

N. J. Microbiology, 2(2), 1982, pp 131 – 140
© The Nigerian Society for Microbiology 1982.

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

BY

O. U. OSOAGBAKA and A.N.U. NJOKU-OBİ
DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF NIGERIA
NSUKKA

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 influenzae* (9.0%), *Staphylococcus aureus* (7%), *Klebsiella pneumoniae* (4%), *Pseudomonas aeruginosa* (4%), *Escherichia coli* (4%), *Nocardia asteroides* in association with others (6.5%), *Neisseria catarrhalis* (1%), and *Peptostreptococcus* sp. (1%). 52% of the infections were caused by single aetiological 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 Aderole 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 aetiological 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 Bacton 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 wastermanni* 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 1×10^7 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 periods for the *asteroides* being Out of the or more than 1 test dilution w aetiologic note

Laboratory health care man oropharyngeal et al. 1961, Le ings of bacteric on expectorate

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 1×10^7 CFU/CM³ 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 aetiological 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
			Str. pneumonia: Pure	10
Lobar/Broncho- pneumonia	25	21	" + 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

FREQU

Micro-org

1 Mycobact
2 Strept. pi
3 H. influer
4 Staph. au
5 Kleb. pne
6 Ps. aerugi
7 E. coli
8 Str. Pyog
9 Peptostre
10 N. catarr
11 Myco. pi
12 Noc. aste
13 Bact. me
14 Str. virid
15 Proteus
16 *C. albic
17 *Paragon

* = Non

Many researchers have found that sputum bacteriology is readily available to the clinician (Louria, 1962; Martin 1972). The answer to the question of whether this method is the answer to the problem of bacterial pneumonia in the Nigerian setting is the reach of many such countries to specialist hospitals.

The concentration of significance has been given to such commensals as Staph. aureus, E. coli, dan et al. (Jordan 1972). The offending micro-organisms in the exudate. Louria (1962) found that the pathogens occurring in the sputum and Martin (1972) found that the examinations and a wet mount

TABLE 2:
FREQUENCY AND INCIDENCE OF MICRO-ORGANISMS ISOLATED IN
BRONCHO-PULMONARY DISORDERS

	Micro-organisms	Frequency		Incidence %	
		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 perhaps only in the teaching or specialist hospitals.

The concentration of micro-organisms in sputum specimens acceptable as of aetiological significance has varied between 1×10^6 and 1×10^7 orgs/cm³. The normal population of such commensals as Staph. epidermis is placed at between 10 and 10^4 /cm³ 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 /cm³ of exudate. Louria (1962) and Monroe et al (1969) found in cases of pneumonia that probable pathogens occurred in numbers of 10^7 organisms/cm³ 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-

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 anaerobic cultures for sputum has long been doubted (Thorsteinsson et al. 1975) but however some workers still advocate it. Bartlett and Finegold (Bartlett and Finegold 1971, 1974) and Lober (1975) isolated significant anaerobes which included *Fusobacterium nucleatum*, *Bacteroides melaninogenicus* and *Peptostreptococcus* sp., with an incidence of 2-3%. In this study we found a 3.0% incidence for *Peptostreptococcus* and *Bacteroides* 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.

LITERATURE CITED

- ADERELE, W.I. 1977. Lung abscesses in Nigerian children. *Nig. Med. J.* 7: 403-407.
- BABER, K. G. 1969. A selective medium for the isolation of *Haemophilus influenzae* from sputum. *J. Med. Lab. Technol.* 26; 391-396.

- BAILEY, W. R. & SCOTT, E.G. 1974. "Diagnostic Microbiology" (4th Ed. 1974. pp. 54-64; 111-226; 377-378. The C.V. Mosby Company - St. Louis, U.S.A.
- BARRETT-CONNOR, E. 1971. The non-value of sputum culture in the diagnosis of pneumococcal pneumonia. *Amer. Rev. Resp. Dis.* 103: 845-848.
- BARSON, A.J. 1971. Fatal *Pseudomonas aeruginosa* bronchopneumonia in a children's hospital. *Arch. Dis. Child.* 46:55.
- BARTLETT, J. G. 1977. Diagnostic accuracy of transtracheal aspiration bacteriologic studies. *Amer. Rev. Resp. Dis.* 115: 777-782.
- BARTLETT, J. G. & FINEGOLD, S. 1974. Anaerobic infections of the lung and pleural space. *Amer. Rev. Resp. Dis.* 110: 56-77.
- BARTLETT, J. G. & S. M. FINEGOLD 1971. Improved technique to process coughed sputum for culture. *Clin. Res.* 19: 183.
- BOAT, T. F. & PETTY, T.L. 1977. Chronic bronchitis and cystic fibrosis: Two chronic obstructive lung diseases of adults (Editorial). *Amer. Rev. Resp. Dis.* 116: 1-2.
- BURNS, M. W. 1973. The significance of *Pseudomonas aeruginosa* in sputum. *Brit. Med. J.* 3: 382-383.
- BURNS, M. W. 1968. Precipitins to *Klebsiella* and other *Enterobacteria* in the serum of patients with chronic respiratory disorders. *Lancet* 1: 383.
- CARGILL, J.S. 1973. The significance of *Pseudomonas aeruginosa* in sputum. *Brit. Med. J.* 3: 594.
- CAUSEY, WILLIAM A. 1974. *Nocardia caviae*. A report of 13 new isolations with clinical correlation. *Appl. Microbiol.* 28: 193-198.
- COWAN, S.T. & STEEL, K.J. 1974. Manual for identification of Medical bacteria—2nd Ed. 1974 Cambridge Univ. Press.
- CRUICKSHANK, R. DUGUID, J.P. MARMION, B.P. & SWAIN, R.H.A. 1975
- I. Bacterial pathogens and associated diseases.
 - II. Identification of specific microbes. Laboratory diagnosis of specific infections. In "Medical Microbiology" (12th Ed. Vols. I & II, pp. 236-366; 356-470. Eds. Cruickshank, Duguid, Marmion & Swain. (E.L.B.S. & Churchill Livingstone, London).
- DARREL, J.H. and HURDLE, A.D.F. 1964. Identification and clinical significance of *Klebsiella* species in chest infections. *J. Clin. Path.* 17: 617-621.
- DEURDEN, B. I. HOLBROOK, W.P. J. G. COLLEE, & B. WATT 1976. The characterization of clinically important Gram-negative anaerobic bacilli by conventional bacteriological tests. *J. Appl. Bact.* 40: 163-188.

- DIALLO, A.A. M. nourished child
- DIXON, J.M.S. and Lancet. 1046-
- FAYINKA, O. A. I. ples in Ibadan.
- GRAYSTON, J. T. 1965. *Mycopl* 102.
- GUCKIAN J. C. & nia. *Amer. Rev*
- GUPTA, S. Et al. 19
- HAAS, H. J. F. M. tory tract in sampling meth
- JORDAN, G. W. & mined by fibre
- LAPINSKI, E.M. from patients
- LAURENZI, G.A. New Engl. J. M
- LEES, A.W. & W. chronic bronc
- LOBER, BENNET Rev. Resp. Di
- LOURIA, D.B. 19 - 1086.
- MAY, J. R. 1975
- * MAY, J.R. 1953.
- MCNEELY, D.J. monia in an i
- MISHRA, S.K. & dia *asteroide*
- MONROE, P.W. nisms in spu
- MULDER, J. 196 bacterial dis

- DIALLO, A.A. M. SILVERMAN and L.G. EGLER 1979. Bacteriology of lung puncture aspirates in malnourished children at ABU Teaching Hospital, Zaria. Nig. Med. J. 9: 421 — 423.
- DIXON, J.M.S. and D.C. MILLER, 1965. Value of dilute inocula in cultural examination of sputum. The Lancet. 1046 — 1048.
- FAYINKA, O. A. 1977. Distribution of different serotypes of *Haemophilus influenzae* in pathological samples in Ibadan. Nig. Med. J. 7: 144 — 148.
- GRAYSTON, J. T. R. ALEXANDER, G.F. KERRY, E. R. CLARKE, J.C. FREMONT & W. MACCOLL, 1965. Mycoplasma pneumoniae infections, clinical and epidemiological studies. J.A.M.A. 191: 97—102.
- GUCKIAN J. C. & W. D. CHRISTENSEN, 1978. Quantitative culture and gram stain of sputum in pneumonia. Amer. Rev. Resp. Dis. 118: 997 — 1005.
- GUPTA, S. Et al. 1974. Clinical study of pulmonary suppuration. Ind. J. Chest Dis. 16: 390 — 399.
- HAAS, H. J. F. MORRIS, S. SAMSON, J. P. KILBOURN & P. J. KIM 1977. Bacterial flora of the respiratory tract in chronic bronchitis. Comparison of transtracheal fiberbronchoscopic and oropharyngeal sampling methods. Amer. Rev. Resp. Dis. 116: 41 — 47.
- JORDAN, G. W. G. A. WONG, & P. O. HOEPRICH, 1976. Bacteriology of lower respiratory tract as determined by fiberoptic-bronchoscopy and transtracheal aspiration. J. Infect. Dis. 134: 428 — 434.
- LAPINSKI, E.M. E. O. FLAKES, & B.C. TAYLOR, 1964. Evaluation of some methods for culturing sputum from patients with bronchitis and emphysema. Amer. Rev. Resp. Dis 89: 760 — 763.
- LAURENZI, G.A. R.T. PORTTER, & E.H. KAAS, 1961. Bacteriologic flora of lower respiratory tract. New Engl. J. Med. 265: 1273 — 1278.
- LEES, A.W. & W. MCNAUGHT, 1959. Bacteriology of lower respiratory tract secretions in 'normals' and chronic bronchitis. Lancet 2: 1112 — 1115.
- LOBER, BENNETT, 1975. "Bad breath" presenting manifestations of anaerobic pulmonary infection. Amer. Rev. Resp. Dis. 112: 875 — 877.
- LOURIA, D.B. 1962. Uses of quantitative analyses of bacterial population in sputum. J.A.M.A. 182: 1082 — 1086.
- MAY, J. R. 1975. The flora of the respiratory tract in chronic disease. Postgrad. Med. J. 51: 144 — 146.
- MAY, J.R. 1953. The bacteriology of chronic bronchitis. The Lancet 534 — 538.
- MCNEELY, D.J. C. S. KITCHENS & R.M. KLUGE, 1976. Fatal *Neisseria (Branhamella) catarrhalis* pneumonia in an immunodeficient host. Amer. Rev. Resp. Dis. 114: 399 — 402.
- MISHRA, S.K. & RANDHAWA, H.S. 1969. Application of paraffin-bait technique to the isolation of *Nocardia asteroides* from clinical specimens. Appl. Microbiol. 18: 686 — 687.
- MONROE, P.W. H.G. MUCHMORE, F. G. FELTON & J. K. PARTLE, 1969. Quantitation of micro-organisms in sputum. Appl. Microb. 18: 214 — 220.
- MULDER, J. 1964. Clinical significance of bacteriologic examination of sputum in cases of acute and chronic bacterial disease of respiratory tract. Adv. Intern. Med. 12: 233 — 252.

- NINANE, G. JOLLY, J.P. PIOT & M. KRAYTMAN 1977. *Branhamella (Neisseria) Catarrhalis* as pathogen. Lancet 2(802a), 147.
- NOAH, M.A. ODUGBEMI, OYEGUNLE & OGUNDIPE, 1976. Bacterial aetiology of acute pneumonia in Lagos children by examination of tracheal aspirates. Nig. Med. J. 6: 3:19 – 323.
- OSOAGBAKA, O. U. 1980. Improved methods for processing sputum for the diagnosis of lower respiratory tract infections. Nig. J. Med. Lab. Technol. (In Press).
- PECORA, D.V. 1963. A comparison of transtracheal aspiration with other methods of determining the bacterial flora of the lower respiratory tract. New Engl. J. Med. 269, 664 – 666.
- PERCIVAL, A. & ROBERTS, C. 1971. Significance of Enterobacteria and Pseudomonas in sputum. In Microbiology of the Seventies. Ed. F. J. Baker pp. 154 – 190. (Butterworths, London).
- PIERCE, A.K. & SANFORD, J.P. 1974. Aerobic Gram-negative bacillary pneumoniae. Amer. Rev. Resp. Dis. 110, 647.
- PIRTLE, J.K. P.W. MONROE, T. K' SMALLEY, J.A. MOHR & E.R. RHOADES 1969. Diagnostic and Therapeutic advantages of serial quantitative cultures of fresh sputum in acute bacterial pneumonia. Amer. Rev. Resp. Dis. 100 : 831 – 838.
- RANDHAWA, H.S. MISHRA, S.K. R.S. SANDHU. R.R. MAZUNIDAR, R.C. JAIN, P.U. RAO ET AL., 1973. Prevalence of Nocardiosis in bronchopulmonary diseases. Ind. J. Med. Res. 61:689 – 699.
- RUBIN, A.H.E. & ALROY G.G. 1977. *Candida albicans* abscess of lung. Thorax 32 373 – 376.
- SCOTT, E. G. 1976. Microbiological and clinical evaluation of expectorated sputum: A diagnostic dilemma. Delaware Med. J. 48: 9 – 11.
- SHOME, S.K. D.K. SIRKAR, S.U. BHAKTU & M.M. SINGH, 1973. Incidence of nocardia among human bronchopulmonary disorders. Ind. J. Med. Res. 61: 30 – 37.
- SUTTER, V.L. & S.M. FINEGOLD 1975. Anaerobic bacteria: Their recognition and significance in the clinical laboratory. Med. Rev. Mex 56 (1198), 236 – 253.
- THORSTEUNSSON, S.B. MUSER, D.M. & T FAGAN 1975. The diagnostic value of sputum culture in acute pneumonia. J.A.M.A. 233.—895
- WARREL, D.A. 1975. Respiratory tract infections in the tropics. Practitioner 215: 740 – 746.
- WILLIAMS, FRASER R. 1977. *Haemophilus influenzae*: A Clinical bacteriologist's point of view. In-take Issue No. 52: Brit. Med. J. Suppl. (Abbot Laboratories Ltd. Kent).
- WILLIAMS D. M. , J. A. KRICK, & J.S. REMINGTON 1976. Pulmonary Infection in the compromised host; Part I: Amer. Rev. Resp. Dis. 114: 359–386.
- WILSON, M.J.B. & D.E. MARTIN 1972. Quantitative sputum culture as a means of excluding false positive reports in the routine microbiology laboratory. J. Clin. Path. 25: 697 – 700.

THE

The bacterial and twenty-four in their vagina. Three for the non- and 16 of the n in 14 of the pre respectively. Bacter the vaginal swabs. Statistically, women investigated

Several labor Menda, Thadepal followed by pept many types of r differed between gnant women wit

Cultures we logy Out-Patient women attendin ment of Gynecol Nsukka. Five of were in their mid 18 and 40 years tics and women v