2
			Nocardia + Str. pyogenes	2
			Str. pyogenes + Paragonimus sp.	1
			Str. pneumonia: Pure	3
			Pseudomonas aeruginosa	3
			N. catarrhalis: Pure	1
Tuberculosis/ Bronchitis	48	38	Mycobacterium species: Pure	12
			" + S. aureus	6
			" + Str. pyogenes	3
			" + Klebsiella sp.	2
			" + Pneumococci	2
			" + Mycoplasma pneumoniae	2
			Str. pneumoniae: Pure	8
			" + H. influenzae	1
			" + E. coli	2
Lobar/Broncho-pneumonia	25	21	Str. pneumonia Pure	10
			" + H. influenzae	1
			" + Ps. aeruginosa	1
			Kl. pneumoniae: Pure	5
			Staph. aureus: Pure	2
			E. coli: Pure	2
Multiple abscesses	12	11	Staph. aureus: Pure	5
			Ps. aeruginosa: Pure	2
			Str. pyogenes: Pure	2
			Noc. asteroides + C. albicans	2
Miscellaneous Chest infections	65	58	Str. pneumoniae: Pure	6
			" + Staph. aureus	5
			" + Pseudomonas sp.	3
			N. asteroides + H. influenzae	2
			Staph. aureus: Pure	7
			" + Bacteroides sp.	2
			Ps. aeruginosa: Pure	4
			E. coli: Pure	5
			E. coli + Mycoplasma pneumoniae	3
			N. asteroides + Candida albicans	1
Miscellaneous Chest Infections			N. tuberculosis + Str. viridans	1
			M. pneumoniae	1
			E. coli + Proteus sp.	2
			Kl. pneumoniae: Pure	3
			Kl. pneumoniae sp. + Mycoplasma sp.	2
			Kl. pneumoniae + Nocardia asteroides	3
			Peptostreptococcus sp. Pure	2
			N. catarrhalis: Pure	1
			*C. albicans: Pure	1
			H. influenzae	4

* = a fungus

TABLE 2:

FREQUENCY AND INCIDENCE OF MICRO-ORGANISMS ISOLATED IN BRONCHO-PULMONARY DISORDERS

		Frequency		Incidence %	
	Micro-organsims	Pure	Mixed	Pure	Mixed
1	Mycobact. tuberculosis	12	26	6	13
2	Strept. pneumoniae	27	15	13.6	7.5
3	H. influenzae	16	22	9.0	11.0
4	Staph. aureus	14	11	7.0	5.5
5	Kleb. pneumoniae	8	7	4.0	3.5
6	Ps. aeruginosa	8	4	4.0	2.0
7	E. coli	7	7	3.5	3.5
8	Str. Pyogenes	2	6	1.0	3.0
9	Peptostreptococcus sp.	2	–	1.0	–
10	N. catarrhalis	2	–	1.0	–
11.	Myco. pneumoniae	1	7	0.5	3.5
12.	Noc. asteroides	–	13	–	6.5
13	Bact. melaninogenicus	–	4	–	2.0
14	Str. viridans	–	3	–	1.5
15	Proteus mirabilis	–	2	–	1.0
16	*C. albicans	2	1	1	0.5
17	*Paragonimus sp.	2	1	1	0.5

* = Non-bacterial species

Many researchers have found the use of washed, homogenized and diluted specimens in quantitative sputum bacteriology to be reliable in reducing oropharyngeal contamination and also readily available to most workers (Bartlett and Finegold 1971; Guckian and Christensen 1978; Louria, 1962; Morroe et al. 1969; Pirtle et al. 1969; Thorsteinsson et al. 1975; Wilson and Martin 1972). The use of tracheal and bronchoscopic aspirates has been accepted by many as the answer to the problem of oropharyngeal contamination (Jordan et al. 1976; Pecora, 1963) and this method is now used by many workers as it obviates the tedium in quantitative sputum bacteriology. However, for our purpose we used quantitative sputum bacteriology because in the Nigerian setting, as in many other developing countries the use of bronchoscopy is outside the reach of many General Hospitals where expectorated sputum is still popularly used. In such countries transtracheal and other aspirates are used perharps only in the teaching or specialist hospitals.

The concentration of micro-organisms in sputum specimens acceptable as of aetiologic significance has varied between 1×10^6 and 1×10^7 orgs/cm^3. The normal population of such commensals as Staph. epidermis is placed at between 10 and 10^4/cm^3 according to Jordan et al. (Jordan et al. 1976). Laurenzi *et al.* (1961) established that in bronchial diseases offending micro-organisms were "invariably present in numbers exceeding 1×10^6/cm^3 of exudate. Louria (1962) and Monroe *et al* 1969) found in cases of pneumonia that probable pathogens occurred in numbers of 10^7 organisms/cm^3 or greater. Pirtle *et al.* (1969), Wilson and Martin (1972) and many others have confirmed this, and it appears to be more acceptable. The examinations of direct sputum smears stained by Gram's and Ziehl-Neelsen's (Z.N) methods and a wet mount, particularly of blood-stained specimens were useful. They provided an in-

sight into the most probable pathogens. The Z.N. and the wet mounts were particularly useful as screeming tests for pulmonary tuberculosis and paragonimiasis. Paragonimus sp. was shown by Nwokolo, as cited by Warrel 1975 to have increased in the Eastern States of Nigeria in 1972, and this increase was attributed to the eating of crabs during the Nigerian civil war. There is therefore the need to routinely examine the wet mount of blood-stained specimens to exclude this condition, as these crabs are still eaten by many people.

In 52% of our test cases the most probable pathogens were isolated pure as single organisms while in 33% polymicrobial infection was obvious. Most of the single pathogens were isolated in cases in which pneumonia and multiple abscesses were clinically suspected. Of the 21 positive cases in pneumonia, only two had more than one organism grown. *Str. pneumoniae* predominated in 12 in which it grew pure in 10. The remaining nine were accounted for by five cases of *Kl. pneumoniae*, two each of *Staph. aureus* and *E. coli*, all growing pure. *Str. pneumoniae* was also isolated in reasonable numbers as aetiologic agent in the other clinical conditions. In their investigation of pneumonia in malnourished children, Diallo *et al.* 1979 suggested that pneumonia in these patients was mostly due to a single aetiologic agent. Many authors stress on the role of *Str. pneumoniae* as the principal aetiologic agent in bacterial pneumonia and the results of this study confirm these views.

H. influenzae appeared most frequently in the 42 positive cases in which chronic bronchitis was clinically diagnosed. It was isolated as a single agent in 14 cases. and mixed in 18 others. Its combination with *Myco. tuberculosis* in 8, and *N. asteroides* in 3 of these 18 cases leaves doubts as to these 11 cases being chronic bronchitis. Six other cases in this group in which *Ps. aeruginosa, Paragonimus* sp. and *N. asteroides* were principal aetiologic agents could also not reasonably be classified as chronic bronchitis. Therefore out of 25 cases of possible chronic bronchitis, *H. influenzae* was of aetiologic significance in 21; thus confirming its principal role in chronic bronchitis in this country as already established for other parts of the world (Haas et al. 1977; May, 1975; May, 1953; Williams, 1979).

Mycobacterium species appeared with the highest incidence of 13% as a mixed infection and 6% as single aetiologic agent. Since this organism is decidedly a very important pathogen in the lung and of more aetiologic significance than most of the others, it is regarded as the principal aetiologic agent in the 36 cases in which it was isolated and given an incidence of 19%. This agrees with the views of Warrel 1975. *Myco. tuberculosis* is still the most dominant pathogen in lower respiratory tract infections in the tropics. The next in incidence as a single aetiologic agent was *Str. pneumoniae* (13.5%). It has been established that in pneumonias, and indeed in lower respiratory tract infections in the tropics, *Str. pneumoniae* is the highest cause next only to *Myco. tuberculosis* (Diallo et al. 1979; Warrel, 1975). *H. influenzae* was next with 9% as a single aetiologic agent and 11% as a mixed infection.

The role of *S. aureus* (7%), *Kl. pneumoniae* (4%), *Ps. aeruginosa* (4%), and *E. coli* (3.5%) as single aetiologic agents in lower respiratory tract infections in this country must be noted. Gupta *et al.* (1974), May (1975), Aderele (1977) and others have stressed on the importance of *S. aureus* as a causative agent for cystic fibrosis, lung abscess, lobar pneumonia and multiple lung abscesses. Diallo *et al.* (1979) reported *S. aureus* as second to *Str. pneumoniae* in incidence and associated it with a diagnosis of Staphylococcal pneumonia. In our experience *S. aureus* was the most frequent single organism in cases of multiple lung abscesses although 2 isolates (1%) were obtained in pneumonia cases. Gram-negative enterobacteria have been associated with bronchopulmonary disorders in increasing proportions (Aderele 1977; Boat and Petty 1977; Burns 1968; Pierce and Sanford 1974). Burns (1968) in confirmation of earlier findings was able to demonstrate by immunoelectrophoresis that precipitins specific to *Klebsiella* species and other enterobacteria were present in such chronic destructive bronchial conditions as bronchiectasis, cystic fibrosis and chronic *Klebsiella pneumonia*, but not in chronic bronchi-

tis. The ready disposition of *S. aureus* and the Gram-negative enterobacteria to antibiotic resistance makes it imperative that their occurrence in acceptable numbers in the sputum be reported early and properly investigated.

Noc. asteroides which had a mixed infection incidence of 6.5% ranked fourth in the mixed infection series. Its role as a primary or secondary infection complicating other bacterial or mycotic infections of the lungs has been documented particularly in the Indian subcontinent and in the United States (Causey 1974; Randhawa et al. 1973; Shome et al. 1973). Its occurrence in the tropics particularly Nigeria, has not been adequately investigated and reported. *N. catarrhalis* a normal inhabitant of the upper respiratory tract and usually regarded as a non-pathogen was isolated pure and in significant concentrations in two cases in which they were also B-lactamase positive. The clinical diagnosis for one of this was pneumoconiosis. The pathogenic role of *N. catarrhalis* in the lower respiratory tract has been reported (McNeely et al. (1976; Ninane et al. 1977). The incidence in this study was 1%. It is no longer very safe to assume that all strains of this organism are non-pathogenic in the respiratory tract, especially when they are numerically significant and betalactamase positive.

The role of anaerobes in lower respiratory tract infections is well reported. The wisdom of putting up anerobic cultures for sputum has long been doubted (Thorsteinsson et al. 1975) but however some workers still advocate it. Bertlett and Finegold (Bartlett and Finegold 1971, 1974) and Lober (1975) isolated significant anaerobes which included *Fusobacterium nucleatum, Bacteroides, melaninogenicus* and *Peptostrephococcus* sp., with an incidence of 2-3%. In this study we found a 3.0% incidence for Peptostreptococcus *and Bactèroides* spp. and suggest that anaerobic cultures of sputum should be put up only for fetid, foul-smelling specimens as these are the most likely types of specimens to harbour anaerobes of aetiologic significance as pointed out by Mulder (1964).

In conclusion we suggest that there is the need for more investigations to determine the nature of lower respiratory tract infections in the tropics and particularly in the developing countries. It is obvious that many of the infections are polymicrobial in nature and that even those that are of a single aetiologic agent are varied. It is important to determine the role and incidence of the various micro-organisms involved in the different types of pulmonary infections. Many of the patients presenting themselves at the hospitals or clinics can rightly be said to be 'compromised' because of the poor standard of living, the uncontrolled and irregular use of broad-spectrum antibiotics,smoking and alcoholism. Consequently no organism isolated from sputum in reasonable number should be lightly dismissed as a nonpathogen.

From the varied types of organisms encountered in this study in relation to the various conditions clinically diagnosed, it seems obvious that proper laboratory investigations are necessary in this country in all cases of pulmonary disorders to establish accurate diagnosis on which effective treatment can be based. Reliance on clinical and radiologic observations alone can lead to wrong diagnosis and wasteful polypharmacy. The need for clinicians to supply more detailed, accurate and precise data about patients being investigated in the laboratory is stressed. This will help in the proper interpretation of laboratory results, the application of correct and effective treatment and will also make research into these diseases more meaningful and beneficial to the practice of medicine.

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