Incidence of Lower Respiratory Tract Infections among Elderly Patients in Federal Medical Centre UMUHIA

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Abstract: *The incidence of Lower Respiratory Tract Infections among elderly patients attending Federal Medical Center, Umuahia, Abia State, South East Nigeria was evaluated. The study was aimed at assessing the various organisms from sputum and to test for their antimicrobial susceptibility. Two hundred and seventy patients who were seen over a six month period who were diagnosed for Lower Respiratory Tract Infections were used for the study. Out of this population, two hundred and fifty(250) sputum samples were examined from symptomatic patients while twenty(20) were from asymptomatic subjects. Sputum samples were examined for Microscopy, Culture and Sensitivity. There were two hundred positive sputum samples from symptomatic patients and twenty positive samples from asymptomatic control subjects. The commonest organisms isolated were Streptococcus pneumonia, Klebsiella pneumonia, Pseudomonas aeruginosa Staphylococcus aureus and coagulase negative staphylococcus aureus. The later was isolated from asymptomatic control group. Their percentage occurrence in sputum samples are as follows: Streptococcus pneumonia 42%, Klebsiella pneumonia 20%, Staphylococcus aureus 16%, Pseudomonas aeruginosa 13%, coagulase negative Staphylococcus aureus 9%. From the study and the laboratory findings, a significant growth was obtained from the total number of sputum samples tested. The incidence of lower respiratory tract infections among the elderly patients (between the ages of 56 and above) visiting the out-patient department of this Hospital was found to be 200 (74%) of the total sputum samples of symptomatic subjects investigated. Antibiotic susceptibility tests showed that most of these isolates were highly resistant to co-trimoxazole. Pseudomonas aeruginosa was resistant to most antimicrobial drugs tested especially Co-trimoxazole and Ampicilin. Gentamicin and Ciprofloxacillin had high levels of sensitivity of 77.1% and 69.8% respectively. The study demonstrated that there was significant incidence (P<0.05) of lower respiratory tract infections in elderly in Umuahia metropolis.*

I.   Introduction

Lower Respiratory Tract Infections (LRTI) is an acute illness (which present for 21 days or less) usually with cough as the main symptom and with at least one other lower respiratory tract symptom (sputum production, dyspnea, wheeze, chest discomfort/pain) and no alternative explanation e.g. sinusitis and asthma. Respiratory Tract Infections (RTI) are the most common reason for primary care consultations (Creer et al., 2006). One third of all RTI's are Lower Respiratory Tract Infections (LRTI) (Huchan et al., 1998) with an incidence rate of 44-50/1000 (Macfarlane et al., 2001). Lower Respiratory Tract Infections treated in hospitals include severe cases of acute exacerbations of chronic bronchitis (AECB) and Community-Acquired Pneumonia (CAP) as well as Hospital Acquired Pneumonia (HAP); the latter consists of Ventilator-Associated Pneumonia (VAP) and Healthcare Associated Pneumonia (HCAP) (Grossman, et al, 2005). Infection of the lower respiratory tract is responsible for

4.4% of all hospital admissions and 6% of all general practitioner consultations (Anderson et al., 1993). They also account for 3% to 5% of deaths in adults, especially over the age of 60 years. The therapy of LRTI is widely empirical (okesola and Ige, 2007). However, increasing antibiotic resistance in frequently isolated respiratory tract pathogens has complicated the selection process of antimicrobial agents (Guthrie, 2001), nevertheless, the choice of antimicrobial therapy for bacterial LRTI is relatively straight forward when the etiologic agents and their antibiotic susceptibility patterns are known. However, the clinical presentation is usually not specific enough to make a firm etiologic diagnosis whether in the community or hospital settings (Fang et al., 1990). Therefore, the initial choice of antibiotic is often based on the knowledge of local epidemiology; the economic analyses have confirmed that bacteriological more effective antibiotics can reduce overall management costs, particularly with respect to consequential morbidity and hospital admission. Application of these principles would positively benefit therapeutic outcomes, resistance avoidance and management costs and will more accurately guide antibiotic choices by individuals, formulary, and guideline committees (Ball P, 2002). This study was, therefore, conducted to determine the bacterial etiology of LRTI in our environment, as well as to update clinicians in the various antimicrobial alternatives currently available for the treatment of LRTI.

Aims and objectives
This study is aimed at:
1. Isolating, Identifying and Characterizing the etiologic bacterial agents of Community Acquired Lower Respiratory Tract Infections among elderly patients in Federal Medical Center Umuahia, Abia State.
2. Determining the antimicrobial susceptibility pattern of the isolates.
3. Estimating the incidence of community acquired lower respiratory tract infections among this group in Umuahia metropolis.

2.0 Materials and methods

2.1 sampling and sample collection

A total number of two hundred and seventy (270) sputum samples were collected from elderly patients, attending Federal Medical Center Umuahia, Abia State (after their due consent in accordance with the ethical requirements of the health facility). Very few of them got admitted during the course of the Research. The sputum was collected separately in sterile container from all patients presenting with lower respiratory tract infection as defined by a new or increasing cough, productive sputum, chest pain, fever, anorexia, hemoptysis, headache and weight loss (Gauche et al., 2000). Sampling was done by probability sampling method using stratified random technique with a view to avoiding bias in the subject recruitment for the research work.

INCLUSION CRITERIA
The three basic inclusion factors for a subject to participate in this study include
- Age ≥ 56 years
- Subjects must not have been on antibiotic therapy for the past 7 days.
- Subjects must have been confirmed of having lower respiratory tract infection, whether symptomatic or asymptomatic by the consulting clinician. This information was freely obtained from their folder before recruiting them for this research having got the clearance from the ethics and research committee of Federal Medical Center Umuahia.

SAMPLE HANDLING
Sputum samples were seeded or inoculated on the correct media and incubated appropriately as soon as they were collected from patients. Sputa were screened by microscopic examination for relative number of polymorph nuclear cells and squamous epithelial cells in a low power (x10) field. Invalid specimens ($\geq$10 squamous epithelial cells and $\leq$ 25 polymorph nuclear cells/field) should not be examined further.

MICROBIOLOGICAL ANALYSIS OF SAMPLES

Chosen sputum samples for the experiment were first cultured (inoculated) unto the appropriate media to avoid contamination or introduction of unwanted organisms from the environment or from experimenter before microscopic procedures. Gram stained smear were examined for all sputum samples. In microscopic examinations, samples showing not less than 10 squamous epithelial cells per low power field confirmed the reliability of the specimen indicating that, it was not contaminated with saliva. Sputum samples were then inoculated on blood agar and chocolate agar. All the inoculated plates were incubated at 37^0c for 24 hours (in candle jar containing 3-5% CO_2). Discrete colonies from culture plates (Petri dishes) were sub-cultured severally at 37^0C for 24 hours to obtain a pure culture. Pure culture were inoculated into agar slants and stored at 40^0C for further identification.

ISOLATION AND IDENTIFICATION OF ISOLATES

Bacterial isolates were identified based on cultural and microscopic morphology, physiological and biochemical test as described by Cheesbrough (2002) were used for identification process. These include catalase, Urease, Coagulase, motility, indole, oxidase, citrate utilization methyl-red, vogas-proskauer test, bile solubility and sugar fermentation test for specific sugar such as glucose, sucrose, lactose, and mannose. Additional tests used includes Gram- staining for morphology, serum bactericidal assay, Hemolysis production test and Assay of B-lactamase production.

GRAM STAINING

Thin smear of young bacterial culture (18-24 hours old) was made on a clean grease free glass slide. It was allowed to air dry, and covered with crystal violet stain (primary stain)for 60 seconds. The stain was quickly washed with clean water. The water was tipped off and the smear was covered with Gram iodine (the mordant) for 30 seconds and further washed with clean water. The smear was then decolorized rapidly for about 20 seconds using 95% ethanol. The smear was quickly washed with clean water and then flooded with dilute carbon fuchsin (secondary stain) for 30-60 seconds. The stain was washed off with clean water and slide was allowed to dry at room temperature. The gram stained slide was examined first with x40 objectives lens to check for the staining and distribution of Gram stained bacteria, then with oil immersion objective lens (x100) to look for the bacteria. Gram positive bacteria appeared purple while Gram negative bacteria appeared red or pink.

MOTILITY TEST

The hanging drop method described by Fawole and Oso (1988), was employed in the motility test. A little petroleum jelly was placed around at the center of a clean grease free welled slide. Then a drop of young bacteria culture (18-24 hours old) was placed at the center of the cover slip. Then a slide was carefully placed on the cover slip and quickly inverted (with the cover slip sticking at the glass slide by means of petroleum jelly). The slide was viewed using x40 objective at reduce illumination for maximum contrast. A directional movement of the organism is a positive test.

CATALASE TEST

This method involves dipping a sterile wooden stick containing a good growth of the test organism into hydrogen peroxide contained in a test tube (2-3ml). The presence of gas bubbles indicated a positive reaction.

OXIDASE TEST

The method described by Cheesbrough (2002) was adopted for the test. Two drops of freshly prepared oxidase reagent (1% aqueous solution of tetra methyl phenylenediamine dihydrochloride) were made on a clean filter papers in a petri dish. Then, a sterile wooden stick was used to collect the test organism and smeared on the position of filter paper damped with oxidase reagent. The appearance of blue-purple colour within 10 seconds indicated a Positive reaction. (a delayed positive reaction is indicated by purple coloration within 10-60 seconds).

COAGULASE TEST

The slide method described by Cheesbrogh (2002) was used for the test. A loop-full drop of normal saline was placed on each end of slide. An 18-24hours old culture of test organism was emulsified in each of the drops to make two thick suspensions. Thereafter, a drop of human plasma was added to one of the suspensions. The mixture was stirred for about 5 seconds. Clumping of the organism within 10 seconds is a positive test. No plasma was added to

the second suspension which is the control to differentiate any granular appearance of the organism from the true coagulase clumping.

UREASE TEST

The nutrient agar medium incorporated with urea and phenol red indicator was incubated with the suspected colony. The culture was incubated for 18-24 hours. A positive urease test change was shown by a change of colour of agar form pale yellow to red.

INDOLE TEST

An aliquot of 1.5% sterile Peptone water in a test tube inoculated with the test bacterium culture is incubated for 48 hours at 30^0c. Then, 0.5ml Kovac reagent was added and then gently shook. A red colour indicated positive indole test.

CITRATE UTILIZATION TEST

Simon's citrate agar was prepared based on manufactures instruction, sterilized and allowed to solidify at an inclined angle of 45degree. The test organism was streaked on the surface of the agar slant and then incubated for about 5 days at 30^0c. A change in the colour of agar medium from green to blue following growth of the organism on the slant indicated a positive test while the absence of growth and colour change indicated negative test.

VOGES-PROSKAUER TEST

Two (2) ml of sterile phosphate peptone water was inoculated with the bacteria culture and incubated at 30^0C for 48hours. Then, 1ml of 40% potassium (KOH) was added, followed by 3ml of 5% alcoholic alpha-napthol. The test tube was shook and observed for colour change. A pink colour within 2-5 minutes showed a positive test.

ACID AND GAS PRODUCTION FORM CARBOHYDRATE FERMENTATION

Bacteria differ in their ability to metabolize carbohydrates. However, most bacteria will ferment a variety of sugar to form one or more acid and products which in some cases is accompanied by the evolution of gas, usually CO_2 and H_2. The acid can be detected by the inclusion of an indicator for example, phenol red in the growth medium. The method described by Cheesbrough (2002) was adopted for this test.

Generally, a 5ml of 1.5% peptone water was added with 5ml of 0.2% phenol red indicator and sterilized by autoclaving at 121^0c for 15 minutes. Thereafter, 0.1ml of 10% of sterile sugar to be used was added to the medium aseptically, the sugar was sterilized separately by filtration. After this, the test organism was inoculated into the growth medium and a sterile inverted Durham tubes introduced into each test tube. It was then incubated for 24 hour at 30^0c, the tubes observed for acid and gas production. Acid production gave a red to yellow colour change, while gas production was indicated by air displacement in the inverted Durham.

Glucose And Lactose

The test organisms were inoculated by stabbing the butt and slope of Kliglers iron agar (oxoid, England). This was incubated at 37^0c for 24 hours . a yellow slope and a yellow butt indicate the fermentation of glucose and lactose.sss

SUCROSE FERMENTATION

Aseptically, 5ml of 10% sucrose solution was added to sterile peptone water containing the indicator, bromothymol blue and mixed well. Using a wire loop, the test organisms were inoculated into the medium and incubated overnight at 36^0c for 24 hours. A Change in colour from blue to yellow indicate sucrose fermentation whereas no colour change indicates a negative test.

Mannitol Fermentation

Aseptically, 5ml of 10% mannitol solution was added to peptone water containing bromothymol blue indicator. The test organism were inoculated into the medium and incubated at 37^0c for 24hours, colour changes from blue to yellow were observed to be due to fermentation of mannitol whereas no colour change indicated that mannitol was not fermented.

Serum Bactericidal Assay

A serial dilution of human serum was made up to 640 dilutions. A colony of the test organism was added into each dilution, the mixture was allowed to stand for 3 hours and the specimens were later streaked onto a nutrient agar plate and incubated for 24hours. Stains were termed sensitive if viable count dropped to 1% of initial value and resistant when 90% of the organisms survived after 3hours (Benje, 1998; Stregfried et al., 1994).

HAEMOYSIN PRODUCTION

The peptone water culture of the test organisms were inoculated into 5% human blood. These were incubated at 37^0c for 24 hours. Haemolysin production was observed by a zone of clearing around the bacterial growth.

ANTIBIOTIC SUSCEPTIBILITY TEST

All the bacterial isolated were tested for antibiotics susceptibility by Kirby Bauer disc diffusion method, and zone size was interpreted following the National Committee for Clinical Laboratory Standard (NCCLS, 2002).

A sterile cotton swab dipped it the broth culture of isolate was streaked evenly all over the surface of Muller Hinton agar and, antibiotic disc was placed aseptically on the inoculated plates using sterile forceps. Also a known positive (sensitive) control culture was used and the result interpretation done on the basis of zone size inhibition. A zone size inhibition within 3mm radius of that of the positive control is interpreted as sensitive. Also a zone size inhibition of not more than 2mm radius of the positive control is interpreted as resistance. A zone size of inhibition falling in between the above two is said to be intermediate. The plates were then incubated for 24 hours at 37^0c. The antibiotics used and their standard concentrations were as follows:

Antibiotics	Standard	Concentration used
Ciprofloxacin	CF	5µg
Chloramphenicol	C	30 µg
Erythromycin	E	15 µg
Gentamycin	G	10 µg
Penicillin G	P	10 units
Streptomycin	S	10 µg
Ampicillin	A	10 µg
Nalidixic acid	NA	30 µg
Trimethoprim	CO	1.25/23.75 µg
Colistin	CL	10 µg
Oxacillin	OX	1 µg
Augmentin	AG	20/10 µg

ASSAY OF β-LACTAMASE PRODUCTION

β- lactase production was assayed using the method of Sng et al (1981). A strip of whaman no 1 filter paper was placed in the bottom of a Petri dish. A few drops of buffered crystalline penicillin bromocresol purple solution were dropped until the paper is almost saturated. The test organisms were transferred to the filter paper to cover an area of 5mm in diameter. The petri dishes were incubated at 37^0c for 30 minutes. A yellow colour indicated production of penicillonic acid from the breakdown of penicillin by the β-lactam producing organisms.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC OF AMPICILLIN AGAINST *Pseudomonas aeruginosa*

The minimum inhibitory concentration of commonly used antibiotic-ampicillin, against the most resistant isolate, *Pseudomonas aeruginosa was* determined using the paper disc method as described by Olok, (2000). Sterile paper discs were dipped into different concentration of ampicillin. Also sterile paper disc were dipped into sterile water used for the dilution of antibiotics was used as a control. Each soaked disc was then layered on nutrient agar plates already seeded with *Pseudomonas aeruginosa* in duplicates. The plates were incubated at 37^0c for 24hours and then examined for zones of inhibition. The lowest concentration of the antibiotics which inhibited growth was taken as the minimum inhibitory concentration.

STATISTICAL ANALYSIS

The differences among isolates were analyzed by the Chi-square and fisher's exact test (Steele and Torrie, 1986). Chi-square was used to observe the frequency of isolates more than two while fisher's exact test was used to observe frequency less than two, P-value less than 0.05 was considered significant for all tests.

3.1 STUDY SUBJECTS

A total of 270 study subjects with LRTI were investigated during the study period. Of these, 20 were asymptomatic. Distribution of study subjects by age and clinical symptoms is shown in table 3. 1 Of the 250 symptomatic subjects, 200 (80%) were males and 50 (20%) were females. The ratio of male to female was 4:1. In asymptomatic patients, the age group85 to 90 predominated and represented 100% of all control group.

INCIDENCE OF LRTI AMONG SYMPTOMATIC PATIENTS

The incidence of LRTIs among the study subjects by age and gender is shown in table 3.2. Significant positive cultures was observed in 200/250 (80%) of symptomatic patients, (p<0.05). The prevalence of LRTI in both symptomatic and control groups was 220/270 (81.4%). The overall prevalence of LRTI was higher in males (80%) than in females (20%). And there was statistically significant difference between the two groups (p<0.05). In this study, LRTI is more common in the elderly patients (aged 86-95 years) among symptomatic patients.

CAUSATIVE ORGANISMS

The type of microorganism, the number and percentage occurrence of each organism isolated from sputum samples in both symptomatic subjects and asymptomatic control group are shown in Table 3.3. A total number of 220 organisms were isolated, 200 from symptomatic and 20 from control patients. *Streptococcus pneumonia* (42%), *Klebsiealla spp,* (20%), *Staphylococcus aureus (*16%) and *Pseudomonas aeruginosa* (13%) were the most common probable bacteria pathogens isolated from symptomatic subjects. In addition, coagulase negative *Staphylococcus aureus* (9%) were isolated only from control subjects. More than one bacterial (mixed type) were isolated in equal proportions (15%) from both groups. The gram positive and negative bacteria constituted 148(67.2%) and 72(32.7%), respectively. There are no statistical significant differences in the isolation frequency of each organism from the control subjects (p>0.05). Details of the morphological and biochemical characteristics of the isolates are presented in table 3.4. *Staphylococcus aureus,* a Gram positive organism, is catalase and coagulase positive, produces acid and gas when it ferments sucrose, mannitol and lactose. *Staphylococcus aureus* also grow well aerobically and in a carbon dioxide enriched atmosphere. It produces yellow to cream or occasionally white, 1-2mm in diameter colonies after young colonies. In MacConkey agar, *Staphylococcus aureus* produces 0.1-2mm colonies after overnight incubation at 35-37 degree centigrade. Following overnight incubation, *streptococcus pneumonia,* a Gram-positive organism forms translucent or mucoid colonies 1-2mm in diameter on blood agar. In young cultures, the colonies are raised but later become flattened with raised edge giving them a ringed appearance (draughtsman). When cultured anaerobically on blood agar, some strains of *Streptococcus pneumonia* show β-haemolysis. In chocolate and lysed blood agar, it grows well and the growth is enhanced when incubated in carbon dioxide enriched atmosphere (candle jar). It gives a positive test to citrate utilization test. *Escherichia coli,* a Gram-Negative motile rod is indole and methyl red positive, and ferments lactose to produce acid and gas. It reacts negatively to VP, Oxidase, citrate and urea tests. *Escherichia coli* produces 1-4mm diameter colonies in blood agar after overnight incubation. Some strains produce hemolytic colonies and their colonies may also appear mucoid. It ferments lactose in MacConkey agar producing pink colonies *Klebsiella Pneumonia* is an aerobic and a facultative anaerobic Gram-negative, non motile capsulated rod. In blood agar, it produces large grey white usually mucoid colonies. Most stains are lactose-fermenting producing mucoid pink colonies on MacConkey agar. It is urease and citrate positive and negative for VP, methyl red and indole tests and ferments lactose and maltose. It produces β-lactamases and it is highly resistant to ampicillin *Pseudomonas aeruginosa,* a Gram negative, non-motile rod, produces large, flat and spreading colonies which are often haemolytic and pigment producing in blood agar. The pigments, usually diffuse into the medium, giving it a dark greenish blue colour. When pigment is left at prolonged room temperature, it becomes more intense. *Pseudomonas aeruginosae* also produces pale coloured colony on MacConkey agar. It is oxidase positive and produces acid only but no gas form glucose.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

The resistance pattern of bacteria isolated from LRTI patients against 15 antimicrobial agents are shown in table 3.5. The main Gram-positive isolates from symptomatic patients showed a high level (>80%) of resitance to C0-trimoxazole, Tetracycline and Ampicillin. Intermediate level (60-80%) of resistance was found against penicillin G, Nitrofurantoin, Nalidixic acid and Streptomycin (62.5%) and low level (<60%) of resistance observed for Oxacillin, Trimethoprim,Erythromicin and Augmentin. Low level (25%) and high level (100%) of resistance to Septrin was also observed among symptomatic patients. The resistance pattern of three gram-positive isolates (one *S.aureus* from symptomatic patient and two coagulase negative *Staphylococci* from asymptomatic control group) showed a high level of resistance (>80%) to ampicillin, Tetracycline and co-trimoxazole. All the strains were sensitive to Ciprofloxacillin, Gentamicin and chloramphenical. The minimum Inhibitory concentration (MIC) of Ampicillin against *Pseudomonas aeruginosa* was also determined (Table 3.6). the MIC ranged between 0.20 to ≥ 250μg for Ampicillin. Out of the 39 isolates of *Pseudomonas aeruginosa* , 20 were found to be positive while 4 isolates were hemolytic on human agar indicating the presence of α-haemolysis. All the isolates were Serum resistant.

RESULT

Table 3.1

DISTRIBUTION OF STUDY SUBJECTS BY AGE NUMBER AND CLASSICAL SYMPTOMS

Age range	Number	Clinical symptoms
56-65	54	High grade fever, cough productive of yellowish sputum, chest pain
66-75	75	,,
76-85	46	,,
85-90	20	Asymptomatic control subjects
86-95	65	High grade fever, cough productive of thick yellowish yellowish sputum, chest pain.
96-above	10	,,

Table 3.2

INCIDENCE OF LRTI BY AGE AND GENDER

Age group (years)	Male	Female	B-lactase activity		Total positive Samples
	Sputum samples studied	Sputum samples positive	Sputum samples positive	Sputum samples positive	
56-65	34	19(56%)	20	9(31%)	28
66-75	48	37(77%)	17	8(33%)	45
76-85	33	28(85%)	13	7(33%)	35
85-90	10	10(100%)	10	10(100%)	20
86-95	53	47(88%)	18	15(83%)	62
96-above	32	29(91%)	1	1(100%)	30
Total	210	170	70	50	220

TABLE 3.3

Type of microorganism, and their % occurrence in sputum samples

Organism	number	% occurrence
Streptococcus pneumonia	93	42%
Klebsiella pneumonia	43	20%
Staphylococcus aureus	35	16.0%
Pseudomonas aeruginosa	29	13%
Coagulase-negative *Staphylococcus aureus*	20	9%
	220	**100**

TABLE 3.4: ISOLATION AND IDENTIFICATION OF BACTERIAL ISOLATES.

Sample Code	Colonial Characteristics	Gram Reaction	Biochemical Tests												Sugar Fermentation					Probable Organism
			CATALASE	URASE	COAGULASE	MOTILITY	INDOLE	OXIDASE	CITRATE	METHYL RED	VOGES PROSKAUR	BILE	SOLUBILITY	GUC	SUC	LAC	MAL	MAN	
A₁B₆	Convex smooth and white	GPC in clusters	+	-	+	-	-	-	-	-	+ -		-	-	+	+	-	+	*Staphylococcus aureus*
A₇B₆	Colony with entire edge																		
C₁₁,X₄	Slightly raised edge colony	GPC in clusters	+	+	-	-	-	-	-	-	+ +		-	-	+				*Coagulase Staphylococcus aureus*

TABLE 3.4: ISOLATION AND IDENTIFICATION OF BACTERIAL ISOLATES.

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			CATALASE	URASE	COAGULASE	MOTILITY	INDOLE	OXIDASE	CITRATE	METHYL RED	VOGES PROSKAUR	BILE	SOLUBILITY	GUC	SUC	LAC	MAL	MAN	
F₃ W⁹ Y₈, O₉ In young cultures, colonies are raised but later become flattered with raised edges giving them a ridged appearance (draughtsman)	Translucent or mucoid Colonies 1-2mm in diameter	GPC in chains	-	-	-	-	-	-	-	-	+		-	-	-	-	-	-	*Streptococcus Pneumonia*

Key: GLU: Glucose, LAC Lactose: MAL Maltose   MAN: Mannose

SUC Sucrose   GNR: Gram Negative Rod

GPC: Gram Positive Cocci   A: Acid,

G: Gas

Sample Code	Colonial Characteristics	Gram Reaction	CATALASE	URASE	COAGULASE	MOTILITY	INDOLE	OXIDASE	CITRATE	METHYL RED	VOGES	PROSKAUR	BILE	SOLUBILITY	GUC	SUC	LAC	MAL	MAN	Probable Organism
R10M11 B10 C5	Smooth light colony with irregular edge	GN Short rods	-	-	-	+	-	+	-	-	-	-		A	-		-	A	-	*Pseudomonas aeruginosa*
R18,	Large creamy colony	GNR	-	+	-	-	-	+	-	-	-	AG	AG	AG	-		AG			*Klebsiella pneumonia*

Table 3.5

Antimicrobal susceptibility Tests on Bacterial Isolates from sputum samples

Antibiotic	(concentration)	**Number (%) of isolates susceptible to antimicrobials tested**				
		S. Pneumonia n= 72	K. Pneumonia N=43	S. aureus n=20	P.aeruginosa n=39	Av.% sensitivity (%)
Ciprofloxacin	Cf (5µg)	38 (61.29%)	30 (83.3)%	26(92.9)%	5(41.7)%	69.8
Chloramphenicol	C (30 µg)	46(74.2%)	18(50%)	14(50)%	6(50)%	56.1
Erythromycin	E(15 µg)	5 (6.5 %)	20(55.6%)	10(35.7)%	5(41.7)%	34.9
Gentamicin	G (10 µg)	32 (51.6%)	32(88.9%)	24(84.7%)	10(83.3%)	77.1
Penicillin G	P (10 units)	0 (0.1%)	9(25%)	0 (0%)	0(0%)	6.2
Streptomycin	S(10 µg)	10(16.1%)	6(16.7%)	2(7.1%)	0(0%)	9.9
Ampicilin	A(10 µg)	0(0%)	0(0%)	1(3.6%)	0(0%)	0.9
Tetracycline	T(30 µg)	0(0%)	0(0%)	1(3.6%)	0(0%)	0.9
Nalidixic acid	Na (30 µg)	0(0%)	1(2.8%)	0(0%)	0(0%)	0.7
Nitrofurantion	NF(300 µg)	1(1.6%)	2(5.6%)	4(14.3%)	0(0%)	5.3
Trimethoprim	Co(1.25/23.75µg)	34(54.8.%)	12(33.3%)	18(64.3%)	2(16.7%)	41.0
Colistin	Cl (10µg)	31(50.0%)	4(11.3%)	5(17.9%)	1(8.3%)	23.0
Oxacillin	OX(1 µg)	20(32.3.%)	12(33.3%)	18(64.3%)	2(16.7%)	41.0
Augumentin	Ag (20/10µg)	40(64.5%)	16(44.4%)	1(3.6%)	14(33.3%)	36.5
Cotrimoxazole	SXT (30 µg)	0(0%)	0(0%)	0(0%)	0(0%)	0.0

Table 3.6 MIC Values of Ampicillin Against Pseudomonas Aeruginosa

Mic (µg)	No of organism	Percentage	B-lactase activity
0.20	1	3.4	-
0.5	0	0	-
1	5	10.3	-
2	2	17.5	-
4-64	28	62	+
128	3	6.8	+
≥250	0	0	-

Discussion

Based on the laboratory findings (i.e. significant growth obtained in culture during this study), the incidence of lower respiratory tract infections among elderly patients (between the age of 56 and above) visiting the out-patient department of Federal Medical Centre, Umuahia was found 200(74%) of the total270 sputum samples investigated. The result of this study was not in line with that conducted by Gauchen *et al* (2006) where their

laboratory findings showed a significant growth of 74(41.1%) only out of the total 181cases they investigated.The greater negative results in their study may be attributed to viral and other etiological agents, apart from bacterial pathogens mainly investigated in this study. Although four different types of bacteria were isolated and identified in sputum samples of symptomatic subjects, greater numbers of the isolates were gram-positive bacteria. The ratio of Gram-positive bacterial infection to Gram-negative bacterial infection was 1.8:1. This findings did not correlate well with that of similar study conducted by Sayami (1995) which has the ratio of Gram-positive bacterial infection to Gram-negative bacterial infection as 1.2:6. The reason could be attributed to high prevalence of *Streptococcus pneumonia* which was the major *Nosocomial* infections seen in Federal Medical umuahia, especially in the chest Clinics of the Health institution. The study showed males (59%) at more risk of lower respiratory tract infection than the females (15%). Reason as such could be because females enrolled in this study comprised largely of aged mothers who, being less mobile, experienced less exposure to respiratory risk factors. It could also be attributed to predisposing factors in males like smoking and alcoholism. Furthermore, in this study, higher incidence of lower respiratory tract infection was observed in elderly population, 95 years and above, accounting for 91% of the total infected patients in that age group than the younger lot 56-65 years, accounting for 56% of the total infected cases in their age group particularly, these patients in higher age group above 95 years were found more prone to lower respiratory tract infection (96%). Similar study showed that pneumococcal infection is common in patient of this age group, who also have an increasing incidence of lower respiratory tract infection as they get older. This result correlated well with the work of Mac Farlane et al .(1993). The reason has been attributed to less effective immune system in older patients, due to either malnutrition or underlying diseases such as Diabetes mellitus, Uremia, emphysema etc. Of the total bacterial isolates, *Streptococcus pneumonia* was the most common isolate (42%) followed by *Klebsiealla pneumonia* (20%) and *Staphylococcus aureus* (16%). *Streptococcus Pneumonia* was of highest incidence among the Gram-positive isolates. The high predominant of *Streptococcus Pneumonia* correlated well with the earlier study of Mac Farlene *et a.l*(1993).It is also worthy to note ,*Streptococcus pneumonia* (and other Gram-negative bacilli) were diagnosed predominantly in elderly population with pre-existing chronic disease in line with that earlier study of Mac Farlane et al. (1993).

In this study, isolates resistant to one or more antibiotics commonly in use was observed to be higher than similar case study conducted by Joshi *et al.* (1999). All isolates, both Gram-positive and Gram-negative showed resistance to Co-trimoxazole. This was attributed to patients who engage in self medications or by careless use of antibiotics in treating respiratory infections by most Clinicians without conducting the required antibacterial susceptibility tests. Similarly, higher percentage of both gram-positive and gram-negative isolates showed appreciable resistance to ampicillin, tetracycline and Nalidixic acid to earlier study. *Pseudomonas aerugniosa and klebsiella pneumonia* were among the bacteria manifesting higher levels of resistance-most notably in developing countries like Nigeria. The incidence in the antibiotic non-susceptibility strains of pathogens in recent years could be attributed to their indiscriminate and promiscuous use especially by clinicians who do not see the need to be patient enough and wait for antibacterial susceptibility tests to be released by the microbiologists. This alarming situation is due, in part, to widespread confusion over the difference between viral and bacterial respiratory infections, which also led to the emergence of resistance *Pneumococci* and *staphylococci*. Similarly, in the case of Gram-positive bacterium like *streptococcus pneumonia,* the use of suboptimal and long duration regimens increases the opportunity for acquisition and/or amplification of resistance strains. Formerly, first line medications were both effective and affordable but with the onset of resistance,However, newer treatments are roving too costly to the vast majority of those living in poor developing Nations; WHO (1996).

CONCLUSION

This study indicates that there have not been any significant changes in the pattern of bacterial involvement causing lower respiratory tract infections. Also, the population and the age group at risk have remained more or less the same over the period of time. However, increasing number of drug resistant pathogens emphasizes the importance of judicious antibiotic use. Education program in this respect for general public and clinicians as well could be useful in checking the emergency of drug resistant pathogens like *Mycoplasma pneumonia* and other respiratory viruses. In

microbial investigation of lower respiratory tract infections, these measures could definitely reduce the unnecessary antibiotic use in humans.

Also, as most developed and developing countries like Nigeria face population aging and the incidence of lower respiratory tract infection increases with age, it is most important to consider preventive and early clinical management strategies for LRTI in the very elderly members of our society. This could be achieved through two approaches, firstly, elderly with changeable risk factors could be advised to reduce exposure to these factors, for instance, very elderly who currently smoke could be advised to stop or reduce smoking, and unnecessary use of glucocorticoids. Secondly, those very elderly at risk could be firmly advised to apply for influenza and pneumococcal vaccination. Before giving this advice and changing current guidelines, however, the effect of both strategies on reducing lower respiratory tract infections in the very elderly people have to be studied in a randomized intervention study.

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