"A STUDY FOR DETECTION OF *LEGIONELLA PNEUMOPHILIA* ANTIGEN IN URINE BY ICT AND GRAM'S STAIN FROM PATIENTS WITH BACTERIAL PNEUMONIA IN A TERTIARY CARE HOSPITAL AT KANPUR".

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Abstract

Background- *Legionella pneumophilia* causes infection having high mortality and morbidity rate presents in non-pneumonic (Pontiac fever) and acute pneumonic (Legionnaires` disease). They are detected in urine by Gram's stain, culture on Buffered Charcoal Yeast Extract (BCYE) , Iummnochromatography Test (ICT) or by Enzyme Linked Immunosorbent Assay (ELISA).

Aim- To detect *Legionella pneumophilia* antigen in urine by using two different methods by ICT and Gram's stain in pneumonia patients.

Material & Methods- The present study was a prospective study conducted in the Department of Microbiology & Medicine from July 2022 -April 2023 at RMCH&RC, Kanpur. Total 40 urine samples were collected from patients having pneumonia and stained with Gram's Stain & antigen detection by ICT (CERTEST Legionella).

Results - Out of 40 urine samples from pneumonia patients 35 were males and 5 were females in which 20 patients were from Non ICU and 20 were from ICU .The age group affected maximum was 60-65 years of age .The risk factors associated were Diabetes mellitus(17.5%), Chronic kidney disease(25%), Low respiratory rate(20%,)Immunocompromised patients(15%), Smokers(7.5%) and alcoholic(5%)were seen highly. According to the analysis, out of 40 patients a total of 2 ICU patients were positive in which 2 (5%) patients were positive for Legionella urinary antigen by card test and 1 (2.5%) patient were positive by Gram's stain .The sensitivity and specificity of ICT, gram's stain was found to be 100%, 97.43%, 50%, 100% and PPV and NPV of ICT and gram's stain was found to be 50%, 100%,100%, 97.43%. Further analysis reports of ICT positive showed that the patients having leukocytosis was (5%), Diabetes mellitus(5%), Chronic kidney disease(5%)and with a history of past smoking(7.5%)& alcoholism(5%)was seen .Other organisms from Gram stain were observed and isolated from culture were *Klebsiella pneumoniae* (40%) which was the most common among patients of ICU and Non- ICU followed by *Pseudomonas aeruginosa* (20%) ,*Acinetobacter baumanii* (12.5%), *Citrobacter koseri*(15%), *Morganella morganii*(2.5%), *Enterococcus spp.(*10%) .The antibiotic susceptibility pattern shows sensitive to Polymyxin-B(100%), colistin(100%) and resistant to drugs ampicillin(20%), cefotaxime(20%) and for Legionella azithromycin(50%) and gentamycin (50%) were observed to be the most sensitive drug .

Conclusion –ICT was found to be the rapid and more effective method when compared to other convectional method Gram's stain with higher sensitivity and specificity. Hence, while investigating severe pneumonia patients requiring hospitilization with risk factors in ICU, Legionella test should be kept as a differential diagnosis for Legionnaire's disease.

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DOI: - 10.48047/ecb/2023.12.si5a.0329

INTRODUCTION-

Legionella pneumophilia is a thin , strictly aerobic ,pleomorphic gram negative bacilli[1]. Most of them are motile by monopolar flagellum and this serogroup 1 is catalase-positive [2] *Legionella pneumophila* is found mostly in environments. The infection is through aspiration of contaminated water, faucets, showers, humidifiers, respiratory devices and nebulizers there is no direct transmission of legionellosis from one person to another [3]. It is the causative agent of Legionnaires' disease which is a severe form of atypical pneumonia in patients with infiltration of chest Xray with an incubation period of 2-10 days which can be characterized by non- productive cough, chest pain and can also cause the Pontiac fever which includes fever, chills, body aches, nausea, sore throat and flu like symptoms which may be caused by viral, bacterial, fungal infections[4]. Clinical diagnosis include samples of sputum, bronchoalveolar lavage fluid, pleural fluid, urine, blood is taken for direct microscopy then culture is performed on Buffered charcoal yeast agar (BYCE) for identification of *Legionella* specifically antigen in urine is detected by card test [5]. .For treatment newer macrolides (especially azithromycin) and the quinolones are now the antibiotics of choice.

No current vaccine are available for prevention of *L.pneumophilus* requires identification of the environmental source of the organism and minimizing the production of aerosols in places and from water that may be contaminated with *Legionella* and it can be eradicated from water by hyperchlorination and by superheating (>700C) of the water before its supply[6]. Therefore, this study was undertaken for the detection of *Legionella pneumophilia* antigen in urine samples of patient with bacterial pneumonia by rapid card test(CerTest kit).

MATERIAL & METHODS-

The present was an observational prospective study conducted in the Department of Microbiology and Department of Medicine at Rama Medical College Hospital & Research Centre Mandhana, Kanpur from July 2022 to April 2023. The study protocol was approved by institutional ethical committee. The patients above 40 years of age both from ICU and non-ICU were taken after the complete history of underlying disease, smoking history, recent travel history and antibiotic use prior to admission in hospital their inclusion criteria were recorded pneumonia as the primary diagnosis in medical record according to the clinical symptoms and signs (fever, cough, chest pain, shortness of breath, myalgia, dyspnea) and infiltration in chest X ray in patients of all age . Exclusion criteria were from Patients with other cause of pneumonia (viral, fungal). 10 ml of urine sample was collected then it was centrifuged and condensed and sent for the test by Gram's Stain and by lateral flow immunochromatographic test was performed by Cer test Biotec Legionella card test.

RESULTS-

In this study, 35 (87.5%) from 40 pneumonia patients were males and 5 (12.5%) were females[Figure no.1]The patients were from ICU 20 (50%) and from Non- ICU 20(50%) [Figure no.2]. The common chief complaints was unproductive cough(25%), chest pain(27.5%) , shortness of breath(12.5%), fever without chills(10%), myalgia(10%) , headache(7.5%), fatigue(7.5%) [Figure no.3]. There was chest infiltration in X Ray in few patients. The risk factors involves diabetes mellitus (17.5%), chronic kidney disease(25%), immunocompromised (15%), patient on steroids(10%) , past history of smoking(7.5%) and had low respiratory rate(20%) over 60 years of age were at risk factors[Figure no.4]. Out of 20 (50%) ICU patients 3 were positive for Legionella in which 2(5%) were positive for Legionella urinary antigen test by rapid card and 1 (2.5%) patient by gram's stain[Figure No.5a and 5b]. as the proper history analysis done, the patient was a past smoker and prior to hospitalization his complete blood count was demonstrated in which he had elevated sodium level, potassium level and had leukocytosis and was immunocompromised.

Furthur analysis of all patients were done by rapid card and also by gram stain and culture but different organisms were isolated by culture in which Gram negative bacilli were 35(87.5%). *Pseudomonas aeruginosa accounts 20%, Klebsiella pneumonia 40%, Acinetobacter baumanii. 12.5%, Citrobacter koseri. 15%, Morganellamorganii 2.5%* and few strains of Gram positive cocci 05(12.5%) *in which Enterococcus fecalis. 5%, Enterococcusfecium 5%*[Figure no.6].The antibiotic susceptibility pattern shows sensitive to Polymyxin-B(100%), colistin(100%) and resistant to drugs ampicillin (20%), cefotaxime(20%) and for Legionella azithromycin(50%) and gentamycin (50%) were the most sensitive drug .[Figure No.7& Figure No.8].

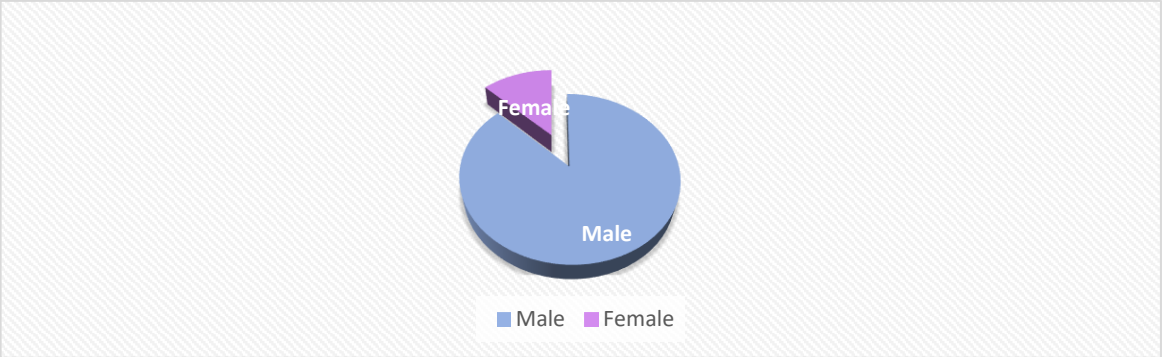

Figure No.1- Gender wise distribution of 40 samples in which males were 35 (87.5%) affected more than females 5(12.5%).

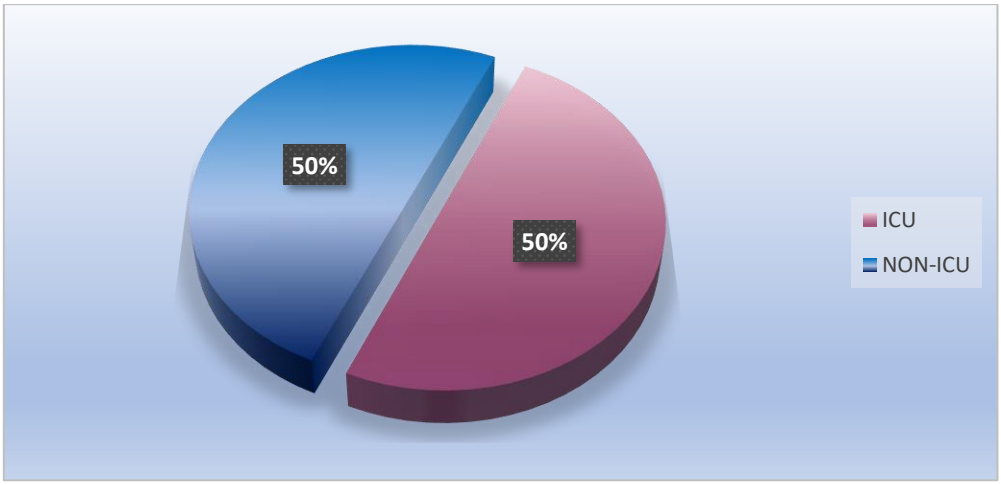

Figure No.2-Ward wise distribution of patients.

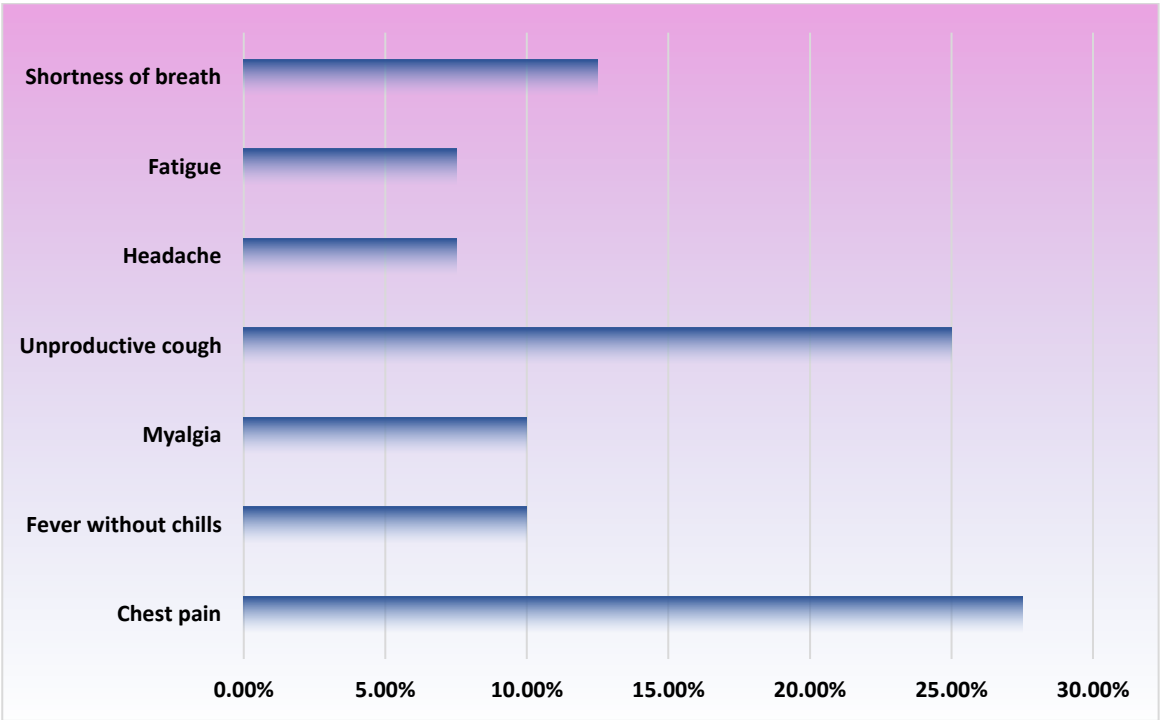

Figure No.3- Shows distribution of patients according to their signs and symptoms in which chest pain (27.5%) , unproductive cough (25%) , shortness of breath (12.5%) , fever without chills (10%) , myalgia (10%) , headache (7.5%) , fatigue (7.5%).

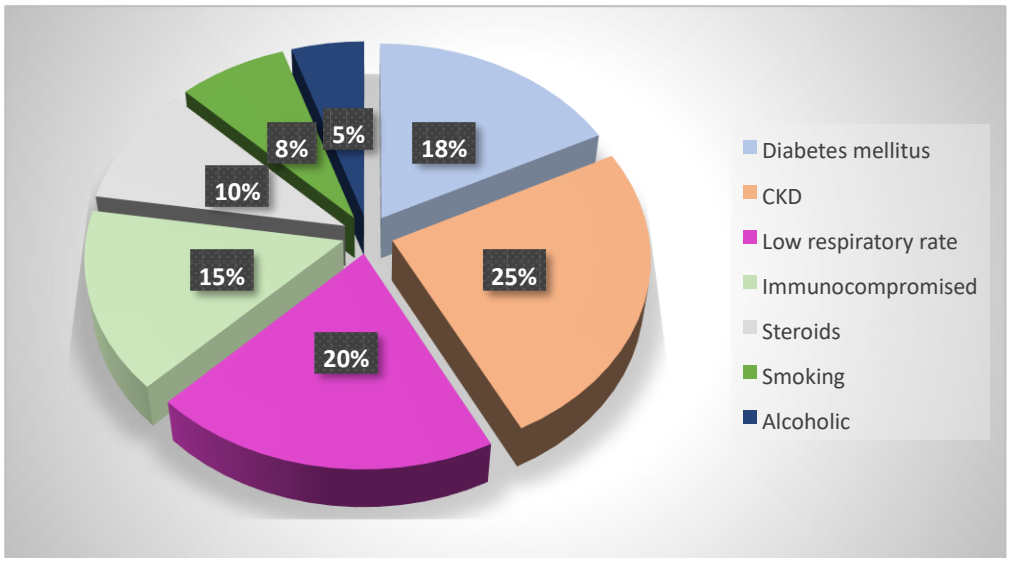

Figure no:4- Shows risk factors of suspected patients .

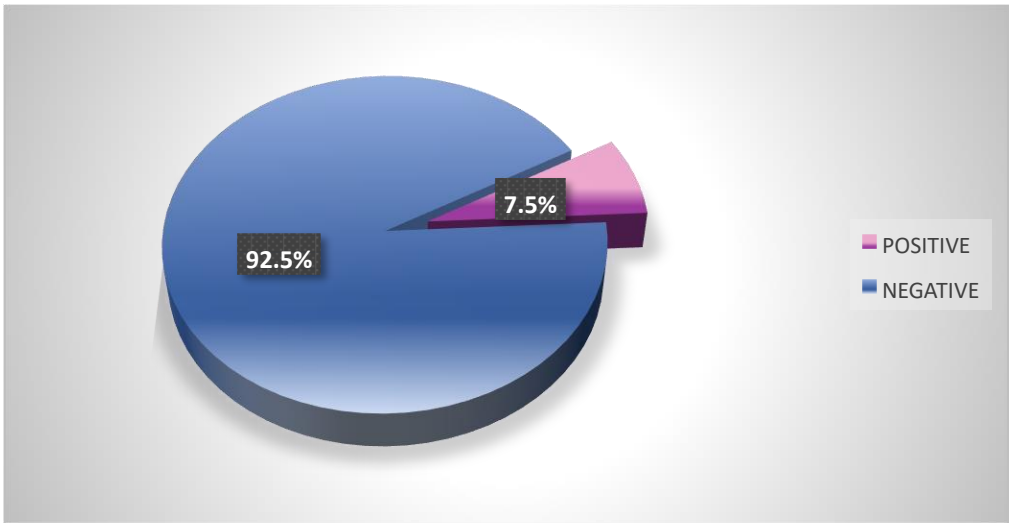

Figure no:5a- Shows number of positive cases of *Legionella pneumophilia*

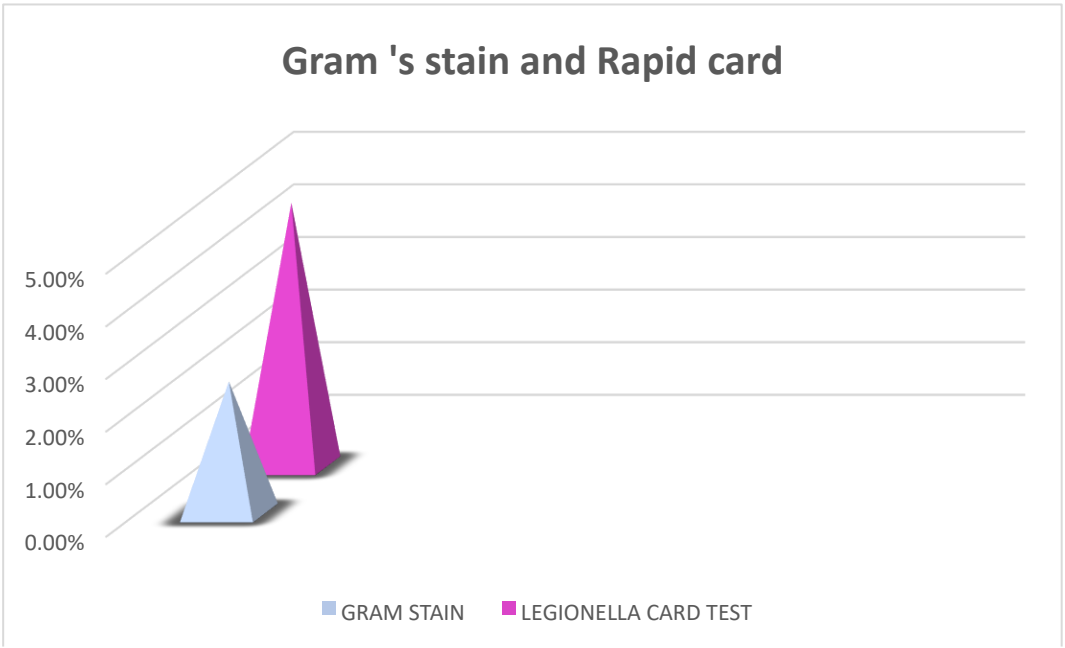

Figure No. 5b- Shows 1 (2.5%) positive by gram stain and 2 (5%) positive by rapid card test.

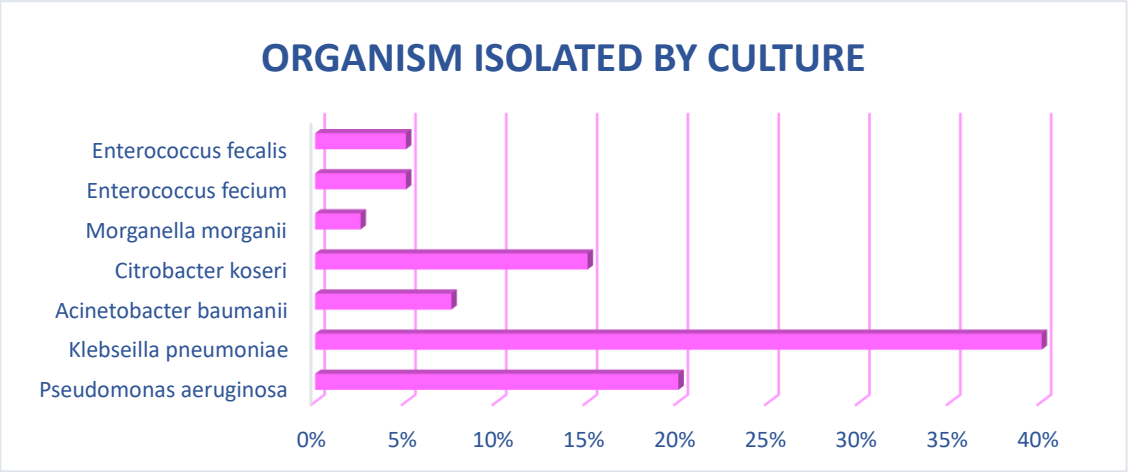

Figure No.6-Shows the organisms isolated after culture in which *Pseudomonas aeruginosa.(20%), Klebseilla pneumoniae. (40%), Acinetobacter baumanii. (7.5%) , Citrobacter koseri.(15%) , , Morganella morganii.(2.5%) , Enterococcus fecalis. (5%), Enterococcus fecium (5%).*

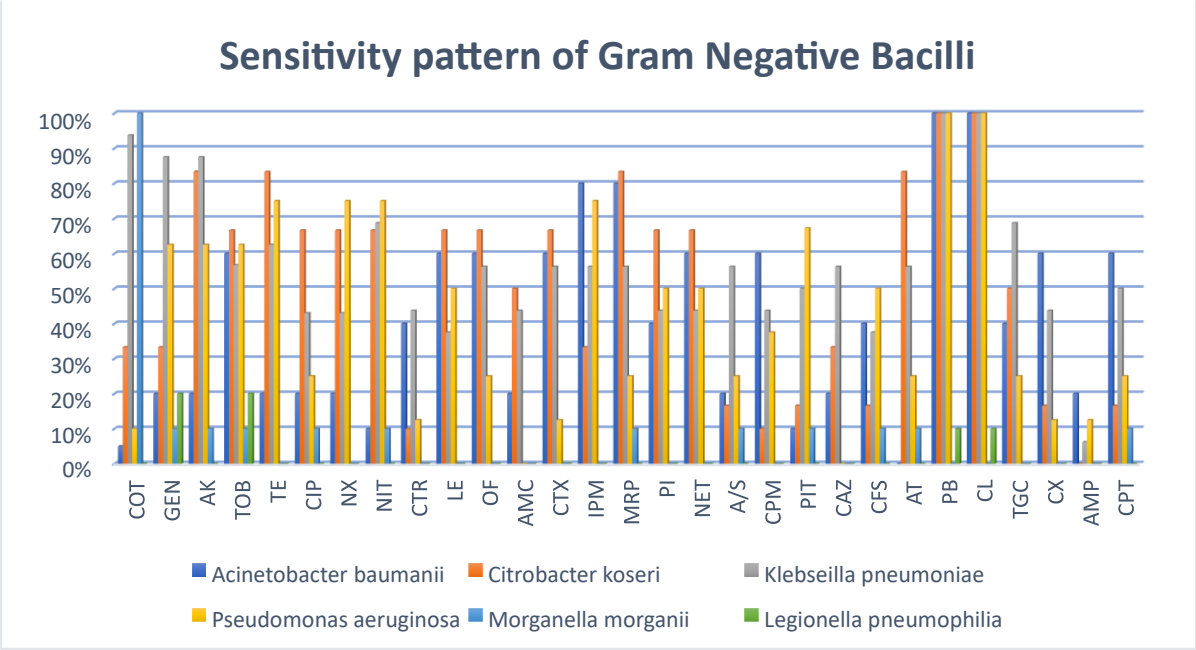

Figure No.7- Shows sensitivity pattern of Gram negative bacilliisolates.

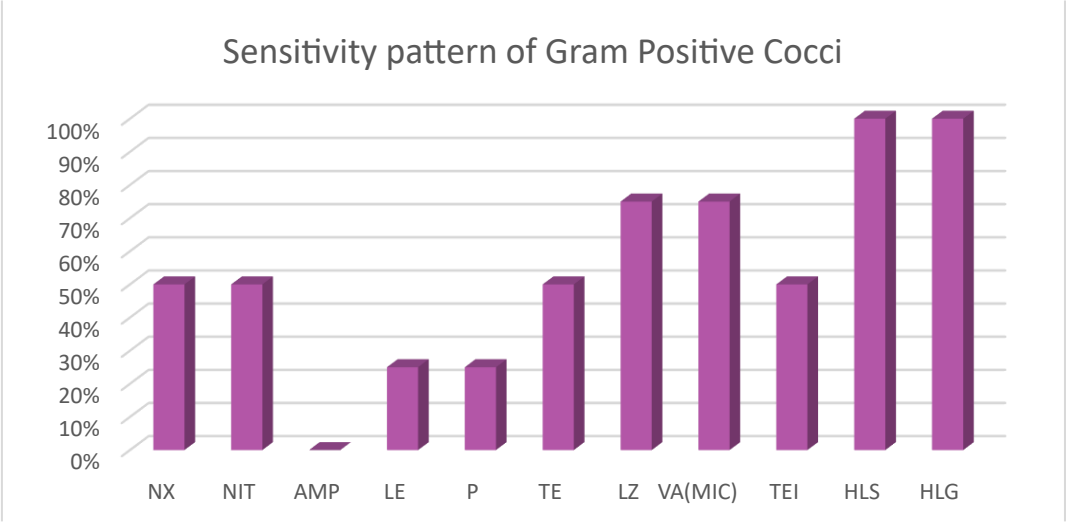

Figure No.8- Shows sensitivity pattern of Gram Positive cocci isolates.

DISCUSSION –

In our study, males 87.5% were more than females 12.5% in the age group of 61-65 years. In a study of Poulose et.al(2005) in Singapore, out of 80 patients with severe community-acquired pneumonia 55 were males and 25 females, mean age was found to be 62 years.[7] The findings were same in accordance to our study. In our study,pneumonia patients having signs and symptoms which includes chest pain (27.5%) , unproductive cough (25%) followed by shortness of breath (12.5%) , fever without chills (10%) , myalgia (10%) , headache (7.5%) , fatigue (7.5%). In the study of Lim WS et.al (2003), the clinical symptoms include systemic inflammation, such as pyrexia, tachycardia and leukocytosis / neutrophilia, combined with localizing chest pain signs such as crepitations and bronchial breathing . The symptoms are frequently accompanied by productive cough and breathlessness[8]. The findings of signs and symptoms were in accordance to our study.

In our study, risk factors of patients were diabetes mellitus(17.5%), chronic kidney disease(25%) , immunocompromised(15%) , patient on steroids (10%) , past history of smoking(7.5%) and had low respiratory rate(20%) over 60 years of age were at high risk. . In the study of Mohammed amin fazeli et.al (2020) age, smoking, heart disease , chronic pulmonary disease, diabetes mellitus, kidney failure, organ transplantation, immunodeficiency, some types of cancer and age over 50 years were risk factors[9]. The findings of other studies risk factors were similar to that in our study but age factor was different as compared to our study.

In our study, out of 40 patients a total of 2 ICU patients were positive in which 2 (5%) patients were positive for Legionella urinary antigen by card test its sensitivity was found to be 100% , specificity 97.43%, PPV 50%, NPV 100% and 1 (2.5%) patient was positive by Gram's stain . In the study of Mohammed amin fazeli et.al (2020) According to the data analysis,out of 140 patients, 5 patients (3.6%) were positive for the Legionella urinary antigen by card test which is 100% sensitive for test[9]. Our findings indicate low prevelance of *Legionella* in patients.

In our study, other organisms isolated after culture were *Pseudomonas aeruginosa.(20%), Klebseilla pneumoniae. (40%), Acinetobacter baumanii. (12.5%) , Citrobacter koseri.(15%) , Legionella spp.(0%) , Morganella morganii.(2.5%) , Enterococcus fecalis. (5%), Enterococcus fecium (5%).* In the study of Mohabow Jemal et.al(2021) the common bacterial pathogens were *Klebsiella pneumoniae* (28.0%), *Strepto coccus pneumo- nia* (24.8%), *Staphylococcus aureus* (18.5%), and *Pseudomonas aeruginosa* (14.0%) [10] .The findings were in accordance to our study by Mohabow jemal study.

In our study the bacterial isolates of gram positive bacteria and gram negative bacteria they were highly susceptible to the drugs Polymyxin B(100%) , Colistin(100%), and gentamycin(80%) exception of *Morganellamorganii* as they are intrinsicly resistant to Polymyxin- B and colistin .The *Enterococcus* species were highly susceptible to high level gentamicin and high level streptomycin isolated from samples of ICU patients and Legionella pneumophilia was sensitive to gentamycin(50%) and azithromycin (50%). In the study of Carolina cruz et.al (2023) Fluoroquinolones was the most active antibiotic, displaying the lowest MIC values in contrast to doxycycline which had the highest sensitive[11]. In other studies fluroquinolones were highly effective but in our study broad spectrum antibiotics were sensitive.

CONCLUSION-

This disease primarily involves smokers, alcoholism, low respiratory rate and people with immunodeficiency, especially those with low lymphocyte count in early admission to hospital. Therefore in patient with severe pneumonia requiring hospitilization in ICU, Legionella test should be kept in mind as a differential diagnostic test for detecting Legionnaire's disease. In our study the incidence of *Legionella pneumophilia* was 7.5%. ICT was observed to be more effective method when compared to other convectional method with higher sensitivity and specificity than the gram stain. Hence, the new ICT kit being rapid, easier and reliable method for commence of treatment.

ETHICAL CLEARENCE- The ethical clearence certificate was taken before starting of study by institutional medical ethical committee.

LIMITATIONS- In our study, less number of samples were studied due to cost constraints.

CONFLICT OF INTEREST- The authors have no conflict of interest.

ACKNOWLEDGEMENT-

The present study was the thesis of a medical student, Maisha Tariq,which was supported by the Department of Microbiology at Rama Medical College Hospital & Research Centre, Kanpur. I acknowledge our Department Professor & HOD DR. R. Sujatha ma'am for her constant support and encouragement during the work.

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