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Microbiology & Pathology

Pneumoniae to Oxacillin

Ofloxacin and Rifampicin

Highlights

Diagnosis of Mycoplasma

Assessment of Clinicians

Discovering Thoughts, Inventing Future

VOLUME 15 ISSUE 4 VERSION 1.0

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Microbiology and Pathology

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Comparative Study of Techniques in the Diagnosis of Mycoplasma Pneumonia among the Patients of Respiratory Tract Infections in Northern Indian Population

By Dr. Abhineet Mehrotra, Dr. S. K. Mehra, Dr. M E Siddque & Dr. Sushil Suri

Geetanjali Medical College, Udaipur

Abstract- *Background:* In the present study an attempt was made to compare the different diagnosis of mycoplasma pneumonia among the patients of respiratory tract infections in northern Indian population. Commonly used techniques in the diagnosis of mycoplasma pneumonia are culture, serology and PCR and different studies assure the different sensitivity and specificity. In the present study author also aim to find the common risk factors of mycoplasma pneumonia.

Material & Methods: The present study was undertaken at Career Institute of Medical Science, Lucknow. The total number of study subjects were 193. The total number of male subjects were 115 (59.6 %) while 78 (40.4 %) were females. Samples were Collected for culture, serology and PCR. All samples of culture & serology were undergone for PCR.

Results: Out of 193 patients 58 (30 %) samples were found positive in culture and 73 (38 %) samples were found to be positive in serology test. 58 patients were found to be positive by both culture and serology which are further tested in PCR and only 10 (17 %) samples were found positive.

Keywords: *mycoplasma pneumonia, culture, serology, PCR & risk factor.*

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Comparative Study of Techniques in the Diagnosis of Mycoplasma Pneumonia among the Patients of Respiratory Tract Infections in Northern Indian Population

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Results: Out of 193 patients 58 (30 %) samples were found positive in culture and 73 (38 %) samples were found to be positive in serology test. 58 patients were found to be positive by both culture and serology which are further tested in PCR and only 10 (17 %) samples were found positive. The chi square test shows that respiratory & renal diseases may be considered as significant risk factors of Mycoplasma pneumonia ($p<0.05$).

Keywords: mycoplasma pneumonia, culture, serology, PCR & risk factor.

I. Introduction

Mycoplasmas (mushroom form) are eubacteria included within the class Mollicutes (from latin mollis = "soft," cutis = "skin"), which comprises the smallest and simplest self-replicating, pleotrophic bacteria that lack cells wall thus, beta-lactam antibiotics, such as penicillin and cephalosporins, are ineffective. The name Mycoplasma, from the Greek μυκής, mykes (fungus) and πλάσμα, plasma (formed), was first used by Albert Bernhard Frank in 1889[1]. Mycoplasmas are primarily mucosal pathogens, living a parasitic existence in close association with epithelial cells of their host, usually in the respiratory or urogenital tracts. M. pneumoniae exclusively parasitizes humans, whereas some of the other human mycoplasmas have also been recovered from nonhuman primates[2]. Mycoplasma pneumoniae is an important cause of respiratory tract infection and is increasingly being associated with other diseases such as asthma and extra-pulmonary complications. Considerable cross-reactivity is known to exist between the whole cell antigens used in the commercial serological testing assays. Usually, mycoplasma infection is a mild illness characterized by fever, cough, bronchitis, sore throat, and headache. In very rare cases, mycoplasma can cause serious illness such as encephalitis (an inflammation of the brain) or meningitis (inflammation of the lining of the brain and spinal cord).

As the diagnosis the clinical symptommatology of a mycoplasma infection does not show pathogen-specific characteristics, the diagnostic differentiation from other pathogens such as viruses and gram-positive bacteria is decisive for appropriate therapy[3]. The diagnosis can be based on direct detection and serology. Detection of the pathogen has been regarded as efficient diagnosis at acute, early stages of disease. Isolation of the pathogen by culture has been considered the reference method. However, it is too insensitive and time-consuming (6-14 days). A good quality pathogen DNA detection system (PCR) is not yet commercially available. To date, serology has been considered the method of choice for diagnosis of infections. The complement fixation test (CFT) represents the classic antibody detection. The CFT cannot discriminate between antibody isotypes. Agglutination tests cannot discriminate between antibody classes either. Both test systems detect mainly the IgM antibody response. In reinfections both CFT and agglutination tests provide predominantly negative results. Nevertheless these test systems currently dominate the ELISA technology[4]. By using ELISA, IgG, IgA, and IgM, antibodies can be differentiated. The crucial factor for a specific and sensitive ELISA is the antigen.[5]

Author α: Research Scholar, Department of Microbiology, Geetanjali Medical College, Udaipur. e-mail: abhineetmehrotra@gmail.com
Author σ: Prof. & HoD, Department of Microbiology, Geetanjali Medical College, Udaipur.
Author ρ: Prof. & HoD, Department of Microbiology Career Institute of Medical Sciences & Research, Lucknow.
Author ω: Associate Professor, Department of Medicine, Career Institute of Medical Sciences & Research, Lucknow.

In the present study we try to evaluate the effective method in the diagnosis of mycoplasma pneumonia among three techniques i.e. Serology, Culture & PCR and an attempt was made to explore the socio demographic and clinical characteristics.

II. Material & Methods

The present study was undertaken at Career Institute of Medical Science, Lucknow. The total number of study subjects were 286. The total number of male subjects were 115 (59.6 %) while 78 (40.4 %) were females. The present study protocol was approved by institutional ethical committee.

a) Culture, Serology and PCR

The expectorated sputum (throat swabs) and blood was collected from all patients. The M. pneumoniae standard strain was revived according to ATCC (American Type Culture Collection) 15531™ guidelines (www.atcc.org) which is commercially available in market. In brief, the lyophilized culture was resuspended in 6 ml pleropnemonia like organism (PPLO) broth. A single drop was used to inoculate PPLO agar; 3 ml suspension was used to prepare glycerol stocks and stored at -70°C. The remaining 3ml suspension was incubated at 37°C and 5 per cent CO2 incubator till growth was observed with change in color from red to yellow. Mycoplasma pneumoniae strains are very slow growing and produce a very light turbidity. Growth in broth is best observed after 10 to 14 days of incubation. Usually it takes at least seven days for the first tubes to start showing growth. Growth is easily recognized by an indicator change from red to orange to yellow. The cells are best transferred when the medium is orange. After medium changes to yellow, cells have started to die. All the patients whose culture was found to be positive were taken for further consideration. The patients having positive culture were marked and further followed for serology. Only serum part which was separated from whole blood of those whose culture was positive. Serum was stored at -20°C till assayed. ELISA (Enzyme-Linked Immunosorbent Assay) kit is used for the accurate qualitative measurement of IgM class antibodies against Mycoplasma pneumoniae in Human serum and plasma. The clinical measurements were recorded. After serological test those patients were again used for follow up (for the purpose of PCR) whose culture as well as serology was found to be positive.as well as all other samples of culture and serology negative were also processed for PCR.

The sputum samples were collected in PBS were centrifuged at 1957 x g for 10 min. Supernatant was decanted and the pellet was resuspended in 0.5 ml PBS and stored at -70oC till further processing. The positive control consisted of relevant purified mycoplasma DNA. The extraction of DNA was done using the organic methods described by Das et al [5]. A 543 bp section of the P1 protein gene of M.pneumoniae was selected for amplification. The primers (Bangalore Genei Pvt. Ltd., Banglore, India) were:

Primer1: 5'CAAGCCAAACACGAGCTCCGGCC-3', which is complimentary to the P1 gene negative strand residues 3666-3688, and

Primer 2 : 5'CCAGTGTCAGCTGTTTGTCCTTCCCC- 3', which is complimentary to the P1 gene positive strand residues 4208-4183.

Amplification was done according to the guidelines of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Banglore, India). Amplified PCR products were subjected to electrophoresis on 3 ethidium bromide stained agarose gel, along with a molecular weight marker. A mixture of 2 μl genomic DNA, 2 μl of 6 X BPB and 8 μl of distilled water was loaded into the 1 per cent agarose gel. The electrophoresis was carried out at a constant voltage of 50 V for 1 h, and a band at 543 bp was taken to be a positive result. The PCR of those patients whose culture and serology was positive was carried out.

III. Results

Table 1 represents the age wise distribution of study subjects. The total number of study subjects was 286. The total number of male subjects were 207 (72.4 %) while 79 (27.6 %) were females. Highest number of subject's i.e. 32.9 % subjects were less than 30 years while second highest number i.e. 30.4 % of study subjects was more than 45 years. Table 2 Gender wise distribution of study subjects. Table 3 shows the test result by different diagnosis technique of mycoplasma pneumonia. Out of 286 patients 168 (58.7 %) samples were found positive in culture and 98 (34.3%) samples were found to be positive in serology test. PCR testing shows only 80 (28 %) samples were found to be positive.

Table 1: Age and sex distribution of the study population (n = 286)

Age Group (years)	Sample	N (%)
<3	15	5.2%
3-5	21	7.3%
6-10	33	11.5%
11-15	19	6.6 %
16-30	94	32.9 %
31-45	87	30.4%
46-60	9	3.1%
>60	8	28 %

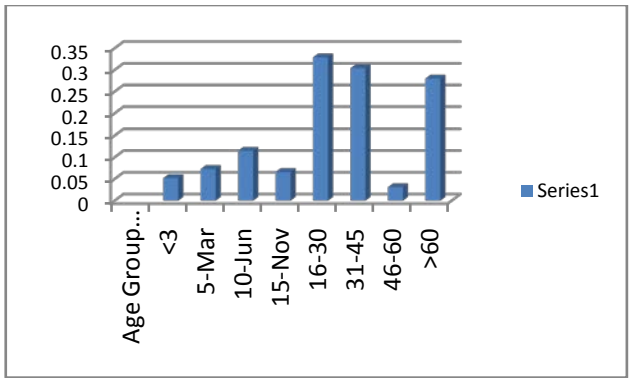

Figure 1

Table 2 : Gender wise distribution

Gender	N	%
Male	207	72.4
Female	79	27.6

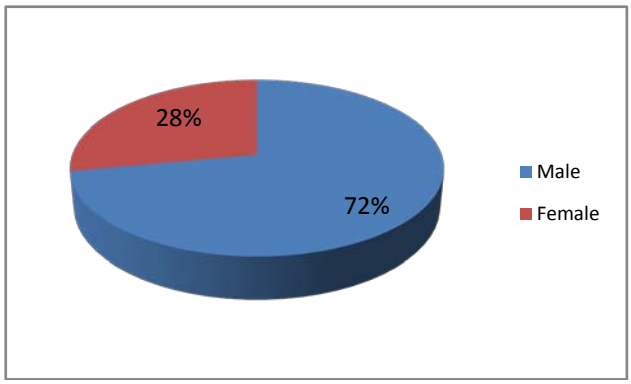

Figure 2

Table 3 : Test results of sample

Test Result	Culture (n=286)	Serology (n=286)	PCR (n=286)
Positive Result	168(58.7%)	98 (34.3%)	80(28%)
Negative result	118(41.3%)	188 (65.7%)	206(72%)

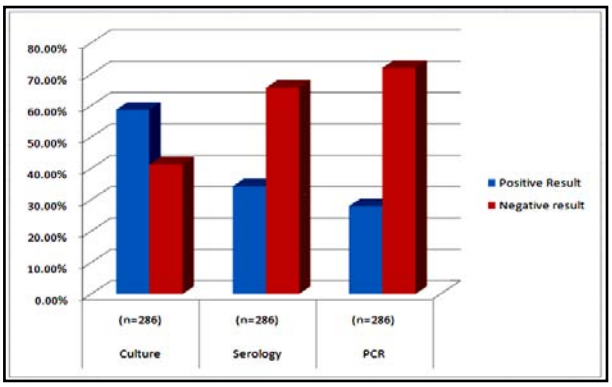

Figure 3

a) Study design

This prospective cross-sectional study design will be used for the present study was conducted on community-acquired pneumonia in the Department of Microbiology, at Career Institute of Medical Science, Lucknow.

b) Sample size calculation

Sample size was calculated by the sample size calculation formula for sing proportion by $n=4pq/d^2$, Where n: sample size, p: prevalence q: 1-p and d is specified absolute precision by assuming prevalence 21 %, with specified absolute precision 0.06 and 10 % data loss, be. Calculated sample size is 260. Adding 10% non-response, the final sample size is 286.

Sample size is 260. Adding 10% non-response, the final sample size is 286.

i. *Inclusion criteria*

Patients clinically diagnosed clinical and radiological proven of atypical pneumonia will be included in the study with,

1. Community acquired pneumonia (CAP):
2. Presence of a new pulmonary infiltrate/ shadow on chest X-ray suggestive of pneumonia at/ within 24 h of hospitalization.
3. Patient residing in community.

ii. *Exclusion criteria*

1. Hospital acquired pneumonia i.e. pneumonia that developed 72 h after hospitalization or within 7 days of discharge.
2. Pulmonary shadow due to a cause other than pneumonia.
3. The serious patients having other disease with atypical pneumonia will be excluded

A written informed consent was taken from the parent/legal guardian of the children before them being enrolled in the study.

Clinical data from the patients were collected using a questionnaire developed and validated and a detailed examination was performed. Routine laboratory investigations were done in all subjects. In the present study culture, serology and PCR technique is used to diagnose the mycoplasma pneumonia.

IV. Discussion

A variety of techniques are used to assess *M. pneumoniae*, each with different advantages and disadvantages. In this study, PCR, ELISA, and culture methods were employed for the detection of *M. pneumoniae* infection in 286 suspected patients. The culture method for the isolation of *M. pneumoniae* requires 2–4 weeks, which limits itsclinical usefulness. Moreover, culturing of *M. pneumoniae* is expensive and time-consuming (7-10). Serological methods are more extensively used than culture, because they are easier to carry out and more affordable (11-13).However, they are generally nonspecific, retrospective, and need 2 samples for titration[7]. It should be noted that in our study, the most reliable result was obtained by the PCR method, which proved to be highly sensitive, specific,

and faster than other methods. The specificity of the culture method was 100%, while its sensitivity barely reached 33%, relative to the results of the PCR method as a gold standard. All of the culture and IgM ELISA test positive patients were PCR-positive, too. The PCR method is more sensitive and is the gold standard currently being used for diagnosis of this organism in some laboratories[14]

V. Conclusion

In the present study it is concluded that only one technique may not be sufficient to diagnose the mycoplasma pneumonia as these techniques do not show higher sensitivity and specificity as many results suggest the same [6]. However, serology and culture is done commonly.

VI. Acknowledgment

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Geographical Distribution of Factor V Laden in Different Regions of India

By Dr. Pushpalatha Manjunatha, Dr. Satish Kumar Amarnath, Arun Kumar H. R, Bala Satish M & S. J. Sabarish Babu

Ecron Acunova, India

Abstract- *Background:* Factor V Leiden is the most common inherited form of thrombophilia with 3 to 8 percent European and American ancestry carrying one copy of the factor V Leiden mutation in each cell, and about 1 in 5,000 people have two copies of the mutation. The mutation is less common in Asian, Hispanic and African populations.

Aim: This study was carried out to know the incidence of Factor V mutation in various regions of India.

Methods: The presence of factor V mutation was determined by PCR- RFLP from patients suspected of thromboembolic etiology from medical, surgical, obstetrics and neonatology departments.

Results: Analysis of 94 patients with coagulation disorders, factor V mutation was seen in 20 cases.

Discussion: Although many reports from India indicate the absence of this mutation or the presence of homozygous mutation in a low level we report about 26 percent of the cases having the homozygous mutation which is the first for India.

GJMR-C Classification : *NLMC Code: QW 4*

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I. Introduction

Factor Van Leiden mutation has been reported in 3 to 8 percent of the Caucasian population with thrombophilia, and absolutely absent in Hispanics, African and Asian populations in the US.

Factor V Leiden named after the city Leiden (The Netherlands), was first identified in 1994 by Prof R. Bertina et al. In normal individuals Activated protein C (APC) prevents blood clots from growing too large by inactivating factor V. In factor V Leiden mutation, there is single nucleotide substitution of adenine for guanine (single nucleotide polymorphism) changes the protein's 506th amino acid from arginine to glutamine, which is the cleavage site for APC (Bertina et al).

Factor V Leiden can be associated with the following complications such as deep vein thrombosis (DVT), superficial thrombophlebitis, sinus vein thrombosis, mesenteric vein thrombosis, Budd-Chiari syndrome, pulmonary embolism (PE), recurrent unexplained miscarriage, preeclampsia and/or eclampsia.

PCR is a simple genetic test that can be done for diagnosis of factor V mutation. The mutation (1691G→A substitution) removes a cleavage site of the restriction endonuclease MnlI, which can be detected by PCR - RFLP.

In Asian-Indian populations 1% to 8.5% has been reported. In Tamil Nadu, south India, 4 out of 72 were reported to be heterozygous for FVL mutation (5.5%), while none were detected for homozygous mutation. Another study to know the association between portal vein thrombosis and FVL, reported 4.1% heterozygous mutation for factor in healthy Indians. However, contradic¬tory results were found when the presence of this mutation was studied in healthy populations from Turkey, Korea, and even from some Indian populations (Wan Zaidah Abdullah et al 2009).

The presence of the mutation markedly increases the risk for renal vein thrombosis, particularly in neonates, and renal transplant vein thrombosis in transplant cases resulting in rejection. Routine screening for factor V Leiden mutation by polymerase chain reaction, and appropriate perioperative and postoperative anticoagulation after renal transplantation might be a valuable strategy to prevent thromboembolic complications in transplant recipients (Craig J Della Valle MD et al).

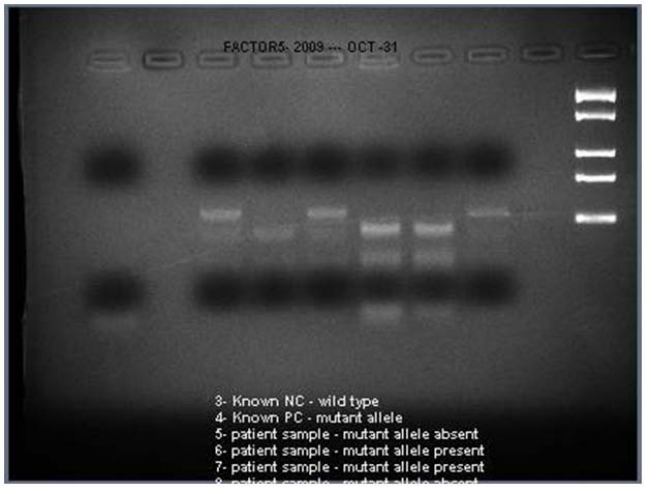
The factor V Leiden mutation is associated are two to three times more likely to have recurrent miscarriages or a pregnancy loss during the second or third trimester (8).

II. Materials and Methods

A total of 94 samples were received from patients attending medicine, surgery, neonatology and OBG departments from April 2007 to December 2010 from different states like Karnataka (2, 24.5%), Andhra Pradesh (43, 45.7%), Punjab (18, 19%) West Bengal (5, 5.9%) and Nepal (2, 2.1%). Patients with history of spontaneous DVT, postsurgical, venous thrombosis, haemorrhaegic infarct, stroke, unexplained DVT, primary DVT, hypercoagulable state. The EDTA blood samples were shipped at – 20°C, DNA extraction was done using Qiagen DNA columns. The samples were analyzed for mutant gene by Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR - RFLP) followed by analysis of fragments of digestion by gel electrophoresis. Positive and negative controls were included in the assay, normal (wild) sample showed 3 bands at 86, 46 and 37 bp region, and positive control

Author α ρ ω ¥: Central Laboratory, Manipal Acunova Ltd, Mobius Towers, SJR i - Park EPIP, Whitefield, Bangalore.
e-mail: pushpa.teju.m@gmail.com
Author σ: Head Quality, compliance and Outreach Services, Manipal Cure and Care, Manipal Towers, 14, HAL Airport Road, Bangalore.
e-mail: Satish.amarnath@gmail.com

showed 2 bands at 123 and 46 bp region, and negative control did not show any bands. The procedure was followed as per Craig J Della Valle MD et al. 2001.



III. Results

Out of 94 samples screened for the mutation, in the age group ranging from zero to 65 years of age. Of the total 59 were males, age ranging from 1 day to 60 years, with a mean age of 33.5 and a stdev of 16.2. Among the 35 females studied, the age range was 1 day to 60 with a mean of 34.7 and stdev of 12.4. Of these 46 (48.9%) were from Andhra Pradesh, 23(24.5%) from Karnataka from southern region, 19.1% from Punjab in the north, 5.3% from west, and. 2.1% from Nepal.

20 samples were positive for heterozygous mutation, 11 were males in the age group in the range of 1 year to 60 years with a mean of 33 and stdev of 16. In the female cases 9 were positive in the age group in the range of 20 years to 40 years with a mean of 29.8 and stdev of 6.6.

A total of 20 patients were positive for heterozygous mutation, of which 11 were males and 9 were females. 7 cases (7.4%) were from Punjab, of which 5 were females and 2 were males constituting 35% of the positives. These cases were obtained by screening 18 suspected cases from Punjab, giving a positive rate of 35%. Even though 43 cases were screened from Andhra Pradesh, 9 were positive of which 5 were males and 4 were females accounting to 18%, with positive rate of 45%, 3 from Karnataka all 3 were males with a positive rate of 15%, 1male from West Bengal with a positive rate of 5%. All the positive samples showed two bands for heterozygous mutation of Factor V Leiden, while the wild type showed 3 bands.

IV. Discussion

The present study was carried out in patients with various clinical conditions such as, spontaneous DVT, post- surgical venous thrombosis, hemorrhagic infarct, stroke, unexplained DVT, primary DVT, hypercoagulable state and pregnant women with history of miscarriages. 20 out of 94 patients had FVL, our findings were similar to the largest series reported from Singapore.

As per Gupta et al both these polymorphisms were totally absent in Indian population, and cannot be considered as independent risk factors or as a predictor for CAD (Gupta N et al. 2003). Factor V Leiden and G20210 prothrombin gene mutations are infrequent in Indian patients with PVT and are unlikely to be responsible for PVT in the Indian population (Sharma S et al. 2006).

There were no association of FVL mutation and prothrombin gene mutation in genetic predisposition to CADs and MI in north Indian population. The discrepancies in studies relating to FVL mutation and FII G20210A allele to CADs may be due to difficulties in estimating low allelic frequency in general population (Gupta N et al. 2003).

The prevalence have been estimated for Poland (Warsaw) 5.0%, Argentina (Buenos Aires) 5.1%, Venezuela (Valencia) 1.6%, Costa Rica (San José) 2.0%, and India (Punjab) 1.3%. Based on worldwide distribution, it can be hypothesized that the factor V Leiden mutation has originated and accumulated in central European Caucasians and spread over the world by migration (Herrmann FH et al 1997). The allele frequency in 618 Europeans was 4.4%, with the highest prevalence among Greeks (7%) and 0.6% in Asia Minor. Factor V Leiden was not found in any of 1600 chromosomes from Africa, Southeast Asia, Australasia, and the Americas, which explains the rarity of thromboembolic disease in these populations (Rees DC et al 1995).

FVL mutations, usually rare in populations other than Caucasians, exceptions do exist. In Asian-In¬dian populations 1% to 8.5% has been reported. In Tamil Nadu, south India, 4 out of the 72 were reported to be heterozygous for FVL mutation (5.5%). Another study

was aimed at finding a possible association between portal vein thrombosis and FVL, and it reported a slightly lower prevalence in its control group of healthy Indians (4.1% were heterozygous for this mutation). However, contradic¬tory results were found when the presence of this mutation was studied in healthy populations from Turkey, Korea, and even from some Indian populations). Singapore report says 10.74% (6.84, 14.64) of Indian pa¬tients were positive for FVL mutation, while less than 0.5% and 1.83% were detected as positive among Chinese and Malay patients respectively (Wan Zaidah Abdullah et al 2009). The factor V Leiden mutation is associated are two to three times more likely to have recurrent miscarriages or a pregnancy loss during the second or third trimester (genetic home reference).

VTE consists of 2 related conditions: deep vein thrombosis (DVT) and pulmonary embolism (PE). Between 20% and 60% of patients with recurrent VTE display APC resistance on laboratory testing, which is mostly due to mutation in the factor V gene. Among the European white population, the factor V Leiden mutation is the most prevalent hereditary thrombophilia. Approximately 4% to 6% of the general population are heterozygous for this trait (which is autosomal dominant), but it is extremely rare among native populations of Africa, Southeast Asia, and Australia (Frederick A et al).

The presence of the mutation markedly increases the risk for renal vein thrombosis, particularly in neonates, and renal transplant vein thrombosis in transplant cases resulting in rejection. Routine screening for factor V Leiden mutation by polymerase chain reaction, and appropriate perioperative and postoperative anticoagulation after renal transplantation might be a valuable strategy to prevent thromboembolic complications in transplant recipients (R P Wüthrich et al).

The prevalence of FVL in the general population varies from 0% to 7%, which is common in Europe and being almost absent in Africa and the Middle East. In patients with venous thrombosis, the prevalence is higher at 19% in the Netherlands and 39% in northern India but is only 3% in Mumbai. In patients with PVT, the prevalence is 7.6% in the Netherlands and 8% in Mumbai. The highest prevalence of 30% has been reported in a study of Egyptian children with PVT (Abraham Koshy et al).

In our study, 20 patients were positive for heterozygous mutation, of which 11 were males and 9 were females who had clinical history of thromboembolic disorders. Particularly 9 females in the reproductive age group most probably with a history of eclampsia. One male baby one day old was positive from AP.

Patients with recurrent thrombotic events related to factor V Leiden mutation have been reported, e.g. deep venous thrombosis in the lower limb followed by cerebral venous thrombosis resulting in quadriplegia. Awareness of the condition and a high index of suspicion is needed to prevent life-threatening complications. It is important to screen all patients less than 50 years of age presenting with spontaneous lower limb deep venous thrombosis are screened for factor V Leiden mutation (Meenakshi et al).

Molecular and epidemiological studies provide evidences that FVL should have occurred as a single event in the past. The Mediterranean region has the highest prevalence of FVL in the world, suggesting the origin of mutation possibly 10,000 years ago, and later spread to other parts of the world (Herrmann FH et al).

Venous thrombosis is a common problem, predominantly affecting people of European origin. This European predisposition has been explained to some extent by the recent characterization of factor V Leiden, and the G20210A prothrombin variant. Analysis of 22 samples for FVL from different non-European countries showed that prothrombin variant is very rare outside Europe except for one case from India (Rees DC et al).

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Assessment of Clinician's Knowledge and Perception on Antimicrobial Resistance a Primary Strategy for Antimicrobial Resistance Control

By Dr. N. Shanmuga vadivoo, Dr. B. Usha & Dr. B. K Padmavathi

Annapoorana Medical College, India

Abstract- *Introduction & back ground:* Antimicrobial resistance (AMR) develops with the inappropriate use, which includes the wrong indication, mode of use, and the poor adherence of the prescribed drugs. Knowledge is the first step in modifying behaviour in relation to physician's adherence to antibiotics prescription practice.

Methods: We did a cross sectional survey of 737 doctors at three tertiary care teaching hospital to assess their Knowledge, perception and attitude regarding Antimicrobial resistance. Anaesthetists, Pre & para clinical doctors who were general practitioners also participated in the survey.

Results: About 93% of doctors strongly agreed /agreed that AMR is a worldwide problem; only 75% rated that it's a problem in their institution. Nearly 85% doctors believed that inappropriate antibiotics use were important cause of Resistance. Also only 81% agreed that poor infection control measures & poor isolation precautions contribute to AMR. Another survey following a CME showed that educational intervention plays an important role in updating clinicians AMR knowledge.

Keywords: *antimicrobial resistance, KAP survey, clinicians & para clinicians.*

GJMR-C Classification : *NLMC Code: QW 4*

Strictly as per the compliance and regulations of:

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Conclusion: Although most doctors view antibiotic resistance as a serious national problem, perceptions about its local importance, its causes and possible solutions vary widely. Overall, most of our clinicians had better understanding and surprisingly Pre & Para clinical doctors also have enough knowledge regarding Antibiotics practice.

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I. Introduction

Antimicrobial resistance (AMR) a global problem is particularly pressing in developing countries where the Health care associated infection (HCAI) burden is high and cost constrains the replacement of older antibiotics with newer, more expensive ones. Several studies [1-4] have identified the inappropriate use of antimicrobials and noncompliance with infection control precautions as the main risk factors associated with an increased probability of colonization with resistant pathogens and there by Antimicrobial resistance. Hence management of common and lethal bacterial infections has been critically compromised by the rapid appearance & spread of these antibiotic-resistant bacteria [5]. The pipeline of antibiotic research and development is nearly dry, especially when it comes to antibiotics active against Gram-negative bacteria [6]. The bacterial disease burden in India is among the highest in the world; consequently, antibiotics play a critical role in limiting morbidity and mortality in the country. This has led to increasing use of newer antibiotics and ultimately ended up with increased prevalence rates of Multi drug resistant bacteria. Though we all know that Abuse, overuse & Misuse of antibiotics have exacerbated Antibiotic resistance, resistance development is a natural unstoppable process. Hence our challenge is to slow the rate at which resistance develops & spreads. Combating Antimicrobial resistance calls for a concerted approach from individuals to global levels involving various organisations like CDC, WHO, Health ministry of India and other organisations [6, 7]. These organizations recommends all the health care facilities to have their own Antibiotic policy based on Local Cumulative antibiogram [8] and to implement antibiotic stewardship programme [9] accordingly to combat the most prevalent MDR pathogens at their own hospital settings.

Author α σ ρ: Associate professor, Department of Microbiology Annapoorana medical college & Hospital Periyaseeragapaadi, SALEM-636 308. e-mails: shanmugavadivoon@gmail.com, dr_ushasekar@yahoo.co.in, bk.padmavathi@yahoo.in

II. Background

Many strategies have been proposed for the rational use of antibiotics, like a formulary replacement or restriction, health care provider education, feedback activities, approval requirement from an infectious disease specialist for the drug prescription [9]. Various studies which were done in India and other developed countries have highlighted the importance of rational drug therapy through educational interventions, strict antibiotic policy and Stewardship [9, 10, 11].

Knowledge is the first step in modifying behaviour in relation to physician's adherence to clinical practice &Guidelines. Therefore a very important primary strategy for framing an Institutional Antibiotic policy is assessment of Clinicians' Knowledge and their perception about Antimicrobial resistance. Also assessment of antibiotics prescription practice &

knowledge about the driving forces behind antibiotics prescription followed by educational intervention plays a very important role. The assessment is usually done by Knowledge, perception & attitude survey based on LIKERTs scale. Studies on clinicians' attitude towards Knowledge, perception of Antimicrobial resistance have been published in both Community and Hospital settings [12-22]. Some of these studies have shown poor correlation between knowledge and practice. Hence the purpose of this study is to conduct a survey to assess and explore Knowledge, attitude & Perception of clinicians' towards antimicrobial resistance at three tertiary care centre.

III. Material & Methods

This study is a cross sectional survey from three tertiary care teaching hospitals during 2014.All the three are located in suburban areas with 530, 900 & 300 beds respectively. Clinicians of above mentioned three tertiary care centres belonging to following specialities like General medicine, Surgery, Obstetrics Gynaecology, Paediatrics, Orthopaedics and super specialities like Nephrology, Urology, Paediatric surgery and Resident doctors working in all the above mentioned speciality participated. Anaesthesia &Para clinical doctors who were general practitioners also participated in the survey.

The Study instrument: The survey was carried out with a structured, validated, anonymous questionnaire encompassing sessions to assess Knowledge & perception of Clinicians towards Antibiotic Resistance. The Questionnaire was reviewed by Institutional Ethical Committee team to assess the relevance & Wordings of questions. The willing participants were approached individually and were requested to fill in the questionnaire anonymously. The Questionnaire was distributed onsite during working hours. No incentives for subjects to participate and no reminders were given.

The response to questionnaire was assessed in FIVE point Likert scale ranging from strongly agrees to strongly disagree. Briefly the questionnaire consisted of

- Professional profile of area of speciality, staff position, experience in that speciality.
- Section-1-Question pertaining to knowledge about Antimicrobial resistance like awareness at Global, national level and community level.
- Section-II-Question pertaining to practices known to contribute to Antimicrobial Resistance.
- Section-III-Questions pertaining to factors involved in antibiotic prescribing practices.
- Section-1V-Questionnaires were Drivers of choice in decision making to prescribe antibiotics.

Following the Questionnaire survey and based on the feedback, a CME on Antimicrobial resistance, Basic infection control practices, Antibiotic policy and Stewardship was organised. Again a Post CME survey in a questionnaire format to assess the Knowledge transfer was given to the Clinicians which consisted of Questionnaire on Necessity for Role of hand hygiene, Isolation precautions, Antibiotic policy & Stewardship programme for reducing Antimicrobial Resistance

IV. Results

A total of 737 doctors filled in the questionnaire. An overview of the professional profile of the 737 participantsare given in Fiure-1. The staff position and years of experience in that particular field shown in Table-1. Table-2 gives the profile of different specialities of physicians & Surgeons from different centres. Anaesthetists and Para clinical doctors were also included in our study because many of them were practicing physicians.

Table 1 : Professional profile of the participants

Staff position	Number %	Years of experience
Prof/Associate prof	170 23%	18-25 yeas
Assistant prof	125 17%	6-10 years
Senior and Junior Residents	361 49%	1-8 years
Consultants	81 11%	10-15 years

Table 2 : Department wise statistics of the participants

Speciality	Total	Percentage
Anaesthesia	30	*4.1%*
General medicine	103	*14.1%*
General surgery	94	*12.5%*
OBG	88	*12%*
Paediatrics	76	*10.5%*
Orthopaedics	63	*8.5%*
Ophthalmology	25	*3.4%*
ENT	21	*2.8%*
Dermatology	23	*3.1%*
Chest/TB	15	*2%*
ICU	81	*11%*
Surgical super speciality	10	*1.4%*
Medical Super speciality	15	*2%*
Pre/Para clinical	93	*12.6%*
TOTAL	737	*100%*

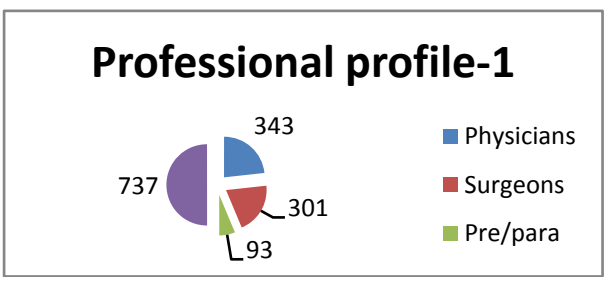

Figure 1 : Overall Demographic profile

Knowledge on Antimicrobial use and Antimicrobial resistance rates is shown in Figure-2. About 93% of doctors strongly agreed /agreed that AMR is a worldwide problem. Response to practices known to contribute AMR is shown in Table-3 .Antibiotic prescription practices and drivers of choice for Antibiotics are shown in table 4 & Table-5 respectively. Data's on Post CME questionnaire survey is shown in TABLE-6.

Table 3 : Practices known to contribute to antimicrobial resistance

	Strongly Disagree	Disagree	Neither	Agree	Strongly Agree
Failure to properly diagnose patients infective conditions	2.5%	16%	9.2%	48.3%	24
Prescribing antimicrobials when they are not needed	4.1%	5.5%	8.4%	37	45
Limited use of laboratory services for infection diagnosis	2.5%	7.1%	8.4%	56%	26%
Poor Adherence to isolation and contact precautions	1%	8.4%	9.6%	58.4%	22.6%
Poor hand hygiene & Poor infection control	1.2%	10.5%	8%	40.3%	40
Patients demand for Antibiotics	3.5%	20.5%	22%	40%	14%
Patients failing to adhere to treatment	0	4.2%	3.3%	45%	47.5%

Table 4 : Antibiotic Prescribing Practices

	Strongly Disagree	Disagree	Neither	Agree	Strongly Agree
Microbiology lab results are efficiently communicated to the treating physician.	2.9%	8.4%	5.5%	41.1%	42.1%
I regularly refer to the susceptibility/sensitivity patterns at this institution (e.g., an antibiogram) when prescribing antibiotics	2.1%	6.3%	12.6%	53%	26%
If medically appropriate IV antibiotics should be stepped down to an oral alternative	3.3%	4.6%	5.6%	58.4%	28.1%
A majority of patients admitted to this institution will be prescribed at least one antibiotic during their hospital stay	1.2%	5.8%	21.8%	52.5%	18.7%
Many of my patients receive 5 or more days of antibiotics during their stay at this institution.	4.2%	9.6%	13.4%	51.2%	21.6%
Only Few of my patients are discharged from this institution on antibiotics.	2.5%	9.2%	19.5%	56.5%	12.5%

Table 5 : Opinion on the Drivers of Choice of Antibiotic for a Particular Infection?

	Strongly Disagree	Disagree	Neither	Agree	Strongly Agree
Severity of infection	1.2%	2.1%	2.9%	50.8%	43%
Likely infecting organisms	0	0.4%	3.7%	63%	32.9%
Lab results	0.7%	2.9%	8.4%	58.4%	29.6%
Effectiveness of antibiotics for patients typically seen	1.2%	3.3%	12.6%	62.1%	20.85
Recommendations' by the pharmacists	27.7%	48.3%	10.5%	3.5%	10%

Table 6 : Post CME Questionnaire Survey

	Strongly Disagree	Disagree	Neither	Agree	Strongly Agree
Essential Infection control practices like Hand Hygiene reduce Health care Associated Infections	0	0	0	37%	63%
Isolation Precautions will significantly reduce Health care Associated Infections	0	0	15%	43%	42%
Do you think Antibiotic policy will help to reduce Antimicrobial resistance in this Institution	0	7%	11%	58%	24%
Do you think Antimicrobial stewardship programs can improve patient care?	0	0	3%	81%	16%
According to you will Antimicrobial stewardship programs reduce the problem of antimicrobial resistance?	0	0	15%	69%	16%
In your opinion will you be able to benefit or update your knowledge by this CME organised by the institution pertaining to Infection Control Programme?	0	0	0	62%	38%

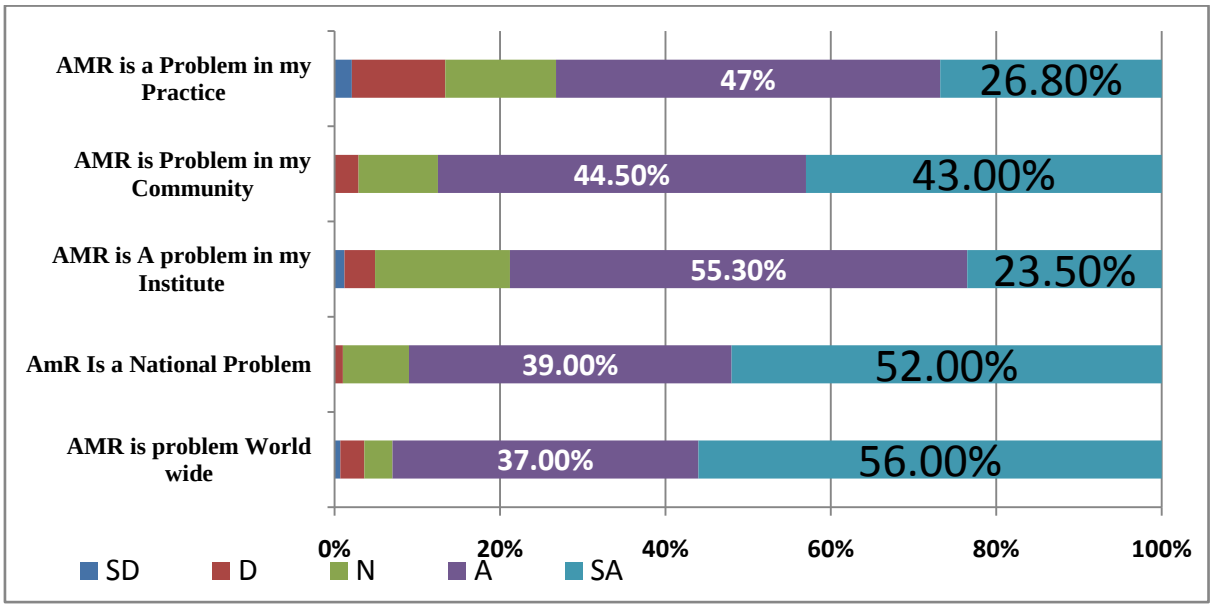

Figure 2 : Antibiotic Resistance -Awareness

V. Discussion

One of the emerging public health problems is AMR and no effective first line drugs exist for resistant pathogens. Inappropriate Antibiotics use & Infection control noncompliance has been primary attributes for dramatic raise in antimicrobial resistance.

The present study describes the results of a KAP-survey among 737 medical doctors (From all the specialities) practicing in three tertiary care teaching hospitals .Our study was done to assess the knowledge, attitude and the perception among the practitioners at a hospital setting towards a rational use of antibiotics.

The awareness of AMR problem worldwide, national & Institutional level and in their practice by clinicians at three centres varied .In our study significant percentage of clinicians (90%) perceived that Antibiotic resistance is a problem Worldwide& national level and less percentage (75%) in their institutional level as shown in Fig-2. In contrast to our study, a high perception that AMR as an institutional problem was shown in studies by Arjun Srinivasan [18] *etal*, and Maha et al [21].Our data is similar to a study by Wester *etal* [21] where in 87% respondents agreed that Antibiotic resistance is a national problem and 55% perceived it to be a problem in their institution. In a study by Giblin *etal* [16] 89% respondent's choice was national problem and 73% in their own institution and 65% in their practice. This disparity among clinician's perception demonstrates a lack of awareness & understanding

regarding the problem. Therefore, until the clinician's perception changes towards the fact that even in their personal practice their patients are also susceptible to AMR they will not have any motivation to change their practice behaviour particularly with respect to antibiotics use.

Regarding our clinician's response to practices contributing to AMR, 93% agreed that patient's failure to adhere to treatment an important contributor of AMR as shown in TABLE-3.In contrast, a study by Maha *etal* [16] showed only 68% agreed that patient's failure to adhere to treatment an important contributor of AMR. Our study also showed only 80% of respondents agreed that poor adherence to infection control practices like isolation precaution & Hand hygiene contributes to antibiotic resistance. In a study by Shah *etal* [20] only 31% respondents agreed that hand hygiene is significant in reducing antibiotic resistance. 54% of respondents agreed that patients demand for antibiotics a contributing factor to Antibiotic resistance. A similar data was shown by Sivagnanam[12] etal and Garcia et al [14] where in 55% of respondents agreed patients demands for antibiotics a contributing factor .

Among the data's on clinicians antibiotics prescribing practice (Table-3)79% agreed that they refer to susceptibility pattern while treating for infections at their respective institution &82% agreed that Micro lab results are efficiently communicated to treating physicians. In a study by Sivagnanametal[12] only 42% of practitioners agreed that they refer sensitivity reports. The necessity of De-escalation to oral antibiotics from IV is needed was agreed by 86% of clinicians.

As shown in Table-5, 74% of respondents disagreed for pharmacist's recommendation for Antibiotics. A similar data was shown in a study by Shah *etal* [20] wherein 73% respondents gave less importance for Pharmacists Recommendations.

Our institution organised a CME which emphasised problems of AMR, and how to combat Resistance by Basic infection control measures like Hand Hygiene, Antibiotic policy &Antibiotic stewardship .A post CME questionnaire survey was done to assess the transfer of knowledge which revealed that almost 100% agreed that basic infection control measures will reduce HCAI & there by Antibiotic resistance as shown in TABLE: 5. 100% of respondents agreed that they will be benefited by CME which will update them in AMR knowledge & Infection control practices. In a study by shah etal 70% agreed that CME will help in updating knowledge.

To our knowledge this is the first time Pre &Para clinical faculties were included in a KAP survey on Antibiotic resistance. This inclusion was done because many of the pre and Para clinical staffs are into General practise and also the necessity of AMR knowledge is essential while treating friends & families.

VI. Conclusion

Antimicrobial resistance accounts for numerous social & economic costs including mortality &morbidity. AMR continues to be a growing problem for all clinicians nationally & at institutional level. A multifaceted problem caused by AMR requires a multifaceted solution. At the institutional level, the assessment of clinician's knowledge on awareness about AMR and to educate them becomes a priority before initiating other strategies.

To summarise, our KAP study on Antibiotic resistance showed that though 95% of clinicians viewed this as a national problem, only 75%agreed that it's a problem in their institution. Also only 81% agreed that poor infection control measures & poor isolation precautions contributes to AMR and 79% refer to susceptibility pattern given by Micro lab. De-escalation of IV antibiotics to Oral antibiotics is a necessity was agreed by 86.5% of respondents.

Finally what have we learnt and understood was that the knowledge& attitude of clinician is crucial to reduce AMR at institutional level. Also as AMR problem is not limited by specialities, a better understanding of practices by all specialities included. Overall, most of our clinicians had better understanding and surprisingly Pre & Para clinical doctors also have enough knowledge regarding Antibiotics practice. Education by workshops & CME play a major role in updating knowledge. Following the study we have framed Antibiotics Policy and stewardship based on our institutional Antibiogram which addressed the susceptibility pattern of the most prevalent drug resistant pathogens.

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Prevalence of Resistant Strains of S*treptococcus Pneumoniae* to Oxacillin, Ofloxacin and Rifampicin in Abraka South-South Nigeria

By Felix O. Enwa, Mercy I Iyamu, Christabel I Eboigbe & C O Esimone

Delta State University, Nigeria

Abstract- The clinical isolate *Streptococcus pneumoniae* is a major cause of illness such as pneumoniae, meningitis, bacteremia and otitis media in children and the elderly. The emergence of drug-resistant strains threatens to complicate the management of these diseases. An hospital-based and community-based surveillance for drug-resistant *Streptococcus pneumoniae* in outpatients with respiratory infection in Abraka, Delta State Nigeria was conducted. Between August – October 2014, a cross-sectional study was conducted in Abraka general hospital and Abraka community to assess the prevalence of drug resistant *Streptococcus pneumoniae* isolated from sputum samples of patients with cough and catarrh (respiratory tract infection). A total of 125 sputum samples of patients with respiratory tract infection were collected and inoculated on 5% sheep-blood agar, incubated at 35oC for 24hours in 5-10% CO2. Susceptibility testing panels of Ofloxacin, Rifampicin and Oxacillin were tested against isolated *Streptococcus pneumoniae*. Of the 125 sputum samples collected 28 (22.4%) was positive for *Streptococcus pneumoniae*.

Keywords: pneumonia; resistance; susceptibility; prevalence.

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Felix O. Enwa [α], Mercy I Iyamu [σ], Christabel I Eboigbe [ρ] & C O Esimone [ω]

Abstract- The clinical isolate *Streptococcus pneumoniae* is a major cause of illness such as pneumoniae, meningitis, bacteremia and otitis media in children and the elderly. The emergence of drug-resistant strains threatens to complicate the management of these diseases. An hospital-based and community-based surveillance for drug-resistant *Streptococcus pneumoniae* in outpatients with respiratory infection in Abraka, Delta State Nigeria was conducted. Between August – October 2014, a cross-sectional study was conducted in Abraka general hospital and Abraka community to assess the prevalence of drug resistant *Streptococcus pneumoniae* isolated from sputum samples of patients with cough and catarrh (respiratory tract infection). A total of 125 sputum samples of patients with respiratory tract infection were collected and inoculated on 5% sheep-blood agar, incubated at 35ºC for 24hours in 5-10% CO_2. Susceptibility testing panels of Ofloxacin, Rifampicin and Oxacillin were tested against isolated *Streptococcus pneumoniae.* Of the 125 sputum samples collected 28 (22.4%) was positive for *Streptococcus pneumoniae*. 18 out of 75male adults (24%) and 10 out of 50female adults (20%) were positive for *Streptococcus pneumoniae*. Also from the result, samples that were positive for *Streptococcus pneumoniae* when considered in terms of age group, showed that adults between 20-25 years had the highest prevalence rate of (31%) when compared to those in patients between 26 -30years which was (13%). Susceptibility studies showed that the highest resistance of *Streptococcus pneumoniae* was observed in the use of oxacillin and rifampicin which gave no zone of inhibition and highest sensitivity was observed in the use of ofloxacin with percentage inhibition 74.3%.

Keywords: pneumonia; resistance; susceptibility; prevalence.

I. Introduction

The discovery of antibiotics and their general use had transformed the patterns of disease and death in many countries (James, 2006 & Sekowska, 2002). Many diseases that once caused high mortality such as tuberculosis, pneumonia and septicaemia became controllable and surgical infections reduced. These successes could hardly be measured when clinical antimicrobial resistance emerged (knothe, 1983) leading to treatment failures. But this antimicrobial resistance was to a single antibiotic at the time, so many researchers (Al-Jasser, 2006) are of the view that the appearance of multi drug resistant pathogens is a recent phenomenon, which has become a worldwide problem.

There are currently 90 known serotypes, with the ten most common causing greater than 60% of worldwide invasive disease (CDC, 2003a). *Streptococcus pneumoniae* is a normal inhabitant of the human upper respiratory tract. The bacterium can cause pneumoniae usually of the lobar type, paranasal sinusitis and otitismedia, or meningitis, *Streptococcus pneumoniae* is currently the leading cause of invasive bacteria disease in children and the elderly. Pneumonia is a disease of the lung that is caused by a variety of bacteria including *streptococcus, Staphylococcus, pseudomonas, Haemophilus, Chlanydia* and *Mycoplasma*, several viruses and certain fungi and protozoans.The disease may be divided into two forms; bronchial pneumonia is most prevalent in infants, young children and aged adults. It is caused by various bacteria, including *Streptococcus pneumonia* and Lobar pneumonia which is more common in younger adults. A majority (more than 80%) of the cases of lobar pneumonia are caused by *Streptococcus pneumoniae*, lobar pneumonia involves all of a single lobe of the lungs, wherein the entire areas of involvement tends to become a consolidated mass, in contrast to the spongy texture of long tissue. (Kenneth, 2003).

Pneumococcal pneumonia is the most common form of pneumococcal infection in adults, and accounts for approximately 36% of community-acquired pneumonia and 50% of hospital-acquired pneumonia. The period of time from infection to presentation of signs and symptoms is short, lasting only one to three days. Mortality rates range from 5-7%, and may be higher in the elderly (CDC, 2003b).

Transmission of *Streptococcus pneumonia* occurs via respiratory droplets from healthy persons carrying the organism in the naso-pharynx or from person with pneumococcal disease. Following exposure, the organism may establish itself in the nasopharynx of its new host usually resulting in asymptomatic colonisation. The organism can be carried for a period of weeks to months. However

Author α ρ: Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Delta State University, PMB 1, Abraka, Delta State, Nigeria. e-mail: felixenwa@yahoo.com
Author σ: Department of Microbiology, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria.
Author ω: Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, PMB 5025, Awka, Anambra State, Nigeria.

sometimes, the newly acquired pneumococcus evades host defensive mechanisms and causes illness (Buttler*et al* 1993).

Antibiotics from different classes can be used in treatment. These are chosen according to the host (age, allergy, localisation of the infection and the epidemiology of antibiotics resistance of pneumococcus strains). Aminopenicillins and penicillins are widely used for the treatment of pneumococcal infection and they are the first line anti-microbial in many countries to treat acute community acquired pneumonia. (Barlett, *et al*., 2004).β-Lactam inhibits the peptidoglycan synthesis of the bacteria call wall by forming a covalent bond with the active site of penicillin-binding proteins leading to hydrolysis of the bacteria. Mutations results in a decrease affinity to β-lactam. With the widespread use antibiotics, starting in the 1940s with penicillin, there has been a steady rise in the number of resistant serotypes or strains of drug resistant *Streptococcus pneumoniae*. The first clinical diagnosis of penicillin-resistant *S. pneumoniae* appeared in the 1967 in New Guinea, and multi-drug resistant strains appeared in South Africa in 1977 (Tomasz, 1997).

The CDC reports that annually there are an estimated 175,000 hospitalized cases of *pneumococcal pneumonia*, 50,000 cases of *pneumococcal bacteremia* and 3,000-6,000 cases of pneumococcal meningitis (CDC, 2003a). Additionally in children less than 5 years there are estimated 5 million case of otitis media every year in this country. Due to the severity of the disease caused by invasive *S. pneumoniae* and the continued rise of antibiotic resistance, several vaccines have been developed in the United State.

The increasing prevalence of resistance to established antibiotics among key bacterial respiratory tract pathogens such as *Streptococcus pneumonia* is a major healthcare problem in Delta State. However, the increasing prevalence of antibiotics resistance bacterial therefore poses a significant problem in selection of drug of choice for treatment of the infection.

Amazingly, the phenomenon of bacteria resistance is now threatening to take us back to a pre-antibiotics era. It has been reported that the growing phenomenon of antibiotics resistance is caused by the use and abuse of antibiotics.

The overall aim of study is to investigate the prevalence of resistance strains of *Streptococcus pneumoniae* to *oxacillin, ofloxacin* and *rifampicin* (fluoroquinolones) and contribute to the existing body of knowledge on the antimicrobial efficacy of antibiotics against the bacteria.

II. Materials and Methods

a) Collection of clinical specimen (sputum)

Specimen collection was carried out from august to October 2014, a total of 125 sputum samples of adults with upper respiratory tract infection in Abraka was collected. The sputum samples were collected from patients with symptoms of lower respiratory tract infection especially cough and who was clinically diagnosed of having respiratory tract infection. A sterile flexible swab was inserted into the patient's nostrils until it touches the posterior wall of the nasopharynx. After performing rotator movements for 5seconds so that secretion could be absorbed, the swab was removed and submerged on Amies medium without charcoal and taken to the microbiology laboratory at room temperature. A single collection was performed for each patient.

b) Isolation of streptococcus pneumonia from sputum samples

Sputum samples was streaked onto nutrient agar plate and incubated at 35-37°c for 24hours and plates that showed growth of organism was futher plated on blood agar prepared with tryptose blood agar base (Difco, Becton Dickson and company, sparks MD USA) Supplement with 5% of defibrinated sheep blood.

c) Identification of test micro-organism

The identification of the test organisms by appropriate cultural methods and gram staining was done following standard microbiological procedures.

d) Biochemical reactions

i. *Bile solubility test*

1. Preparation of Bile salt

2% bile salt was prepared by dissolving 2g in 100mls of sterilized normal saline, making a solution of bile salt.

2. Inoculum preparation

Bacterial inoculum was prepared by inoculating 0.25mls of 24hours overnight culture into sterilized test tubes.

0.25mls of the bile salt was added into broth test tube. 0.25mls of normal saline was dispensed into another test tube to serve as the control. 0.25mls of broth organism was added into both test tubes and incubated for 2hours. A clear solution indicated a positive test for *Streptococcus pneumonia*.

e) Susceptibility studies

Susceptibility testing panels that include antibiotics used in treating patients with pneumonia in Abraka which includes Ofloxacin, Rifampicin and Oxacillin were treated against isolated strains of *Streptococcus pneumonia* using disc diffusion method.

Briefly a Mueller-Hinton agar plate was prepared following manufacturer's instruction, test organisms equivalent to 0.5 Mcfarlene equivalent standard was inoculated on the surface of sterile agar plate and was allowed for 15mins to prediffuse, then antibiotics disc as mentioned above was placed on the

agar plates with sterile forceps and incubated for 18-24hours at 37oc after which the inhibition zone diameter was taken millimetre. Susceptibility result was interpreted as resistance or susceptible or intermediate according to the definitions of the national committee for clinical and laboratory standard institutes.

Other biochemical test such as Carbohydrate Fermentation, Iodine and Coagulase were carried out as described by Monica 2002.

III. Results

a) Identification of collected test micro-organisms

The identification test carried out on the clinical isolates as presented in Table 3.1 showed that *Streptococcus pneumonia* presented a characteristic alpha haemolytic reaction (greenish zone of inhibition) on blood agar which differentiates *Streptococcus pneumonia* from the group A (beta haemolytic) *Streptococcus*, but not from commercial alpha haemolytic (viridans).

Motility test results showed that *Streptococcus pneumonia* is non-motile. Catalase test results showed that they are catalase negative (they lack catalase) and fermentation test result showed they ferment glucose to lactic acid. Gram-staining reaction test results showed that they are gram positive showing a lancet shaped cocci (elongated cocci with a slightly pointed outer curvature), they were seen as pairs of cocci (diplococci) but they also occur singly and in short chains.

In bile testing, results showed that they undergo lysis by bile salt (egdeoxycholate). Virtually all clinical isolates of pneumococci harbour the autolysis and undergo deoxycholatelysis.

Table 3.1: Results of identification

	Identification Tests	Results
1	Cultural Characteristics	Colonies produce a zone of green (alpha) haemolysis when cultured on blood agar. They grow as glistening colonies on agar about 1mm in diameter
2	Motility Tests	They are non-motile
3	Staining Reactions	They appear as dark purple gram positive cocci occurring in pairs, singly or short chains.
4 I Ii Iii	Biochemical Reactions Coagulase Indole Sugar Fermentation	 Negative (They lack coagulase) Negative (They lack indole) Show a yellow colour with bubbles in a tube (they ferment glucose to lactic acid.
5	Bile Solubility Test	Show a clear solution in the table containing 2.5ml of bile salt and 2.5ml of broth culture of organism.

b) Antimicrobial sensitivity test results

i. *Population of streptococcus pneumoniae isolated*

From Table II, the prevalence rate of *Streptococcus pneumoniae* carries among the adult studied was 22.4% (28/125). Eighteen out of seventy-five male adult (24%) and 10 out of 50 female adults (20%) had positive cultures for *Streptococcus pneumoniae*.

A significant difference was found when the positive result of pneumococcal isolation was arranged according to age group (p=0.0005), with a higher rate of isolation in the adult aged from 20 – 25 years (31%; 20/65). Among 40 adults, from 26 – 30 years old (13%, 8/60) had positive cultures for pneumococcus. 60 patients were treated with antimicrobial drugs in the last month with a higher rate of isolation is (25%).

Table 3.2 : Association of epidemological data with the isolation of *streptococcus pneumoniae* of 125 adults studied

Variable	Negativity, N (%)	Positive, P (%)
Population	97 (77.6%)	28 (22.4%)
Sex		
Female	40 (80%)	10 (20%)
Male	57 (76%)	18 (24%)
Age		
20 – 25 Years	45 (69%)	20 (31%)
26 – 30 Years	52 (86%)	8 (13%)
Use of Antibiotics (prior to sputum collection)		
No		
Yes	34 (84%)	10 (16%)
Not Reported	45 (75%)	15 (25%)
	18 (86%)	3 (14%)

c) *Antimicrobial sensitivity result*

The results of penicillin susceptibility tests of pneumococci using rifampicin and oxacillin, determined that of 28 specimens of confirmed *Streptococcus pneumoniae* all were resistant to rifampicin and oxacillin. All isolates were sensitive to ofloxacin which gave a mean zone of inhibition of 18.68 and standard deviation of 2.75 (18.68 ± 2.75) with percentage inhibition of 74.3%.

IV. Discussion

The result obtained in the present study described the epidemiology period in Abraka, Delta state where the prevalence of adults colonized with nasopharynegeal *Streptococcus pneumoniae* was 22.4% from which 24% male adults and 20% female adults are for *Streptococcus pneumoniae*. This study confirmed that age is an important factor for pneumococcal isolation with high prevalence of isolates in the age group between 20-25 years (31%) and low prevalence of isolates in the age group between 26-30 years (13%) which is agreement with Austrian (1986).

According to Borer *et al*.2001, the alterations in the mucosa of the respiratory tract of allergic patients can result in impaired mucociliary activity, predisposing to bacteria colonization in this site. The interpretation of susceptibility of the drugs used in this study showed that pneumococcal resistance rate was higher with rifampicin and oxacillin as they showed no zone of inhibition indicating no susceptibility and a high susceptibility (74.3%) was observed in ofloxacin.

Previous antibiotic exposure has been documented as a risk factor for antibiotic resistance in many studies and also, antibiotic therapy may increase the rate of isolation of penicillin-resistant nasopharyngeal pneumococci (Kaplan and Mason 1998).This fact may explain the high prevalence of oxacillin resistant pneumococcal strains found in this study, since 60(48%) of the 125 adults had been treated with antimicrobial drugs in the month prior to the study.

The high rate of resistance to rifampicin and oxacillin found in this study is similar to that described in other studies in and outside Nigeria (Jacobs *et al*.., 1978).

Ofloxacin can be used as an alternate drug of choice in the treatment of *Streptococcus pneumoniae* infections, although the optional therapy for infections with drug-resistant pneumococci is not well defined.

There are reports that children 6years of age were more likely than older children and adults to be infected with multi-resistant isolates. However in this study, a disturbingly high incidence of drug resistant pneumococcal infections among adults was found.

Although the overall prevalence of pneumococcal infections in Abraka community is low 22.4% recommendations for empirical therapy are needed particularly in communities in which the prevalence of drug resistant is high. The geographic variation in the prevalence of drug resistant strains of pneumococci in Nigeria highlights the importance of community based monitoring of pneumococcal susceptibility to antimicrobial agents to guide therapy. In addition, the increased prevalence of drug resistant *Streptococcus pneumoniae* emphasizes the critical need for preventive strategies in populations at risk from serious pneumococcal infections.

Frequent and prophylactic use of antimicrobial drugs has also been associated with a risk of drug resistant pneumococcal infections and such was observed in this study of 125 adult sputum collected 44 adults had not been treated with antibiotics prior to the collection while 60 adults had been treated and 21 adult cases not reported. A significant difference was observed with adults who had been treated with antibiotics prior to sputum collection and those who had not been treated. Of the 28 *Streptococcal pneumonia* isolated, 15 (25%) out of 60 adults who had had antibiotics prior to collection was positive for *Streptococcus pneumonia* while 10 out of 44 adults who had not had antibiotic prior to collection of sputum were positive. (10; 16%). Despite a low incidence of pneumococcal infection in Abraka especially in patience between the ages of 26 – 31 years, age groups of 20 – 31 years was found to be associated with drug-resistant *Streptococcus pneumoniae*. Rural residence and poverty may be responsible for poor health states and little or no access to medical care. As a result, people tent to see the roadside chemist/pharmacy dealers and procure cheap drugs without doctor's prescription because there is no restriction or guidelines to the use of antibiotics in Nigeria. A critical component for the control of drug-resistant *Streptococcus pneumoniae* will be community wide educational programs for clinicals and the public on the importance of appropriate antibiotic use.

The vast majority of *Streptococcus pneumoniae* isolates had a relatively high resistant to rifampicin and oxacillin. The continuing spread of drug resistant strains of pneumococci treatment options will become more limited and prevention measures will become critical.

From this study, possible reasons for this low prevalence in populations infected with *Streptococcus pneumonia*, include the fact that the survey only adults, the subjects were enrolled for a very short time, winter (a period of frequent respiratory illness) was not the season of enrolment, and the fact that human genetic traits may play a role.

The identification and confirmatory tests carried out on the organism to establish their identity yielded results that were in agreement with previous studies. For example *Streptococcus pneumonia* appeared as alpha haemolytic when cultured in blood agar, colonies characteristically produced an alpha (green) hemolysis.

Solubility in bile salt agar solution, also in agreement with previous studies, *Streptococcus pneumonia* fermented glucose producing acid and gas, were coagulase negative and purple gram-positive cocci in clusters and single when viewed from the microscope.

Antimicrobial susceptible test is an essential technique used in pharmacology to determine the efficacy of novel antimicrobial agent against micro-organism.

Susceptibility studies was carried out using oxacillin, rifampicin and ofloxacin against isolated strains of *Streptococcus pneumoniae*. The experiment was replicated twice and an average zone of inhibition reading was taken. Studies were conducted to investigate the prevalence of *Streptococcus pneumoniae* infection in adults in Abraka community and also to investigate the prevalence of resistance of *Streptococcus pneumoniae* to oxacillin, rifampicin and ofloxacin and to know the potential of these listed drugs as a broad spectrum antimicrobial agent in the treatment of nasopharyngeal infection.

The data collected were subjected to analysis, mean deviation, standard deviation and percentage deviation of the zones of inhibition exhibited by ofloxacin as no zone of inhibition was observed with rifampicin and oxacillin indicating non-sensitivity towards *Streptococcus pneumoniae*. This study emphasizes the importance of antimicrobial susceptibility testing of all *Streptococcus pneumoniae* infection in adults in Abraka, as well as crucial need for community based programs of surveillance for drug resistant pneumococcus to aid clinicians in their choice of therapy for pneumococcal infections.

From the study the following conclusion can be drawn;

The overall prevalence of *Streptococcus pneumoniae* infection in Abraka community is low.

- The standard antimicrobial agent ofloxacin was significantly more effective than rifampicin and oxacillin.
- Rifampicin and oxacillin antibiotics are not sensitive to *Streptococcus pneumoniae* as the bacteria were totally resistant.
- The study data suggest an urgent need for consensus guidelines to prevent development of multi-drug resistant strains of *Streptococcus pneumoniae* in this region in future.
- The prevalence of fluoroquinolone resistance in *Streptococcus pneumoniae* is low.

V. Recommendation

From the result obtained the following recommendations are made;

- Ofloxacin should be used as a first-line drug alone or in combination to treat pneumococcal infection as well as infection of the upper respiratory tract in Abraka metropolis.
- Strategies to encourage judicious antibiotics use should be implemented this will enhance prevention of infections with *Streptococcus pneumoniae*.

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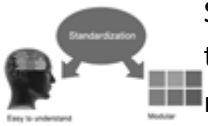

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It is mandatory to read all terms and conditions carefully.

AUXILIARY MEMBERSHIPS

Institutional Fellow of Open Association of Research Society (USA) - OARS (USA)

Global Journals Incorporation (USA) is accredited by Open Association of Research Society, U.S.A (OARS) and in turn, affiliates research institutions as "Institutional Fellow of Open Association of Research Society" (IFOARS).

The "FARSC" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., FARSC or William Walldroff, M.S., FARSC.

The IFOARS institution is entitled to form a Board comprised of one Chairperson and three to five board members preferably from different streams. The Board will be recognized as "Institutional Board of Open Association of Research Society"-(IBOARS).

The Institute will be entitled to following benefits:

The IBOARS can initially review research papers of their institute and recommend them to publish with respective journal of Global Journals. It can also review the papers of other institutions after obtaining our consent. The second review will be done by peer reviewer of Global Journals Incorporation (USA) The Board is at liberty to appoint a peer reviewer with the approval of chairperson after consulting us.

The author fees of such paper may be waived off up to 40%.

The Global Journals Incorporation (USA) at its discretion can also refer double blind peer reviewed paper at their end to the board for the verification and to get recommendation for final stage of acceptance of publication.

The IBOARS can organize symposium/seminar/conference in their country on behalf of Global Journals Incorporation (USA)-OARS (USA). The terms and conditions can be discussed separately.

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We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.
The board can also take up the additional allied activities for betterment after our consultation.

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Note :

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- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

"

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The Author can submit the paper either online or offline. The authors should prefer online submission.Online Submission: There are three ways to submit your paper:

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1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also.Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

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32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.

- Do not give permission to anyone else to "PROOFREAD" your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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