CHAPTER ONE

1.0 INTRODUCTION

Pneumonia is referred to by the United Nations International Children's Emergency Fund (UNICEF) as "the forgotten killer of children". It is a severe form of acute respiratory infection that specifically infects the lungs. Under nutrition accounts for 53% of all deaths among children under five and pneumonia is the second major killer of children accounting for 19% of deaths. It is a more dangerous killer compared to malaria and measles (UNICEF, 2006). Approximately 10 million children die yearly around the world before they are five years old and about 97% or more of these deaths occur in developing countries. Almost 2 million deaths are attributed to acute respiratory infections (ARI), particularly pneumonia accounting for almost 1 in 5 under-five deaths (UNICEF, 2006). Pneumonia is also the cause of 13% of all infectious illnesses in infants younger than 2 years. Though the deaths due to pneumonia within the first few weeks of life (neonatal period) is not included in the 2 million deaths documented, it has been estimated that it accounts for about 29% if when added gives a total of 3 million under-five deaths annually (WHO 2016). In Nigeria for instance, the executive director of the National Primary Health Care Development Agency (NPHCDA) stated during the World Pneumonia Day (12th, November, 2012) that about 130,000 Nigerian children die each year from pneumonia (NPHCDA, 2012).

1.1. Statement of problems

Staphylococci pneumonia predominantly affects people with underlying lung disease including those on mechanical ventilators (CDC, 2011). *S. aureus*particularly *MRSA* is one of the most frequently isolated pathogens in nosocomial pneumonia and its resistant nature is due to its ubiquity (with up to 50% persistent or intermittent colonized adults and colonized persons being at increased risk for subsequent infection) and its production of extracellular

enzymes and toxins, which function as virulence factors (Steven *et al.*, 2015). In addition, *S. aureus* accounts for 10-20% of theillness with pneumonia. The bacteraemia often arises in *S. aureus* pneumonia and is often associated with both increased morbidity and mortality(Schreiber*et al.,* 2011). The prevalence of *Staphylococcus aureus* and the increase in resistance of *Staphylococcus aureus* to different antibiotics is becoming rampant(Arunava*et al.,* 2013). There is therefore need to evaluate the prevalence of enterotoxins and biofilms produced by *Staphylococcus aureus.* Studies of the epidemiology of *S. aureus* –caused pneumonia in Nigeria is very scarce (Igor*et al.*, 2008; Schaumburget *al.*, 2014). Till date there is no published study on pneumonia induced by *S. aureus*in South-South Nigeria. Adequate information is needed for treatment of these infections/ disease and for the purpose of formulating effective infection control measures as well as sound antibiotic-use policies. As a result, the following **research questions** will be considered.

Reasearch Questions

- How does the resistance patterns of *S. aureus*isolates affect clinically diagnosed pneumonia subjects in the studied zone?
- Does *S. aureus*affect the lungs tissue of clinically diagnosed pneumonia subjects?
- Is there a correlate between the *S. aureus* pneumonia and methicine resistant *S. aureus*?
- How has *S. aureus*isolates influence the haematological and clinical chemistry parameters?
- Does *S. aureus*produce biofilm and enterotoxin?

1.2. Aim and objectives of the study

To ascertain the prevalence of *Staphylococcus aureus* associated with pneumonia in the South –South Nigeria. This study was designed to achieve the following specific objectives:

- Toinvestigate the resistance patterns of *S. aureus*isolates in the studied zone.
- Toascertain the effect of *S. aureus*isolates on the lungs of Albino Wistar rats.
- To check if the observed resistance is chromosomally or plasmid mediated.
- Toinvestigate the effect of *S. aureus*isolates on the heamatological and clinicalchemistry parameters.
- To determine the prevalence of biofilms and enterotoxins produced by *Staphylococcus aureus.*
- To check the activities of the Garlic, Ginger and Tumeric plants extracts on clinical isolates

1.3 Research Hypothesis

H_o = Null Hypothesis

There is no significant difference between *Staphylococcus aureus* associated pneumonia and *Streptococcus pneumoniae*

H_I = Alternate Hypothesis

There is no significant difference between *Staphylococcus aureus* associated pneumonia and *Streptococcus pneumoniae*

Justification of the study

Due to the current public health burden of pneumonia in Sub- Saharan Africa, knowledge on the prevalence patterns, geographical distribution and characterization of this disease will be of great importance as these data may be used to devise mechanisms to stem the emergence and subsequent spread of infections and drug resistance by the organism.

CHAPTER TWO

LITERATURE REVIEW

2.1. Acute Respiratory Infections

The International Classification of Diseases (ICD) defined acute respiratory infection as "any infection that affects the upper or lower respiratory system". Acute lower respiratory infections refer to infections that affect the airways below the epiglottis and include severe infections such as pneumonia. A significant proportion of the disease burden of acute lower respiratory infection is attributed to pneumonia (UNICEF, 2006).

2.2 Pneumonia

Pneumonia is an acute or chronic infection of one (or both) of the lungs characterized by swelling of the inner parts (air sacs/alveoli) of the lungs resulting from direct inflammation of the lung tissue (Shah, 2012). The air sacs are filled with fluid which is the cause of the respiratory difficulties associated with pneumonia. The swelling is usually caused by an infection from bacteria, virus, fungi or other pathogens such as Mycoplasma, Legionella and Chlamydia. It can also be caused by irritation from chemical (chemical injury due to gastric acid/aspiration of food/hydrocarbon and lipid or radiation-induced pneumonia) or physical agents. The causative agents may reach the lung through the bloodstream or direct inhalation into the lung alveoli. The World Health Organization (WHO) definition of clinical pneumonia classifies it into two namely: **very severepneumonia** and **severe pneumonia**.

2.3 Types of Pneumonia

Pneumonia is one of the commonest conditions observed among hospitalized patients and is represented as either **community-acquired pneumonia** (CAP) or **hospital-acquired pneumonia** (HAP). However, certain forms of pneumonia may fall into a gray area between CAP and HAP, such as those diagnosed in patients with pneumonia found in nursing homes.

These patients on laboratory examination are often found to have different organisms other than those commonly responsible for true CAP, and thus perhaps a better term for this condition is **healthcare-associated pneumonia**(Alan *et al.,* 2014). The types of pneumonia commonly encountered are named according to the settings where it was acquired or the causative agent and include the following:

2.4. According to setting of acquisition

a. **Community-Acquired Pneumonia**

Community-acquired pneumonia (CAP) is a clinical term used to describe signs and symptoms of pneumonia in a previously healthy person who acquired the infection outside a hospital or healthcare setting. Alternatively, it is defined as an acute infection of lung parenchyma in a patient who has acquired the infection in the community, as distinguished from hospital-acquired (nosocomial) pneumonia (File, 2012). It is one of the commonest and most serious childhood infections, with an incidence of 34 to 40 cases per 1,000 children in Europe and North America (UNICEF/WHO, 2006). Although death from CAP rarely occurs in developed nations, lower respiratory tract infection is one of the leading causes of childhood mortality in developing nations outside of hospitals and other health care settings. CAP is the most common type of pneumonia with most cases occurring during the winter. About 4 million people get this form of pneumonia each year and about 1 in 5 people who have CAP need to be treated in a hospital. Community acquired pneumonia is associated with various significant co-morbidities (File, 2012).

b. Hospital-Acquired Pneumonia

The term hospital-acquired pneumonia (HAP) refers to pneumonia not present and without evidence of incubation at the time of admission of the patient to a hospital. The risk of getting HAP increases especially for patients who are placed on a

ventilator to mechanically aid their breathing. This leads to the development of a type of hospital-acquired pneumonia known as **ventilator-associated pneumonia** (VAP). It is defined as "HAP arising > 48-72 hours after endotracheal intubation (Kalil *et al*., 2016). This subset of HAP is important because of the increase in length of hospitalization, hospital costs, and mortality attributed to it. HAP is regarded as being more serious compared with CAP because the patient is already sick and has reduced level of immunity. Also, the presence of resistant organisms particularly to first-line antibiotics used in the treatment of pneumonia particularly bacterial pneumonia further increases the associated severity. Another type of HAP is **Healthcare related pneumonia** (HRP). It is defined as "HAP occurring ≥ 48 hours after admission to a health care facility (Kalil*et al*., 2016).

c. Health Care-Associated Pneumonia

Pneumonia acquired in other health-care settings, such as nursing homes, dialysis centers, and outpatient clinics is referred to as health care-associated pneumonia (HCAP). It is pneumonia that fulfills any of these conditions: arising in patients hospitalized within 90 days of infection; residing in a nursing home or long-term care facility; IV delivery of antibiotics; chemotherapy; wound care; or attendance at a hospital or hemodialysis clinic (ATS & IDSA, 2005). The pattern of pathogens causing hospital-associated pneumonia is characteristically different from that causing community-acquired pneumonia, with greater representation of gram-negative bacteria such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and greater prevalence of multiple antibiotic resistance (Richard *et al.,* 1999). Thus, these types of pneumonia can be further differentiated on the basis of their causative organisms.

2.5 According to causative organism

a. Bacterial Pneumonia

In simple terms, this refers to pneumonia caused by bacteria particularly *Streptococcus pneumoniae* and *Staphylococcus aureus*. Other bacteria that cause pneumonia include: *Streptococcus pyogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Neisseria meningitides, Moraxella catarrhalis*, and *Haemophilus influenza etc.* Bacterial pneumonia attacks individuals of any age and can develop on its own after exposure to cold or flu. Bacterial pneumonia develops inhalation or aspiration pathogens by children (Horan *et al.,* 2008).

b. Viral Pneumonia

This used to describe pneumonia caused by viruses including: Adenovirus type 14, Herpes simplex virus, Influenza subtype H5N1, Coronavirus, and Respiratory syncytial virus. The main target of most respiratory viruses is the upper respiratory tract. However, some of them cause pneumonia especially in children. Viral pneumonia arises primarily from inhalation of infected droplets from the upper airway into the lungs. Most of these pneumonias being of viral origin are acute in nature and last for short periods but others can be severe and sometimes fatal especially those caused by influenza virus. This is because the virus invades the lungs and multiplies rapidly and may present with almost no physical signs of the lung tissue becoming filled with fluid. This pneumonia is most serious in people who have pre-existing cardiovascular or hepatic disease and also in pregnant women (McCullers, 2006).

c. Atypical Pneumonia

This refers to pneumonia caused by microorganisms other than bacteria and viruses such like *Legionella pneumonia, Mycoplasma pneumonia,* and *Chlamydophila pneumonia.* Also, Rickettsia (also considered an organism somewhere between viruses and bacteria) cause Rocky Mountain spotted fever, Q fever, typhus and psittacosis, diseases that may have mild or severe effects on the lungs causing atypical pneumonia. It is a type of CAP that is passed from person to person and is often referred to walking pneumonia (that is pneumonia that doesn't prevent the patient from moving about). Mycoplasma is one of the smallest free-living agents of disease in humankind. They are not classified as to whether they are bacteria or viruses, but they have traits of both. They cause a mild and widespread pneumonia, affect all age groups, but occur most often in older children and young adults (McCullers, 2006).

2.6. Other Types of Pneumonia

a. Tuberculosis pneumonia

Tuberculosis can also cause pneumonia and the organism usually implicated is *Mycobacterium tuberculosis*. It is a very serious lung infection and extremely fatal unless identified and treated early.

b. Opportunistic Pneumonia

This refers to pneumonia commonly seen in patients with impaired immunity. They are usually caused by organisms that are normally harmless to people with healthy immune systems. These organisms include: *Pneumocystis carinii,* renamed *Pneumocystis jiroveci* in 2002 which is the most common cause of pneumonia in HIV/AIDS patients. Pneumonia caused by this organism is termed Pneumocystis carinii pneumonia (PCP). Other organisms that cause opportunistic pneumonia are

fungi, such as *Mycobacterium avium*, viruses, such as cytomegalovirus (CMV), Immunosuppressant increase the risk of these pneumonias. PCP can be successfully treated in many cases. It may recur a few months later, but treatment can help to prevent or delay recurrence. Other less common pneumonias may be quite serious and occur more often. Various special pneumonias are caused by the inhalation of food, liquid, gases or dust, and by fungi.

c. Occupational and Regional Pneumonia

Chemicals that on inhalation irritate the lungs can cause inflammation and pneumonia. Also, exposure to farm animals such as cattle, pigs, sheep, horses etc. who are host of pathogens such as Anthrax, Brucellaand *Coxiella burnetii* (which causes Q fever) increases the risk of the workers to pneumonia especially on inhalation of these organisms.

2.7. Other Common Types of Pneumonia

a. Aspiration Pneumonia

Aspiration is defined as the inhalation of foreign material either oropharyngeal to gastric contents into the lungs or lower airways. The quantity and nature of aspirated material, the frequency of aspiration and the host factors that predispose the patient to aspiration and modify the response determines the number of syndromes caused. This type of pneumonia can occur if food, drink, vomit, or saliva from your mouth is inhaled into your lungs. The probable causes may be brain injury, swallowing problem, or excessive use of alcohol or drugs which leads to stimulation of the gag reflex. Aspiration pneumonia can cause pus to form in a cavity in the lung and this is described as lung abscess (Krishnan *et al.*, 2011).

b. Lobular Pneumonia (Bronchopneumonia)

Lobar pneumonia refers toacute pneumonia that affects one or more lobes of the lung and characterized by sudden onset, chill, fever, respiratory difficulties, cough, and blood-stained sputum, marked by consolidation, and normally followed by resolution and return to normal of the lung tissue.

Among these types of pneumonia, nosocomial pneumonia (NP), healthcare-associated pneumonia (HCAP) and community-acquired pneumonia (CAP) are of major concern due to the morbidity and mortality attributed to them (Guest and Morris 1997).

2.8. Epidemiology

The epidemiology of pneumococcal pneumonia in most areas of the world is poorly defined and documented particularly in the Pacific islands and African countries. This is due to inherent difficulties in diagnosis of the disease in these regions and in establishing a specific etiology. For instance, diagnosis of childhood pneumonia is normally done using basically clinical parameters particularly cough and raised respiratory rate (Biederer *et al.,* 2012). However, this method of diagnosis is non-specific and depends highly on the context in which it is applied thereby making it unsuitable for epidemiological purposes(Connallon *et al.,* 2012). Moreover, since other organisms apart from bacteria (such as virus, atypical microorganisms etc.) cause pneumonia and few cases are bacteremia, the use of blood cultures to investigate the causative organisms in cases of pneumonia proves futile and inconclusive(Don*et al.,* 2010). Although the associated risk of pneumothorax limits its use, lung aspirates are a better diagnostic option. Also, chest x-rays can also be used as they have proven in time past to be helpful in differentiating between the different causes of pneumonia. Aside these, no new method is specific enough to be used in diagnosing pneumococcal pneumonia thereby providing useful data for epidemiological studies or vaccine clinical trials (Frenck and Yen, 2012)

2.9. Mortality for Pneumonia in the General Population

Mortality of pneumonia in the general population of European countries ranges from 5 per 100,000 in Italy, up to 50 per 100,000 in Finland and UK(Rajaratnam*etal.,* 2010). Also, mortality in newborns, when compared with the mortality in the general population, can be interpreted as a measure of the general sanitary situation in each country. It is important to note that the mortality for pneumonia may show relevant changes in a short period of time, so that the comparison of data among countries may be affected by the availability of statistics for the same calendar years in each country(CDC, 2007).

Acute respiratory infections are a major cause of hospital admission and death in Nigerian children. Between the years 2000 and 2003 it was estimated that pneumonia accounted for 20% of deaths in children under the age of 5 years in Nigeria. In a prospective cohort study in Ilorin, the rate of acute respiratory infection was three episodes per child per year with pneumonia being responsible for 1.3 episodes per child per year. In another hospital-based study in Ibadan, 28.4% of children admitted to the hospital with acute lower respiratory tract infection had acute bronchiolitis with respiratory syncithial virus being the most common viral aetiologic agent. There are scanty data on the bacterial aetiology of pneumonia in Nigerian children. There is a seasonal variation in acute respiratory infections in Nigerian children with more episodes occurring during the rainy season. Pneumonia is also associated with measles infection, and this has been recognised as the major cause of death from measles in sub-Saharan Africa (Walter *et al.,* 2004).

2.9.0. Pneumonia Incidence in children

Childhood pneumonia is an important cause of morbidity in both the developed and developing world, and also mortality in the developing world. World Health Organizationstated that about 150-156 million cases of pneumonia occur yearly in children younger than age 5 years and as many as 20 million cases are severe enough to require

hospital admission resulting in the death of about 2 million children worldwide (Rudan *et al.,* 2008). The most of these deaths occur in Africa and Southeast Asia. Mortality is much lower in the United States, but morbidity is substantial. The prevalence of pneumonia and person-person spread of pneumonia in temperate regions is higher during winter due to decrease in mucociliary clearance from dry air and overcrowding. In the developed world, the annual incidence of pneumonia is estimated to be 33 per 10,000 in children younger than five years and 14.5 per 10,000 in children 0 to 16 years (Rudan *et al.,* 2008).

The first global estimate of the incidence of clinical pneumonia in children aged less than 5 years for the year 2000 was calculated and published by Rudan *et al*. (2005). This estimate had its basis on data analyzed from 28 selected community-based longitudinal studies done in developing countries that were published between 1969 and 1999(Bartolome*et al.,*2004). These studies were the only sources meeting the predefined set of minimum-quality inclusion criteria in the analysis.The median incidence for developing countries was estimated as 0.28 episodes per child-year, with interquartile range 0.21–0.71 episodes per child-year. The large variation in incidence between the selected studies was most probably due to the distinct study designs and real differences in the prevalence of risk factors in the various study settings(Igor *et al.,* 2008). Given the substantial uncertainty over the point estimate, a triangular approach was used to check for plausibility of assessment of pneumonia incidence. An overlap between the values of 148 and 161 million of new episodes per year obtained by the main appraisal and two ancillary assessments was noticed. This gave more weight to the estimate obtained through the main approach and the analyses also suggested that the incidence of clinical pneumonia in children aged less than 5 years in developing countries worldwide is close to 0.29 episodes per child-year. This equates to 151.8 million new cases every year, 13.1 million (interquartile range: 10.6–19.6 million) or 8.7% (7–13%) of which

are severe enough to require hospitalization. Furthermore, 4 million cases occur in developed countries worldwide.

About one-half of children younger than five years of age with community-acquired pneumonia (CAP) require hospitalization. Hospitalization rates for all causes of pediatric pneumonia among children younger than two years in the United States decreased after introduction of the pneumococcal conjugate vaccine to the routine childhood immunization schedule in 2000 (from 12 to 14 per 1000 population to 8 to 10 per 1000 population). Furthermore, more than half of the world's new pneumonia cases each year are concentrated in just five countries where 44% of the world's children aged less than 5 years live: India (43 million), China (21 million) and Pakistan (10 million) and in Bangladesh, Indonesia and Nigeria (6 million)(Igor *et al.*, 2008).

Mortality

The mortality rate in developed countries is lower (<1 per 1000 per year) than in developing countries where disease patterns may be affected by malnutrition, the absence of widespread immunization programmes, and the lack of early medical attention, greatly increase the mortality rates (Wardlaw *et al.*,2006). In developing countries, respiratory tract infections are not only more prevalent but more severe, accounting for more than 2 million deaths annually; pneumonia is the number one killer of children in these societies(Hoyert and Xu, 2012). Consequently, more than 98% of pneumonia deaths in children occur in 68 countries where progress in reducing under-five mortality is most critical. The inequalities are overwhelmingly exacerbated by the burden that pneumonia places on families and the health system in low-resource countries(Berman,1991).Thus, children who are hungry due to poverty and living in remote areas are most likely to be visited by this "*forgotten killer*".

2.9.1. Pneumonia incidence in adults

The rate of CAP increases with increasing age with the overall rate of community-acquired pneumonia (CAP) in adults being approximately 5.16 to 6.11 cases per 1000 persons per year this vary seasonally, with more cases occurring during the wet season. The higher rates of pneumonia are observed for men than for women and for people of African descent compared with Caucasians. There is a geographical variation in the etiology of CAP and *Streptococcus pneumoniae* is implicated as the most common cause of pneumonia worldwide(Antoni*et al.*, 2016). A combination of Pneumonia and influenza areamong the eight leading cause of death in the United States and the most common cause of infection-related mortality. Precisely, in 2007, about 52,700 persons died as a result of these disease conditions(Bonafede *et al.*, 2012). Overall, the annual incidence of CAP ranges from 5-11 per 1,000 persons, with more cases occurring during wet season. Also, in 2006, approximately 4.2 million ambulatory care visits for CAP were documented in the United States, with *Streptococcus pneumoniae* being the leading causative pathogen of the disease. The estimated yearly burden of CAP in the United States exceeds $17 billion(Jackson *et al.*, 2004).

Mortality

In 2005, a combination of pneumonia and influenza was described as the eight most common cause of death in the United States and the seventh most common cause of death in Canada. About 60,000 deaths were documented as due to pneumonia in the United States. Mortality is highest for Community-acquired pneumonia (CAP) patients increased on hospitalization, with a 30-day mortality rate of up to 23 percent in such patients. The percentage of mortality due all causes of CAP is documented to be as high as 28 percent within one year and given the aging population in North America, it is expected that the burden of CAP will increase in the coming years. CAP is a common and potentially serious illness. It is frequently associated with considerable morbidity and mortality, particularly in elderly patients and those with significant comorbidities. (Tomczyk *et al*., 2016)

2.9.2 Etiology of *Staphylococcus aureus* induced *pneumonia*

Staphylococcus aureus is a Gram-positive aerobic bacteriumimplicated as one of the common causes of infections in the human body.*S. aureus* is part of the normal human flora and does not usually cause infection(Woodhead*et al.,* 1987). About 30% of humans are most often colonized with *S.aureus* in their noses and it is also found on the skin and other body sites (CDC, 2011). Over time, 20% of the population will almost always be colonized with *S. aureus*, 60% of the population will be colonized with *S. aureus* off and on, and another 20% are almost never colonized with *S. aureus* (MDH, 2010). It has a significant number of virulence factors that facilitate adherence and invasion to host tissues in addition to structures that disable host defenses and toxins that induce septic syndromes (Archer, 1998). In addition, *S. aureus* has acquired genes that promote resistance to several classes of antibiotics the most important being the *mec*A gene that confers resistance to methicillin and almost all β-lactams (Zetola, *et al.,* 2005*).* Most of staphylococcal strains are methicillin-resistant and many strains contain toxins that are likely responsible for the severity of *Staphylococcus aureus* illness. It appears that the genetic element for methicillin resistance has been introduced into multiple highly virulent methicillin-susceptible strains with great potential for further spread (Bradley, 2005).

Staphylococci pneumonia predominantly affects people with underlying lung disease including those on mechanical ventilators (CDC, 2011). Methicillin-resistant *S. aureus* (MRSA) is viewed clinically as the primary pathogen of skin and soft tissue infections, though, invasive infections also occur (Zetola *et al.,* 2005;Kowalski *et al.,* 2005*).Staphylococcus aureus* particularly MRSAis one of the most frequently isolated pathogens in nosocomial pneumonia and its resistant nature is due to its ubiquity (with up to 50% persistent or intermittent colonized adults and colonized persons being at increased risk for subsequent infection) and its production of extracellular enzymes and toxins, which

function as virulence factors. In addition, *S. aureus* accounts for 10-20% of the *illness with pneumonia.* Also, due to the limited therapeutic options to treat MRSA and because MRSA tends to be not only resistant to all β-lactams but also to other antibiotic classes such as the fluoroquinolones, it has become even more problematic (Meyer *et al.,* 2010).

MRSA that causes nosocomial pneumonia and community acquired pneumonia is designated as nosocomial MRSA (HA-MRSA) and community acquired MRSA (CA-MRSA). Most strains of *Staphylococcus aureus* associated with nosocomial pneumonia/health care—*associated* pneumonia (NP/HCAP) and community acquired pneumonia (CAP) have distinct characteristics. The former contains the staphylococcal cassette chromosome SCC*mec* type I–III, while the latter contains SCC*mec* type IV and V (Gosbell, 2005*).* CA-MRSA can also cause a serious necrotizing pneumonia, among other uncommon infections (Nathwani and Urquhart 2010). The findings from a study carried out at Pulmonary and Critical Care Medicine, Washington Hospital Center, Washington, USA in 2010 concluded that bacteremia often arises in *S. aureus* pneumonia and is often associated with both increased morbidity and mortality. Furthermore, several simple clinical factors to determine clinical features identified patients with *S. aureus* pneumonia as likely to have simultaneous bacteremia (Schreiber *et al.*,2006).

2.9.3. Epidemiology of *Staphylococcus aureus* associated pneumonia

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first observed a few years after the introduction of methicillin in 1959. Since that time, MRSA has increased in prevalence worldwide both as a nosocomial and more recently, a community-acquired pathogen (Enright, 2002). A study was carried out in Germany between 2005 and 2009 and generated sufficient data based on a network of 568 German ICUs. The data obtained from the German national nosocomial infection surveillance system shows that about 20,000 ventilator associated lower respiratory tract infections can be expected annually in German intensive

care units and among them about 20% of these cases are due to *S. aureus*, and 37% of them are methicillin resistant(Elizabeth*et al.*, 2010). Thus, about 1,200 ventilator-associated pneumonia (VAP) cases due to MRSA can be expected every year in German ICUs. A projection of these figures to the whole European Union would result in about 7,500 VAP cases with MRSA in European ICUs annually (Pujol *et al*., 1998).

Nosocomial pneumonia is currently the second most common hospital infection with an incidence of 7.8 to 68.0% and is the leading cause of death from hospital-acquired infections. This is influenced by the following factors, duration of hospital and ICU stay, the type of diagnostic method used for detection of the pathogen, and the sample size of patient studied (Andrew *et al*., 2005). Over the past 2 decades, there has been a steady increase in the rate of nosocomial pneumonia secondary to *Staphylococcus aureus*. In a review of three major studies which examined the etiology of ventilator-associated pneumonia (VAP), *S. aureus* was the most frequently isolated Gram-positive organism and the second-most isolated organism only behind *Pseudomonas aeruginosa*(Chastre and Fagon., 2002). Thus, it is estimated by most studies that *S. aureus* accounts for 15 to 35% of all nosocomial pneumonia cases; however, the true incidence depends on many factors, such as patient demographics, local susceptibility patterns, and methods of diagnosis(Andrew, 2005).

Though *S. aureus* is increasingly being recognized as a major pathogen causing nosocomial pneumonia, there are few studies with good descriptive data that specifically evaluates patient outcomes of *S. aureus* pneumonia. In addition, in the last decade, evidence from various studies have shown that initial inappropriate antibiotic treatment is an important independent predictor of excess mortality in patients with nosocomial pneumonia(Trifiro *et al.*, 2010). Apart from causing uncommon infections, CA-MRSA can also cause a serious necrotizing pneumonia and despite presently occurring in only a small proportion of patients presenting with community-acquired pneumonia (CAP), the associated significant morbidity and mortality makes this an important diagnosis for both respiratory and infection specialists to consider.Current evidence is based on case series and *in-vitro* studies because of the relatively low incidence (Nathwani and Urquhart, 2010).

2.9.4 Pathophysiology

A broad understanding of the anatomy of the lungs gives a better picture of the effect of pneumonia on the respiratory system. The nose and mouth begin the respiratory system and are sites for breathing in (inspiration) and out (expiration). The air tube extending from the nose (nasopharynx) directs air into the lungs while the tube carrying air breathed in through the mouth (oropharynx) also carries swallowed food, water, and salivary secretions through the food tube (esophagus) and then into the stomach(Beasley, 2010). The nasopharynx and oropharynx merge into the larynx, which is protected by a trap door called the epiglottis. The functions of epiglottis are prevention of substances that have been swallowed, as well as substances that have been regurgitated (vomited), from heading down through the larynx into the lungs(Hogg and Timens, 2009).

The respiratory system and its parts can be clearly illustrated as an upside down tree. The larynx flows into the trachea, which is the broadest part of the respiratory tract and is likened to a tree trunk. The trachea divides into the right and left bronchi, each branching off into

several smaller bronchi that course throughout the lung tissue(Davies*et al.*,2003).. Each bronchus divides into tubes of smaller and smaller diameter, finally ends in the terminal bronchioles. The alveoli, which resembles the leaves of a tree are clustered at the ends of the bronchioles and is the site where oxygen and carbon dioxide are exchanged. Lung stroma which is the tissue of the lung, serves only as a supportive role for the bronchi, bronchioles, and alveoli(Friedene*t al.,* 2003).

2.9.5 Invasion of the respiratory system by Bacteria

Bacteria invade the circulatory system rapidly after the onset of pneumonia (Bubeck *et al.,* 2008). The means by which the infecting organism gains entry into the respiratory tract is by inspiration or aspiration of oral secretions. Basically, Staphylococci and Gram negative bacilli reach the lungs via the circulatory system. The defense mechanism of the body particularly pulmonary defense mechanism in case of lungs springs into action and protects the body using the following mechanisms: a cough reflex, mucociliary transport and pulmonary macrophages try to protect the body against the infection(Friedene*t al.,* 2003). However, some people with either suppressed or overwhelmed defense system they become overwhelmed by the invading agent and this leads to development of infection. Thereafter, multiplication of the invading organism occurs and damaging toxins are released causing inflammation and edema of the lung parenchyma. The resultant effect is accumulation of cellular debris and exudes within the lungs (King,2009). The lungs particularly the alveoli is then filled with pus and fluid causing interference in the oxygen absorption and dyspnea (UNICEF, 2006). Soon the hypoxic state of the lungs changes to a consolidated state due to the fluid and exudate filling up. Damage to the ciliated epithelial cells occurs in case of viral pneumonia. The inhaled airborne droplets transport the virus to the lungs. Immediately after entry into the lungs, invasion of the cell lining of the airways and alveoli begins. This result in cell death by direct action of the virus or through a cell controlled self-destruction called

apoptosis. Further damage to the lungs due to the body's response to the invasion occurs as the fluid is leaked into the alveoli. Viral infection of the lungs damages the lungs and increases their susceptibility to bacterial infections.

The symptoms caused by the invading organism are partly due to stimulation of an immune response by the immune system in the lungs which in the bid to ward of infection, kicks into such a high gear, that it damages the lung tissue making it more predisposed to infection. Due to the damage to the lung capillaries, they become leaky and protein-rich fluid seeps into the alveoli. The resultant effect is a less functional area for oxygen-carbon dioxide exchange. The patient becomes relatively hypoxic, while retaining potentially damaging carbon dioxide. The patient's respiratory rate increases rapidly in an effort to breathe in more oxygen and blow off more carbon dioxide.

Production of mucous increases and the mucous is tinge with blood from the leaky capillaries. The efficiency of gaseous exchange is further decreased by mucus plugs. Fluid and debris from the large white blood cells being produced to fight the infection further accumulates in the alveoli. A common feature of bacterial pneumonias known as consolidation occurs when the alveoli, which are normally hollow air spaces within the lung, become solid due to huge quantities of fluid and debris. Consolidation is absent in viral pneumonias and Mycoplasma pneumonias as this pneumonias primarily affect the walls of the alveoli and the stroma of the lung.

The Stages of Pneumonia

Pneumoniaoccurs in four primary stages namely: the 24-hour congestion stage, the red hepatisation stage, the gray hepatisation stage and the resolution stage. Each of these stages exhibits specific physical findings.

24 hour Congestion Stage

The first stage presents as capillary bed engorgement within the alveoli with leakage of serious fluid into the alveolar spaces. Symptoms such as fever, chills, chest pain or ache, general malaise and difficulty breathing may be experienced and also clear, watery phlegm may be produced. Also, elevated white blood cell count may also be present (Atkuri & King, 2006; Steyl, 2007)

Red Hepatisation Stage

In this stage where the red blood cells and fibrin will begin to enter the alveoli. The lung tissue will appear reddened and firm. The patient may experience difficult or rapid breathing.

Gray Hepatisation Stage

The gray hepatisation stage is characterized by collection of fibrin and dying red and white blood cells in the air spaces. Sputum from productive cough may be tinged with blood or purulent discharge. During this time, a reduction in the available area within the lung for gas exchange (atelectasis) may also occur(Atkuri and King, 2006; Steyl, 2007).

Resolution Stage

At this stage, the materials causing the inflammation would be broken down by the lung enzymes. Then, the white blood cells control the infectious agents and any remaining material may be coughed up. Dead lung tissue may also be present (Merget *et al.*, 2008).

2.9.6 Clinical Presentation of *S. aureus* induced pneumonia

The most common clinical presentation of CA-MRSA is skin and soft-tissue infection (Nathwani and Urquhart, 2010). Staphylococcal pneumonia is most commonly observed in infants, young children, and patients who are debilitated.In most infants, young children, and debilitated patients the clinical presentation of *S. aureus* induced pneumonia is a short prodrome of fever followed by rapid onset of respiratory distress which may include tachypnea, retractions, andcyanosis; prominent GI symptoms may also occur(Jeffrey *et al.*,

2006).*Staphylococcal* pneumonia is a rapidly progressive disease and may also develop after influenza infection, which seems to occur preferentially among young adults with a mortality rate of 50%. Typically, the child seems to recover from an influenza infection characterized by febrile illness only to once again develop an increasing fever and the symptoms mentioned above (Tolan, 2013).

2.9.7. Age Specific Clinical Features of Pneumonia

- **Neonates**

These present with refusal to feed,respiratory distress, tachpnoea, grunting temperature instability, cyanosis, retractions, and lethargy (Shah, 2012).

- **Infants**

Infants with pneumonia in the first several months of life may appear febrile, tachypnoeic or apneic, and irritable. Other features include cough, retractions, refusal to feed, wheezing and noisy breathing (Shah, 2012). Most times, fever and tachpnoea may be the only findings in a young infant or toddler with pneumonia. In addition, atypical pathogens (i.e. *C. trachomatis*) causing pneumonia can lead to an afebrile illness accompanied by cough, tachpnoea, or wheezing.

- **Preschoolers/Toddlers**

In mild cases, toddlers present with chest pain, cough, post tussive vomiting, fever, abdominal pain, and in severe cases tachypnoea, cyanosis, grunting, and retractions.

- **Older Children**

Generally, older children normally present with cough, chest pain, fever, dyspnoea Otalgia/Otitis, vomiting, and diarrhoea. Symptoms of bacterial pneumonia include: abrupt onset of fever, cough, and malaise. Minimal fever, malaise, and lingering cough are typical

symptoms of atypical pneumonia. Furthermore, features observed in anyone with pneumonia are poor oral intake, nausea, vomiting, and abdominal pain.

- **Adolescents/Adults**

Generally, cough is the predominant symptom in both infants and adults. Adolescents also experience similar symptoms as in younger children. In addition, they may have other constitutional symptoms, such as headache, pleuritic chest pain, and vague abdominal pain(Lodha *et al.,* 2013).

2.9.8. Clinical presentation according to type of pneumonia

a. Community-acquired pneumonia (CAP)

The necessary features that enable proper diagnosis areCough,fever, CXR infiltrates

b. Hospital-acquired pneumonia (HAP)

Variable clinical features: cough, fever, CXR infiltrates,leukocytosis, increased respiratory secretions.

2.9.9.0. Laboratory Findings

The patient's history such as history of present illness and past medical history are essential in determining the possible cause of the pneumonia.: Greater than or equal to 60 breaths/min Children aged 2-11 months, Greater than or equal to 50 breaths/min Children aged 12-59 months: Greater than or equal to 40 breaths/min During the physical examination of a child suspected to have pneumonia, the respiratory rate should be determined for a full minute particularly in younger patients and compared with normal values for the child's age group. This is because tachypnea is the most sensitive and specific sign of pneumonia with evidence of lung necrosis. In addition, nine (9) of 15 had evidence of pleural effusions early in their hospital course, and five of the nine required at least one pleural drainage procedure. Seven (7) patients out of 15 were immunocompromised (three HIV, one acute lymphocytic leukemia [ALL], one high-dose steroids, and two immunoglobulin deficiency) with an

additional three patients with diabetes. The observed mortality rate was only 13% (two of 15) and both deaths occurred in patients with severe immunocompromised (ALL post chemotherapy and AIDS). Finally, 14 patients of 15 patients were treated with antimicrobials that inhibit exotoxin production (such as clindamycin or linezolid) (Lobo *et al.*, 2010).

Prognosis

Approximately 5-10 million people get pneumonia in the United States annually, and more than 1 million of cases due to the condition require hospitalization(Lodha *et al.*, 2013). Based on these findings, pneumonia is the fourth most frequent cause of hospitalizations. Although the majority of pneumonias respond well to treatment, the infection kills 40,000 - 70,000 people yearly. Men with community-acquired pneumonia are more affected compared to women. They are 30% more likely than women to die from the condition, even if the severity of the illness is the same. This has led researchers to believe that there may be some genetic reason for the disparity in the effect of the disease on the different sexes. (Fine *et al.*, 1996).

2.9.9.1 Treatment guidelines

Empiric therapy for MRSA is recommended, pending sputum and/or blood culture results, for hospitalized patients with severe community-acquired pneumonia defined by one of the following: a requirement for admission to the intensive care unit, necrotizing or cavitary infiltrates, or empyema. Treatment options for health care–associated MRSA or community-associated MRSA pneumonia include seven to 21 days of intravenous vancomycin or linezolid, or clindamycin (600 mg orally or intravenously three times per day) if the strain is susceptible. In patients with MRSA pneumonia complicated by empyema, antimicrobial therapy should be used with drainage procedures.

Children

In children, intravenous vancomycin is recommended for treating MRSA pneumonia. If the patient is stable without ongoing bacteremia or intravascular infection, clindamycin (10 to 13 mg per kg intravenously every six to eight hours for a total of 40 mg per kg per day) can be used as empiric therapy if the clindamycin resistance rate is low. Patients can be transitioned to oral therapy if the strain is susceptible. Linezolid is an alternative option(Jeffrey *et al.*, 2006).

2.9.9.2.Enterotoxins

Bacteria of the *Staphylococcus* genus comprise various species and subspecies that are widely distributed in nature and found mostly in the skin and mucous membranes of birds and mammals.

Staphylococcus aureus is a Gram positive facultative anaerobiccocci (round shaped) bacteria that appears in a grape-like cluster that can thrive in a high salt and low water activity habitat. Pathogenic strains of *Staphylococcus aureus* often promote infections by producing potent protein toxins (enterotoxins) and expressing cell –surface proteins (biofilms) that bind and inactivate antibiotics. The emergence of antibiotic-resistant forms of *Staphylococcus aureus*such as methicillin-resistant *Staphylococcus aureus* (MRSA) is a worldwide problem in clinical medicines(Linda*et al.,* 2006).

Generally, significant host compromise is required for *S. aureus* infection, such as a break in the skin or insertion of a foreign body (for example, wounds, surgical infections, or central venous catheters), an obstructed hair follicle (folliculitis), or a compromised immune system. *S. aureus*disease may be:

- Largely or wholly the result of actual invasive infection (that is, colonization), overcoming host defense mechanisms, and the production of extracellular substances which facilitate invasion;

- A result of toxins in the absence of invasive infection.
- A combination of invasive infection and intoxication.

Staphylococcal enterotoxins are a potential biological threat because of their stability at high temperature (100^{o}C for 1 hour) and ability to incapacitate individuals for several days to two weeks (Linda*et al.*, 2006).

Salman *et al* (2012)carried out a research on characterization of genetically different clones of MRSA in the production of biofilms where he determined whether the ability of *Staphylococcus aureus* to produce biofilm is consistently similar among isolates variation of methicillin resistant *Staphylococcus aureus*

Victoria and Tajudeen(2011) worked on crystal violet binding assay which revealed that bacteria possesses high capacity of biofilm formation on three surfaces (wood, glass and steel).

These previous research findings are associated with biofilms and enterotoxins formation of bacterial but this research is centered on the prevalence of biofilms and enterotoxins produced by *Staphylococcus aureus*associated with pneumonia.

Pathogenic virulence factors are the genetic, biochemical, or structural features that enable an organism to produce disease. The clinical outcome of an infection depends on the virulence of the pathogen and the opposing effectiveness of the host defense mechanisms. *S. aureus* expresses many potential virulence factors. (Note: Coagulase is generally not considered a virulence factor because coagulase-negative mutants are as virulent as the corresponding parental strains; the association between coagulase positivity and virulence in nature is probably fortuitous.

Many bacterial pathogens and nosocomial infections are the cause of acute and chronic infections due to their ability to form biofilms (Stoodley*et al.,* 2002). Even though biofilm-forming properties have been well demonstrated by the members of the *Staphylococcus* genus such as *S. epidermidis* and *S. aureus*, it is less studied in modern methicillin-resistant *Staphylococcus aureus* (MRSA), which has evolved from several clonal lineages of methicillin-susceptible *S. aureus* strains via acquisition of a mobile genetic element called *Staphylococcal* cassette chromosome *mec* (SCC*mec*). The ability of MRSA to produce biofilm has resulted in difficultly in understanding its high clonal diversity, including its enhanced propensity to spread and cause opportunistic human infections in various parts of the world. The initial bacterial monolayer that sticks to a polymeric surface changes to a common biofilm that includes bacteria and an extracellular slime substance(Hall-Stoodley *et al* 2004). The proliferation of the bacteria and the formation of the slime results in a higher resistance to antibiotics because drugs are prevented from reaching the bacteria that are protected by biofilm (Heilmann*et al.,* 1996).

Many studies have concluded that the formation of the biofilm is caused by adherence at late stages of bacterial growth. In this process, the organisms stick to each other through polysaccharide intercellular adhesion (PIA), which is synthesized by products of the *ica* ADBC operon (Chaieb*et al.,* 2005). Thus, it is important to study the ability of different *Staph aureus*clones to produce biofilms in order to address the complexity of biofilm formation. The study carries out phenotypic and genotypic investigation to test this hypothesis and to discover factors that affect the differences in adherence and biofilm production rate and characteristics. Differences occurring due to clonal variation would indicate a need for accurate clonal identification for effective biofilm management upon infection.

Biofilm are defined as microbial derived sessile communities characterized by the cells that are irreversibly attached to a substratum or to each other (Donlan and Costerton, 2002). Biofilm isdensely packed multicellular communities of microorganisms attached to a surface or interface. Bacteria seem to initiate biofilm formation in response to specific environmental cues, such as nutrient and oxygen availability. Biofilm are the source of persistent infections of many pathogenic microbes(Lear and Lewis, 2012). They are responsible for much nosocomial infection and also associated with many medical conditions including indwelling medical device, dental plaque, upper respiratory tract infection and urogenital infection. (Hall-Stoodley*et al.*,2004)

They are embedded in a matrix of extracellular polymeric substances that they have produced and exhibit an altered phenotype with respect to growth rate and gene transcription. Within a biofilm, bacterial communicate with each other by production of chemotactic particles or pheromones, a phenomenon called quorum sensing. Factors that may influence biofilm formation include: Availability of key nutrients, chemotaxis towards surface, and motility of bacteria, surface adhesions and presence of surfactants.

All microbes like Gram positive and Gram negative bacteria have capacity to synthesized biofilm. Bacteria commonly involved include *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus viridans, Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, Proteus mirabilis and Pseudomonas aeruginosa.* (Donlan, 2001)

2.9.9.3 Staphylococcal enterotoxins characteristics

Staphylococcal enterotoxins are a group of single - chain, low-molecular weight (27,000-34,000) proteins produced by some species of staphylococci, primarily *S.aureus, but* also by *S. intermedius, S. hyicus, S. xylosus and S. epidermidis.* To date, 14 distinct enterotoxins have been identified based on their antigenicity and they have sequentially been assigned a letter of the alphabet in order of their discovery (SEA to SEO).

Studies on SEs started from the analysis of *S. aureus* strains involved in *Staphylococcal* food poisoning. In the first SEs identified, the peptide sequence was available before the nucleotide sequence. This was the case for SEA (Maria*et al.*, 2010) SEB and SEC. The abundance of literature on SEs varies considerably among the types, according to the chronology of their identification and their importance in staphylococcal food poisoning. To date, 14 different SE types have been identified, which share structure and sequence similarities. Subsequent translation leads to the generation of a precursor protein, containing N-terminal leader sequence that is cleaved during export from the cell to form the mature enterotoxin protein. Slight variations in processing or post-translational modification may occur as evidenced by the existence of three SEA isoforms with three different isoelectric points. They are rich in lysine, aspartic acid, glutamic acid, and tyrosine residues. Most of them possess a cystine loop required for proper conformation and which is probably involved in the emetic activity. They are highly stable, resist most proteolytic enzymes, such as pepsin or trypsin, and thus keep their activity in the digestive tract after ingestion. They also resist chymotrypsin, rennin and papain. Nevertheless, SEB and SEC1 have been cut in the cystine loop by mild trypsin digestion. *Staphylococcal* enterotoxin B can be destroyed by pepsin digestion at pH 2 but it is pepsin resistant at higher pH, which are normal conditions in the stomach after food ingestion (Martha, 2012).

The genes responsible for enterotoxin production have been described since 1984, when SEA was first cloned by the transfer of chromosomal DNA from an enterotoxigenic *S. aureus* in *Escherichia coli* strain (Milene*et al.*, 2013). Genes SEA and SEE, responsible for the production of SEA and SEE, respectively, are carried in prophages. SEA is composed of 257 amino acid residues and is expressed in the stationary phase of the growth, whereas genes SED and SEJ, which are determinants of toxins SED and SEJ, have plasmid origin. SEJ presents sequences similar to those of SEA, SEE and SED, corresponding to 64, 63 and 51%,

respectively. PCR amplification suggests that gene SEJ may be present in all plasmids encoding SED. SECs are a group of highly preserved proteins of chromosomal origin that present three distinct subtypes: SEC1, SEC2 and SEC3, based on differences among antigenic determinants.

2.9.9.4. Turmeric (*Curcuma longa*)

Turmeric (*Curcuma longa*) is an intenselycoloured culinary spice – a close cousin of ginger and the essential ingredient in curry. It's a primary ingredient in Indian curry. Curcumin is the bioactive compound found in turmeric and is commonly referred to as "Holy powder."(Barry *et al.,* 2009). As a natural polyphenol (antioxidant), it has been used for centuries as both a food and a medicine, treating a variety of inflammatory health problems, including infections and wounds(Kai *et al.,* 2013). Because of its superhero-like anti-inflammatory properties, today, research is showing that turmeric proves to be an honest to goodness miracle spice. In recent studies, turmeric has proven to outperform many pharmaceuticals in the treatment of a wide range of chronic, degenerative diseases, with promising prevention against cancer, Alzheimer's, and Parkinson's disease (Jill, 2012). In 2007, an Advanced Experimental Medical Biology overview stated, "Curcumin has been shown to exhibit antioxidant, anti---inflammatory, antiviral, antibacterial , antifungal, and anticancer activities and thus has a potential against various malignant diseases, diabetes, allergies, arthritis, Alzheimer's disease, and other chronic illnesses. Turmeric's anti-bacterial, anti-viral and anti-fungal agents can help our body fight against colds, cough and flu (Kuldeep, 2014).

2.9.9.5 Garlic

It is a bulbous plant of the allium genus. For over 7000 years garlic has been used by humans and a native to central Asia (Seo *et al.,* 2012).It has been known to ancient Egyptians and has been used as for both culinary and medicinal purpose (Simonetti, 1990). It is known to have a

characteristic strong odour and are sometimes called “stinking rose”. Freshly crushed garlic yields alliin, ajoene, diallylpolysulfide, vinyldithiins which are sulphur-containing compounds, and non-Sulphur – containing compounds such as saponin, flavoniods,and maillard reaction product.

Garlic has a wide antimicrobial spectrum against many species of bacteria, virus, protozoans and fungi (Verma *et al.,* 2012). Mahsa *et al.,* 2015 reported that allicin, an oxygenated sulfur compound was responsible for the antibacterial effect of crushed garlic clove. Alliin is transformed following crushing to allicin by the enzyme allinase.Aged garlic lack allicin but may have some activities due to the presence of s-allylcysteine (Block, 2010).

Preparations made from garlic have been shown to have wide antibacterial activities against gram positive and gram negative bacteria including species of *Escherichia coli, Samonella, Staphylococcus, Streptococcus, Klebsiella, Proteus, Bacillus And Clostridium*.

2.9.9.6 Ginger (*Zingiber officinale roscoe*)

It is a flowering plant in the family zingiberaceae that is widely used as a spice or a folk medicine. It has a characteristic odor or flavour caused by a mixture of zingerone, shogaols, and gingerols, volatile oils. A study on laboratory animals, showed that gingerols increase motility of the gastrointestinal tract, and have sedative, anagelsic and sedative, antipyretic, and antibacterial properties (Nkere *et al.,* 2009). Ginger has been shown to be effective against the growth of both gram-postive and gram-negative bacteria including *Escherichia coli, Proteus vulgaris, Salmonella typhi, Stapylococcus areus,* and *Streptococcus viridans* (Kamrul*et al.,* 2014).In general,the resulting allergic reaction to ginger is rashes(Suzan*et al.*,2010) although it can also be associated with heart burn and other side effect.

2.9.9.7. Clinical Chemistry

Clinical chemistry, a branch of Clinical medicine that deals with body fluids, is based on the basic principle that a disease causes changes in the biochemistry of the body. It may cause either increase in concentration, or decrease in concentration of certain biochemical parameters or even may cause a different substance to appear. Hence, clinical biochemistry deals with changes in the composition of blood and other body fluids which are associated with the diagnosis of disease or monitoring the therapy (Sharda and Sanjaya, 2012).

The Hematological and blood clinical chemistry could aid in the determination of the health status, diagnosis and prognosis of disease (Arunee *et al*., 2012).Physiological, biochemical parameters can be correlated with pathological situations. Screening of haematological characteristics is sensitive in accessing the health of organisms, although not very specific; according to Hsieh *et al.,* 2014, such tests should be supplemented with clinical and biochemical analyses for diagnostic purposes (Baggish*et al.*,2006). Some clinical chemistry parameters evaluated include;Blood urea nitrogen (BUN), glucose, chloride, cholesterol, creatinine, calcium, albumin (immune-nephelometric), triglycerides, sodium, potassium, aspartate aminotransferase(AST),total protein, transferrin, creatine kinase, inorganic phosphate, lipase, bicarbonate, alanine transaminase(ALT) and bilirubin.

2.9.9.8. Heamatology

Haematological parameters are parameters related to blood and its forming organs (Etim *et al.,* 2014). It has been shown that the investigation of the haematological parameters can be used in the evaluation of the health status of an animal providing information that would detect diseases, hence the choice of the parameters of Haemoglobin (Hb),Packed Cell Volume (PCV), Red blood cell count(RBC),Mean Corpuscular Volume(MCV),Mean Corpuscular Haemoglobin Concentration (MCHC), White Blood Cell count(WBC), Neutrophils, lymphocytes and platelets for the study (Yakubu *et al.*,2007; Orji, 2015).

Red blood cells:

It is the main cellular blood component and occupies about 45% of the total blood volume (Cheesebrough, 2012). Haemoglobin transport is a major function of the erythrocyte. The erythrocyte usually have a short life span of about 120days which is due to the absence of nuclei and other organelles that would enable them reproduce and maintain their structure for long and they are destroyed in the spleen or liver(Arthur *et al.*,2001). The erythrocytes are flexible, biconcave disc that lacks nucleus measuring about 8µm in diameter which is composed of haemoglobin surrounded by a flexible protein membrane and lipid bilayer. Its shape changes as the cell squeezes itself through the capillaries Red blood cell serves as a means of delivering oxygen to the blood tissues via blood flow.
Red Blood Cell is produce in the yolk sac during the first few weeks of embryonic life. As development of the embryo occurs, the liver becomes the organ of production of red blood cell during the third trimester, the spleen and lymph node are also found to produce large amount of RBC. The bone marrow of all bones are capable of producing RBC until the age of 5,therafter RBC continues to be produced only in membranous bones e.g. sternum, ribs etc. (Guyton and Hall,2006)

White blood cells (WBC):

Leukocytes or White Blood Cells are less numerous than red blood cell. Leucocytes contain nuclei and all other cellular organelles. White Blood Cells are the major component of the inflammatory process and they generally play important roles in body defense. The normal WBC count in adult is 4.0-10x^{9}/l (Cheesebrough, 2012). WBC count serves as an indicator for inflammation, infection and immune-competence (Arewa, 2011). An increase or decrease in total white blood cell could be due to abnormal bone marrow pathology. The granulocytes, monocytes and a few lymphocytes are produced in the bone marrow while the lymphocytes and plasma cells are produced in the lymph tissue (Guyton and Hall, 2006).

Human has about 7000 WBC per microlitre of blood. The normal percentages of the different types of WBC are as follows; neutrophils 62%, eosinophils 2.3%, basophils 0.4%, monocyte 5.3%, and lymphocyte 30%.

Some disease condition associated with WBC would include; Leucopoenia a condition that occurs when the bone marrow produces few WBC thus leaving the body defenseless against invading agents, Leukemia is a condition due to uncontrolled production of WBC. (Guyton and Hall, 2006). Leukocytosis refers to an elevated white blood cell count i.e. WBC count above the normal ranges. WBC counts become elevated in response to an inflammatory or infectious process. (Abramson and Becky, 2000).

CHAPTER THREE

MATERIALS AND METHODS

3.0. Materials

The materials used includes universal bottles, incubator (model: GP/50/CLAD/250/HYD), autoclave, hot-air oven (model: GP/50/CLAD/250/HYD),Cut glass slides, forceps, UV-visible spectrophotometer (LABTECH-2805), spirit lamp, centrifuge (model: 80-2) micro pipette, measuring cylinder, lid, microtitre plate, Beam balance (Harvard trip 140/1500 series) and Wire loop. Others include nutrient broth, mannitol salt agar, sterile distilled water, glacial acetic acid, methanol, water, crystal violet and *Staphylococcus aureus* Enterotoxin detection kit (Oxoid Toxin Detection Kit, UK), Abacus 380 Hematology Analyzer (USA), Aibino Wistar rats.

Gene	Primer sequence	Size	Ref
MECA	F: 5 “GTA GAA ATG ACT GAA CGT CCG ATA A 3" R: 5" CCA ATT CCA CAT TGT TTC GGT CTA A-3"	293 bp	
FemA	F CGA TCC ATA TTT ACC ATA TCA R ATC ACG CTC TTC GTT TAG TT	450 bp	
mecA	F ACG AGT AGA TGC TCA ATA TAA R CTT AGT TCT TTA GAG ATT GA	293bp	
16S rRNA	F: 5" AAC TCT GTT ATT AGG GAA GAA CA-3"		

R: 5" CCA CCT TCC GGT TTGTCA CC-
3"

3.1 Methods

Collection of plant materials

Garlic bulbs and fresh rhizomes of ginger and turmeric were purchased from Oba market, Benin City during the month of November, 2015. The plants were identified by Dr. (Mrs) M. Ilondu in the Department of Botany, Delta State University, Abraka.

3.2. Sample size/collection

A purposive sampling technique was used to collect samples from clinically diagnosed pneumonia patients over a period of six (6) months (April – September 2015)from fifteen (15) health institutions (ranging from primary to tertiary) in South-South Nigeria. Subjects were instructed to deposit sputum into sterile universal bottles after they have been duly informed about the purpose of the study and their consent obtained through the assistance of Medical laboratory scientists. The Samples were then cultured using selective medium for *Staphylococci* (i.e., Mannitol salt agar).

3.3. Ethical approval

Ethical approval was granted by the ethical committee body before collecting samples (Sputa) from clinically diagnosed pneumonia patients.

3.4. Identification of *Staphylococcus aureus*

. All Gram-positive cocci isolates that were in clusters and that fermented mannitol were subjected to the following standard characterization tests for *Staphylococcus aureus*.DNase, catalase and coagulase (Cheesbrough, 2006).

DNase test: The DNase test was also used to identify the isolates. This test was performed on DNase agar plates (Koneman, 2005). DNase is an enzyme produced by *S. aureus* that cleaves DNA. The DNA present in the agar is hydrolysed by DNase if this enzyme is produced by the organism. After incubation of the DNA agar plate, the plate was flooded with 1M hydrochloric acid (HCl), which precipitates any unhydrolysedDNA, producing cloudiness. A zone of clearance is visible where the DNA has been hydrolysed. The plate was then incubated aerobically overnight at 37°C. A zone of clearing around the inoculum was taken as a positive result. *Staphylococcus aureus* is DNase positive.

Coagulase test (Slide method): Few drops of physiological saline were dropped on a clean slide and a colony of the test organism was emulsified on the slide using a sterile wire loop. A drop of plasma was dropped on the smear and mixed using an applicator stick, then clumping was checked for within 10 secs.

Catalase test: Exactly 3 ml of hydrogen peroxide was poured into a sterile test tube, using a glass rod a colony from the cultured plate was picked into the solution and the presence of bubbles were checked.

3.5 Antibiotic susceptibility testing

The antibiotic sensitivity testing of the isolates were determined using the Kirby-Bauer disk diffusion method (Bauer *et al.,* 1966) following the definition given by the Clinical And Laboratory Standard Institute (CLSI, 2007), using antibiotics containing disc (Oxoid, UK), 0.2m1 of the standardized test organism was aseptically pipetted into a sterile Petri dish,

thereafter; 19ml of molten Mueller-Hinton agar was then added. A uniform mixed of the test *S. aureus* isolate was done by rocking the petri-dish gently but firmly. The antibiotic disc was gently removed from the cartridge and firmly placed on the agar plate using a sterile pair of forceps. The agar plates were left at room temperature for I hour to allow for diffusion of the antibiotic into the agar medium. The plates were incubated by inverting at 37°C for 24hrs. The zones of inhibition were measured to the nearest millimetre and recorded. The antibiotic discs used were Gentamycin (l0μg);Rifampicin (30μg); Vancomycin (l0μg); Ofloxacin (30μg); Oxacilin(30μg); Erytbromycin (l0μg),Clindamycin, Tetracycline (10μg) because they were the commonly used in the studied zone. Isolates were classified as either resistance or intermediate or sensitive according to the Clinical and Laboratory Standard Institute (CSLI, 2007).

3.6. Plant extraction

The spices were washed, dried and chopped into small bits with the aid of a grater and subsequently divided into two portions of equal weight (20g each). Extraction was carried out by cold maceration method using 70% ethanol and distilled water as extracting solvents. Each portions of chopped plant materials were placed in a beaker containing 100ml 70% ethanol and 100ml distilled water respectively and kept at room temperature for 7days. The supernatant was filtered through a white muslin and the filtrate was concentrated by freeze drying technique. For the ethanol extract, 3.5g of powder was recovered giving a % yield of 17.5% while the aqueous extract gave a yield of 2.9g which gave a % yield of 14.5%.

3.7. Serial dilution of extracts

A 600mg/ml of each extract was prepared using the appropriate solvent. A two-fold serial dilutions was carried for 5 concentration with a5ml pipette into different test-tubes ranging from 300mg/ml to 18.75mg/ml using the dilution formula $C_1V_1 = C_2V_2$. Water and 70% ethanol were used as a negative control for the research carried out.

3.8. Screening for antibacterial activity

The susceptibility of the different *Staphylococcus aureus*isolates to ethanol and aqueous extracts of ginger, turmeric, and garlic was determined using agar well diffusion method. Fourty eighty (48) petri dishes were placed on an already disinfected working bench. A marker pen was used to divide each petri dish into six halves with each petri dish appropriately labeled with respect to the concentration using a masking tape. A 20ml of sterilized Muller-Hinton agar were poured into each of the petri dishes and allowed to solidify.

Each *S. aureus* isolates inoculated on each solidified agar using a swab stick. After which, a well was made on the solidified agar on each petri dish using a sterile 6mm cork borer. With the aid of a sterile 2ml pipette, few drops of each concentration of extract were added to their respective wells in the petri dish. After 15 minutes, all petri dishes were incubated for 24 hours at 37^{o}C. Zones of inhibition were observed and recorded after incubation.

3.9. Determination of Minimum Inhibitory Concentration of the extracts

The minimum inhibitory concentration of each extract was carried out to determine the lowest concentration that can inhibit the visible growth of *Staphylococcus aureus*. This was done using the agar dilution method. An overnight culture in nutrient brothof each organisms were prepared. Twenty one petri dishes were placed on an already disinfected working table and appropriately labeled with respect to the different `*Staphylococcus aureus*isolates. A 1ml of the different concentrations of extract was poured into each of twenty one dishes, sterilized Muller-Hinton agar was asceptically poured into each petri dish to a depth of approximately 4mm containing the extracts to solidify. Upon pouring, petri dishes were rocked properly to achieve an even distribution of the extract.

A sterile inoculating loop was used to pick the *Staphylococcus aureus*isolates from the broth to inoculate each medium representing a particular concentration of the extract with the

organism as labeled and 15minutes after inoculation, the petri dishes were carefully packed and place in the incubator at a temperature of 37^{o}C. After 24hours of incubation, observation for growth was made and recorded.

Control tests

Distilled water and 70% ethanol were used as negative control for the research carried out. A positive control test result for rifampicin was employed for comparism with results from the research.

3.9.1 Biofilm detection (crystal violet binding assay)

Nutrient broth (25ml) was measured and poured into different sterilized universal bottles. The cut glass slides were picked aseptically using forceps and four were dropped into each of the universal bottle containing nutrient broth. The universal bottles containing the broth were inoculated with *Staphylococcus aureus* isolated from different locations and labeled accordingly. One sterile universal bottle containing nutrient broth was not inoculated with *Staphylococcus aureus*,this served as a control. The inoculated broths were incubated for 24 hours at 37^{o}C. After 24 hours incubation, the set of cut glass slides were aseptically removed from the broth culture for biofilm quantification. Each set of glass slide was washed three times with 5ml sterile distilled water and then fixed with 3ml methanol per slide. Each glass slide was stained with crystal violet for 20minutes and flushed with water under running tap. The glass slides were air dried and resolubilized with 2.5ml of 33% glacial acetic acid in test tubes. The resolubilized liquid was poured into cuvette for determination of absorbance against optical density of blank reading without inoculation of *Staphylococcus aureus* (control) at a wavelength of 620nm using double beam UV-visible spectrophotometer. The absorbance of negative control was subtracted from the absorbance of the resolubilized solution containing *Staphylococcus aureus* to determine the actual absorbance value.

3.9.2. Enterotoxin detection (reverse passive latex agglutination method)

Nutrient broth (5ml) was poured into each of the sterilizeduniversal bottle and inoculated with *Staphylococcus aureus* with the aid of a wire loop. The inoculated broths were incubated for 24hours at 37^{o}C. The centrifuge tubes, microtiter plate, lid and pipette tips were disinfected using disinfectant. The overnight broth culture of *Staphylococcus aureus* was poured into different centrifuge tubes, placed in the sample compartment and centrifuged for 20minutes at 900rpm. After centrifugation, the culture supernatants were poured into different test tubes. The microtiter plate was arranged so that each row consists of 8 wells and each sample needs the use of five rows. Using a micro pipette, 0.25ml of enterotoxins detection diluents was dispensed in each well of the five rows; 0.25ml of test *Staphylococcus aureus* was added to the first well of each of the five rows. Using a micro pipette and starting at the first well of each row, 0.25ml was picked up and doubling dilution was performed along each of the five rows; it was stopped at the 7th well to leave the last well i.e. the 8th well containing diluents only (positive control). To each well in the first row, 0.25ml of latex sensitized with anti-enterotoxin A was added. To each well in the second row, 0.25ml of latex sensitized with anti-enterotoxin B was added. To each well in the third row, 0.25ml of latex sensitized with anti-enterotoxin C was added. To each well in the fourth row, 0.25ml of latex sensitized with anti-enterotoxin D was added. To each well in the fifth row, 0.25ml of latex control was added. The microtiter plate was agitated by hand to mix the content of each well while taking care to ensure that no spillage occurs from the wells. The plates were covered with a lid to avoid evaporation and allowed to stand on a vibration free surface at room temperature for 24hours. After 24hours, each well in each row were examined against a black background for agglutination

3.9.3. Preparation of the tissues for microscopic examination

Prior to tissue examination, the animal model (Wistar rats) used were allowed acclimatization for 1 (one) week. Thereafter, the rats were intra peritoneally administed with the clinical isolates and monitored 10 (ten) days before theywere sacrificed and the lungs carefully harvested.

The process of preparing harvested tissues for histological analysis was separated into the following number of stages: fixation,tissue processing, staining, and photomicrography.

3.9.3.1. Fixation

This is the process of using chemicals to prevent autolysis and putrefaction of tissues thereby maintaining the tissue chemistry and architecture.

They were fixed in 10% formal saline for 48 hours, using plastic cassettes.

3.9.3.2. Tissue processing

The examination of tissues using a microscope usually requires a slice of the tissue thin enough to transmit light. Preparation of such thin slices is called microtomy. The tissues undergone treatment before being sectioned, entailing impregnation of the specimen with embedding medium to provide support and suitable constituency for microtomy. This preparatory treatment is known as “tissue processing”

3.9.3.3. Dehydration

This is the process of removing water. Graded solutions of alcohol are used with concentrations ranging from 70% to 100% if dehydration is not complete, it will lead to poor sectioning.

3.9.3.4. Clearing

This involves removing absolute alcohol and replacing it with a solvent which is miscible with both alcohol and paraffin wax.

3.9.3.5. Embedding

This is the process of burying a tissue in molten paraffin wax. The paraffin becomes solid when it is cold. This forms a solid support for the tissue during microtomy.

3.9.3.6. Sectioning/mounting

Sections of the lungs were cut using a rotary microtome and floated in a hot water bath. The floated sections were picked and mounted on microscopic slide for staning.

3.9.3.7. Staining procedure

The stain used in this study was Haematoxylin and eosin (H&E). The technique included. Dewaxing and dehydration, then staining in Erhlich's haematoxylin. The section tissue was then rinse in water for 15 minutes and differentiated in 1% HCL in 70% alcohol for 1 minute following another rinsed in water befor bluing in tap water for 2 minutes. It was then counter stained with 1% eosin for 1 minute with another rinsed in water and finally dehydrated, cleared and mounted for photomicrogry. (Godwin *et al*., 2010).

3.9.3.8. Photomicrography

The stained tissue images were captured using a digital microscopic eyepiece X 400 magnification.

3.9.4. Clinical Chemistry.

The animals were re-weighed at the end of the 14^{th} day after been infected with *Staphylococcus aureus*. Lithium heparin sample bottles were prepared and labelled according to the samples. Blood was then collected with the aid of a capillary tube. The tip of the capillary tube was inserted into the medial canthus which on reaching the orbital sinus of the eye, was quickly rotated. Blood flow was by capillary action through the capillary tubes which was collected into the already labelled Lithium heparin bottles. Also blood was collected with the aid of syringes and needles into the Lithium heparin sample bottles which was immediately mixed with the anticoagulant to prevent clotting which may affect results.

The Clinical Chemistry was evaluated in terms Potassium, Chloride, Sodium, Urea and Creatinine using Colorimetric Method.

3.9.5. Haematology (Abacus 380 Haematology analyser)

Abacus 380 Haematology analyser which works on the principle of volumetric impendence method and light absorbance for haemoglobin measurement was used to analyse the following haematological parameters: White Blood Cell (WBC) count, Red Blood Cell(RBC) count, Haemoglobin concentration (HGB), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Platelet counts (PLT) and 3-part differential White Blood Cell-Lymphocytes (LYM), Granulocyte (GRA) and Monocytes (MID).

3.9.6 Molecular biology studies

Conventional Polymerase chain reaction (PCR):

DNA Isolation

The whole chromosomal DNA was extracted by boiling according to the method of Zhang*et al.* (2004). The isolates were grown in Nutrient broth at 37^{o}C for 24 hours. Then 1ml of the liquid culture was transferred into 1.5 ml volume microfuge tube. Bacteria cells were harvested by centrifugation at 12, 000 g for 5 minutes. The supernatant was discarded and the pellet washed twice with Ultra-pure water and re-suspended in 1 ml Ultra-pure water. The bacteria suspension was boiled for 10 minutes to lyses the cells and releases the DNA followed by a 'cold shock' treatment in ice for 10 minutes. The suspension was then centrifuged at 12,000 g for 5 minutes and the clear supernatant containing the DNA was

transferred to a new microfuge tube and used directly in specific PCR to detect and confirm the genus and species of isolates and the presence of Mec A gene.

Preparation of PCR reaction Mixture:

PCR reaction mixture was prepared in a 25 µl reaction volume, containing 12.5 µL of PCR mix (Promega USA), 9 µLsterile distilled deionized water, 0.25 µL each of the forward and backward primers and 3 µL of DNA.

DNA Amplification:

The isolated DNA was amplified with the primers in the table below.

Gene	Primer sequence	Size
MECA	F: 5 “GTA GAA ATG ACT GAA CGT CCG ATA A 3" R: 5" CCA ATT CCA CAT TGT TTC GGT CTA A-3"	310 bp
FemA	F CGA TCC ATA TTT ACC ATA TCA R ATC ACG CTC TTC GTT TAG TT	450 bp
mecA	F ACG AGT AGA TGC TCA ATA TAA R CTT AGT TCT TTA GAG ATT GA	310 bp
16S rRNA	F: 5" AAC TCT GTT ATT AGG GAA GAA CA-3" R: 5" CCA CCT TCC GGT TTGTCA CC-3"	

MecA1 (5_-GTAGAAATGACTGAA CGTCCGATAA-3_) and MecA2 (5_-CCAATTCCACATTGT TTCGGTCTAA-3_) was used to determine methicillin resistance using MEC A gene. For femA-F CGA TCC ATA TTT ACC ATA TCA femA-R ATC ACG CTC TTC GTT TAG TT (Inqaba Biotechnical Company Pty, South Africa). The PCR was expected to yield a fragments of the expected sizes of, 310 bp and 450bp for the *mecA and Fem* genes

PCR was carried out in a thermal cycler (A & E Laboratories UK, Version 7.0) with the reaction cycles consisting of an initial denaturation 94 ºC for 4 min; 30 cycles of 94 ºC for 30 seconds, 50 ºC for 45 min and 68 ºC for 1 min. A final extension step at 68 ºC was continued for another 10 min. The PCR products were analyzed on 1. 5% agarose gels containing 0.5 µg/mL ethidium bromide and visualized on UV transilluminator (Edvotek, USA).

CHAPTER FOUR

RESULTS

4.0 RESULTS

Table 4.1.The Health Institutions and number of *S. aureus* isolated.

HEALTH INSTITUTIONS	CODES	NO OF SAMPLES	NO OF +VE SAMPLES FOR *S.AUREUS*
Federal Medical Centre Asaba	ASB	120	6
General Hospital Sapele	SAP	95	7
University of Benin Teaching Hospital	UBT	140	9
Irrhua Specialist Hospital	OT	130	7
DESUTH Oghara	OGH	130	8
Central Hospital Benin	CB	95	5
Central Hospital Warri	CW	80	4
Central Hospital Ughelli	CU	97	5

Central Hospital Agbor	CA	100	4
General Hospital Sabongida	GS	77	4
Central Hospital Yenagoa	CY	76	5
Central Hospital Ogwashi-ukwu	CO	65	4
Federal Medical Centre Yenagoa	YEN	107	5
Central Hospital Sagbama	CSA	88	3
Stella Obasanjo Women and Children Hospital Benin	SOH	100	3
	TOTAL =	**1500**	**79**

Key:

Central Hospital Benin = (CB)
Irrhua Specialist Teaching Hospital = (OT)
University of Benin Teaching Hospital = (UBT)
Delta State University Teaching Hospital = (OGH)
Federal Medical Centre Yenagoa = (YEN)
Stella Obasanjo Women and Children Hospital = (SOH)
Central Hospital Yenagoa = (CY)
Federal Medical Centre Asaba = (ASB)
General Hospital Sapele = (SAP)
Central Hospital Ughelli = (CU)
General Hospital Sabongida = (GS)
Central Hospital Ogwashiukwu = (CO)
Central Hospital Agbor = (CA)
Central Hospital Warri = (CW)
Central Hospital Sagbama = (CSA)

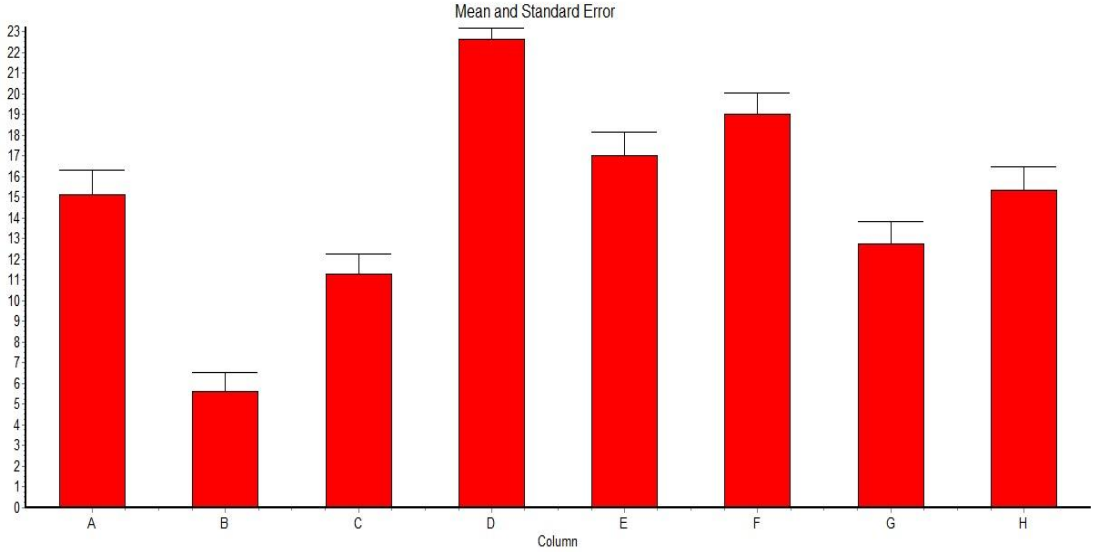


Figure 4.1. A chat of the different antibiotics.

KEY

RD = A **RIFAMPICIN** OX = B **OXACILIN**

VA = C	**VANCOMYCINE**	OFX = F	**OFLOXACIN**
CN = D	**GENTAMYCINE**	E = G	**ERYTHROMYCINE**
DA = E	**CLINDAMYCIN**	TE = H	**TETRACYCLINE**

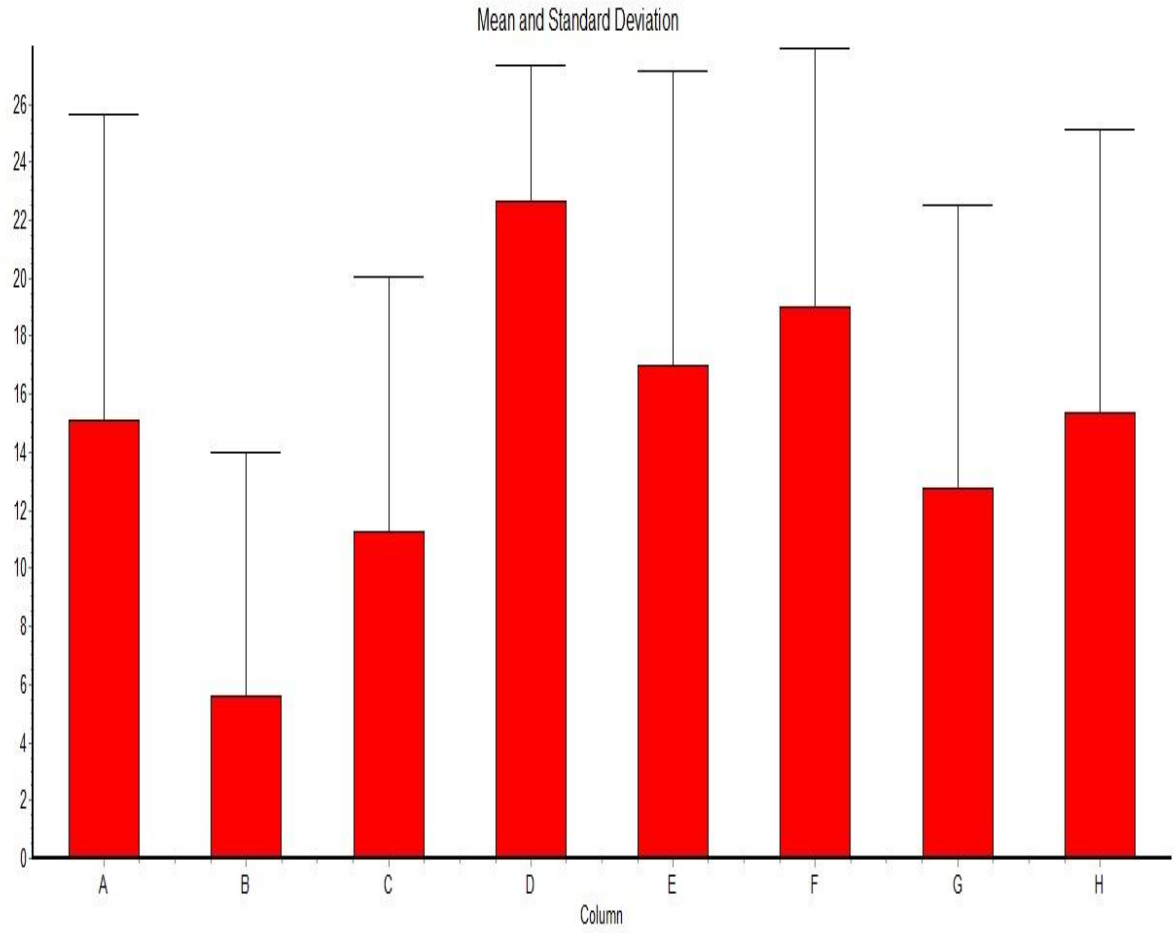


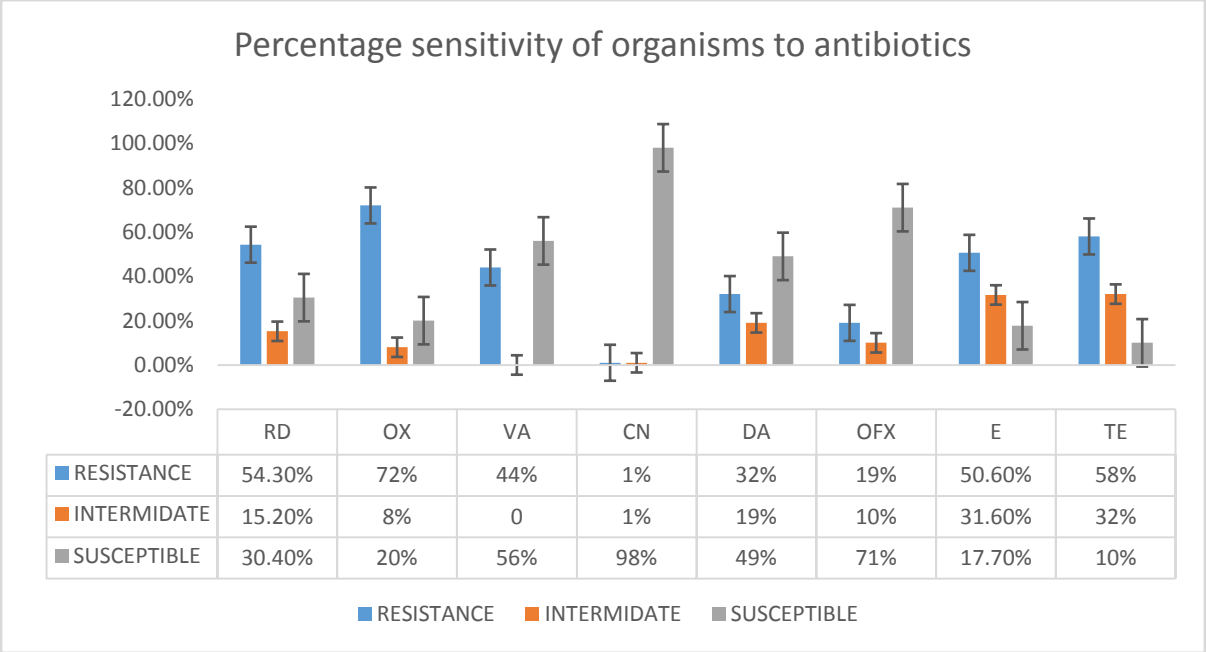
Figure 4.2: A chat on the Mean and Standard of the different antibiotics

KEY

RD = A	**RIFAMPICIN**
OX = B	**OXACILINE**
VA = C	**VANCOMYCINE**
CN = D	**GENTAMYCINE**
DA = E	**CLINDAMYCIN**
OFX = F	**OFLOXACIN**

E = G **ERYTHROMYCINE**

TE = H **TETRACYCLINE**



	RD	OX	VA	CN	DA	OFX	E	TE
RESISTANCE	54.30%	72%	44%	1%	32%	19%	50.60%	58%
INTERMIDATE	15.20%	8%	0	1%	19%	10%	31.60%	32%
SUSCEPTIBLE	30.40%	20%	56%	98%	49%	71%	17.70%	10%

Figure 4.3. Percentage sensitivity of *S. aureus*s to antibiotics

KEY

RD = A Rifampicin
OX = B Oxacillin
VA = C Vancomycine
CN = D Gentamycine
DA = E Clindamycin
OFX = F Ofloxacin
E = G Erythromycine
TE = H Tetracycline

Table 1. Statistical relationship among antibiotics used

	RD	OX	VA	CN	DA	OFX	E	TE
RD	-	S*	NS	S*	NS	NS	NS	NS
OX	-	-	S	S*	S*	S*	S*	S*
VA	-	-	-	S*	S	S*	NS	NS
CN	-	-	-	-	S	NS	S*	S*
DA	-	-	-	-	-	NS	NS	NS
OFX	-	-	-	-	-	-	S*	NS
E	-	-	-	-	-	-	-	NS
TE	-	-	-	-	-	-	-	-

KEY:
- Means not applicable or expressed
S Mean significant difference at P<0.01
S* Means significant difference at P<0.001
NSMeans there is no significant difference at P>0.05

Table 4.2. MIC ranges of the different antibiotics used on the*S. aureus* isolates from the different locations

LOCATION	ERYTHROMYCINE (mg/ml)	TETRACYCLINE (mg/ml)	CIPROFLOXACIN (mg/ml)	OXACILLIN (mg/ml)
ASB	3.75 - 7.5	1.25 - 5	1.25 – 5	ND
SAP	7.5 – 15	1.25 - 10	2.5 – 5	ND
UBTH	7.5 – 15	1.25 - 10	2.5 – 5	ND
OT	3.75 – 30	1.25 - 10	2.5 – 10	1
OGH	3.75 – 30	10	1.25 – 5	1
CB	3.75 – 30	5.0 -10	1.25 – 10	ND
CW	3.75 - 7.5	5	ND	ND
CU	7.5	2.5	ND	ND
CA	3.75 – 30	1.25 - 5	ND	ND
CS	ND	2.5 - 10	ND	ND
CY	7.5 - 15	2.5 - 10	ND	ND
CO	30	1.25	5.0 – 10	ND
YEN	ND	ND	ND	ND
CSA	30	1.25	ND	ND
SOH	ND	ND	1.25	ND

Key:

ND - Not Determine

4.1 Antimicrobial activites of the plant extracts.

TABLE 4.3a: Zone of Inhibition (mm) of Turmeric (*Curcuma longa)* Ethanol Extract against the *S.aureus* isolates

	Concentration(mg/ml)				
SAMPLE	300	150	75	37.5	18.75
CA79	14	9	9	7	6
SOH85	6	6	5	5	4
CSA49	9	7	8	7	3
ASB60	7	6	6	5	4
CO22	8	7	5	4	4
YEN54	7	6	7	4	4
SAP41	9	8	6	5	5
CB4	10	7	7	6	5
CW9	10	10	5	6	4
SAP82	9	8	8	8	6
OGH82	8	7	7	6	4
CU70	14	10	8	6	6
UBT35	9	7	6	4	5
CS41	6	5	5	4	3
OT87	4	4	3	3	2
CY79	10	9	8	8	7

TABLE 4.3b: Zone of Inhibition (mm) of Garlic (*Allium sativum)* Ethanol Extract against the *S.aureus*isolates

	Concentration(mg/ml)				
SAMPLE	300	150	75	37.5	18.75
CA79	3	5	nz	nz	nz
SOH85	5	3	2	nz	nz
CSA49	nz	nz	nz	nz	nz
ASB60	6	4	4	3	nz
CO22	6	nz	nz	nz	nz
YEN54	nz	nz	nz	nz	nz
SAP41	5	3	4	4	2
CB4	nz	nz	nz	nz	nz
CW9	nz	nz	nz	nz	nz
SAP82	nz	nz	nz	nz	nz
OGH82	nz	nz	nz	nz	nz
CU70	nz	nz	nz	nz	nz
UBT35	6	4	5	2	3
CS41	4	5	5	4	4
OT87	5	4	4	5	3
CY79	nz	nz	nz	nz	nz

Key:

nz no zone of inhibition

TABLE 4.3c: Zone of Inhibition (mm) of Ginger *(Zingiber officinale)* Ethanol Extract against the *S.aureus*isolates

	Concentration(mg/ml)				
SAMPLE	300	150	75	37.5	18.75
CA79	2	4	5	6	5
SOH85	nz	nz	nz	nz	nz
CSA49	3	3	4	4	3
ASB60	3	4	5	8	8
CO22	4	3	4	2	2
YEN54	nz	nz	nz	nz	nz
SAP41	6	5	3	2	3
CB4	nz	nz	nz	nz	nz
CW9	4	4	3	4	3
SAP82	nz	nz	nz	nz	nz
OGH82	9	6	6	5	5
CU70	8	7	6	4	5
UBT35	nz	nz	6	5	4
CS41	6	4	7	8	6
OT87	nz	nz	4	4	6
CY79	nz	5	4	6	3

Table 4.3d: Zone of inhibition (mm) of oxacillin against *S.aureus*isolates as control

	ZONE OF INHIBITION(mm)			
SAMPLE	1^{ST}	2^{ND}	3^{RD}	AVERAGE ±SD
CA79	10	9	8	9 ± 1
SOH85	11	11	10	10.7 ± 0.58
CSA49	0	0	0	0
ASB60	27	25	26	26 ± 1
CO22	12	12	12	12 ± 0
YEN54	32	33	32	32.3 ± 0.58
SAP41	16	16	15	15.7 ± 0.58
CB4	10	10	10	10 ± 0
CW9	12	12	13	12.3 ± 0.58
SAP82	8	8	8	8 ± 0
OGH82	32	33	31	32 ± 1
CU70	12	12	13	12.3 ± 0.58
UBT35	0	0	0	0
CS41	0	0	0	0
OT87	19	18	18	18.3 ± O.58
CY79	8	8	8	8 ± 0

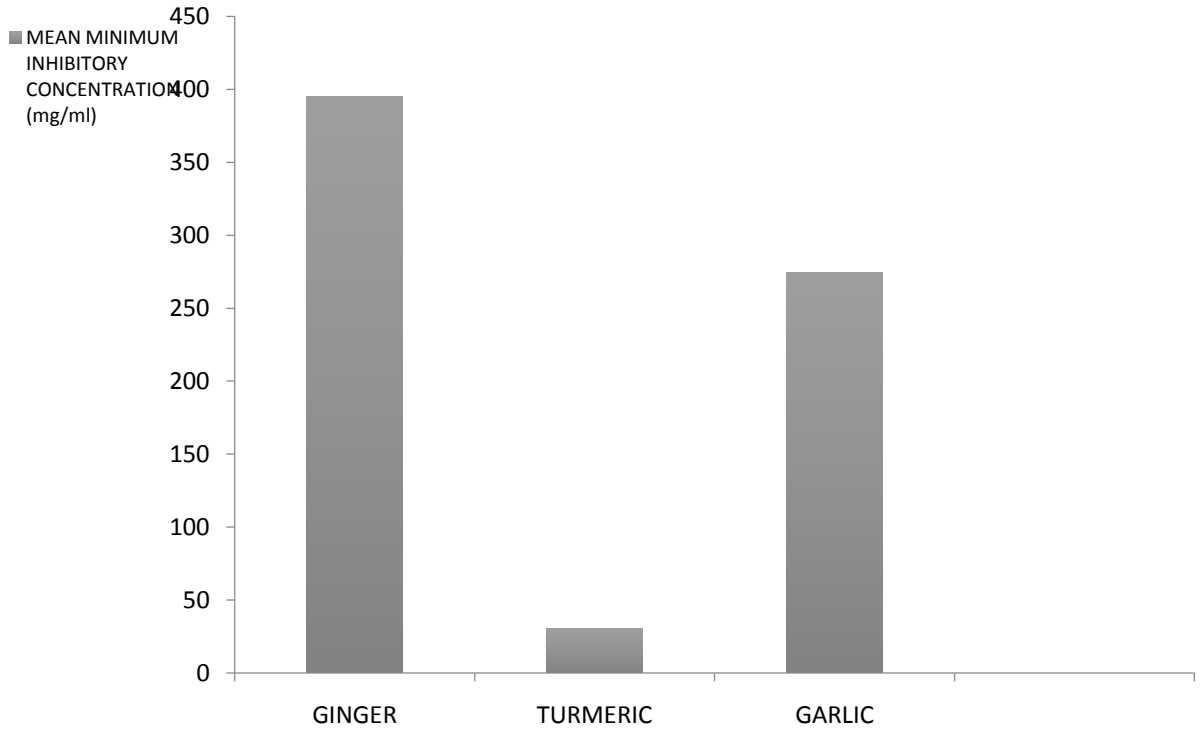


Fig 4.4: Bar chart showing the difference in the mean minimum inhibition concentration of the extracts.

4.2. Biofilm Detetion (Crystal violent binding assay)

Table 4.4 Biofilm formation by *Staphylococcus aureus* from different location.

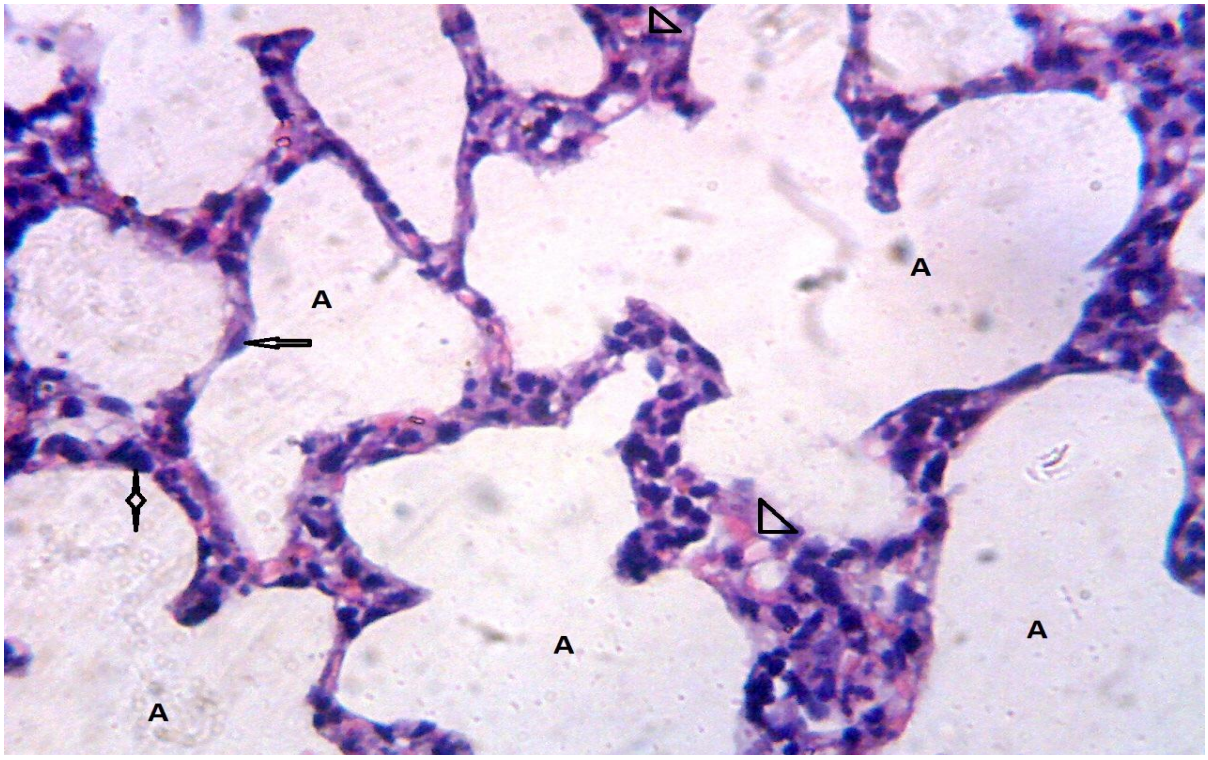
S/N	Sample Location	Total Thickness of Biofilm at each Location	Mean Thickness	Standard Deviation (S.D)	Percentage (%) Thickness
1	Central Hospital Benin	1.359	0.34	0.08	7.81
2	Irrhua Specialist Teaching Hospital	2.322	0.331	0.1	7.6
3	University of Benin Teaching Hospital	2.51	0.279	0.05	6.41
4	Delta State University Teaching Hospital	2.745	0.343	0.08	7.9
5	Federal Medical Centre Yenagoa	1.646	0.329	0.09	7.56
6	Stella Obasanjo Women and Children Hospital	1.432	0.358	0.06	8.22
7	Central Hospital Yenagoa	1.307	0.261	0.09	6
8	Federal Medical Centre Asaba	1.494	0.249	0.06	5.72
9	General Hospital Sapele	2.11	0.301	0.06	6.91
10	Central Hospital Ughelli	1.162	0.232	0.03	5.33
11	Central Hospital Sapele	1.36	0.34	0.08	7.81
12	Central Hospital Ogwashi-ukwu	1.197	0.299	0.04	6.91
13	Central Hospital Agbor	0.851	0.212	0.06	4.91
14	Central Hospital Warri	0.847	0.211	0.07	4.9
15	Central Hospital Sagbama	0.797	0.265	0.02	6

4.3. Enterotoxin detection studies

TABLE 4.5: *Staphylococcusaureus* enterotoxins produced by the clinical isolates.

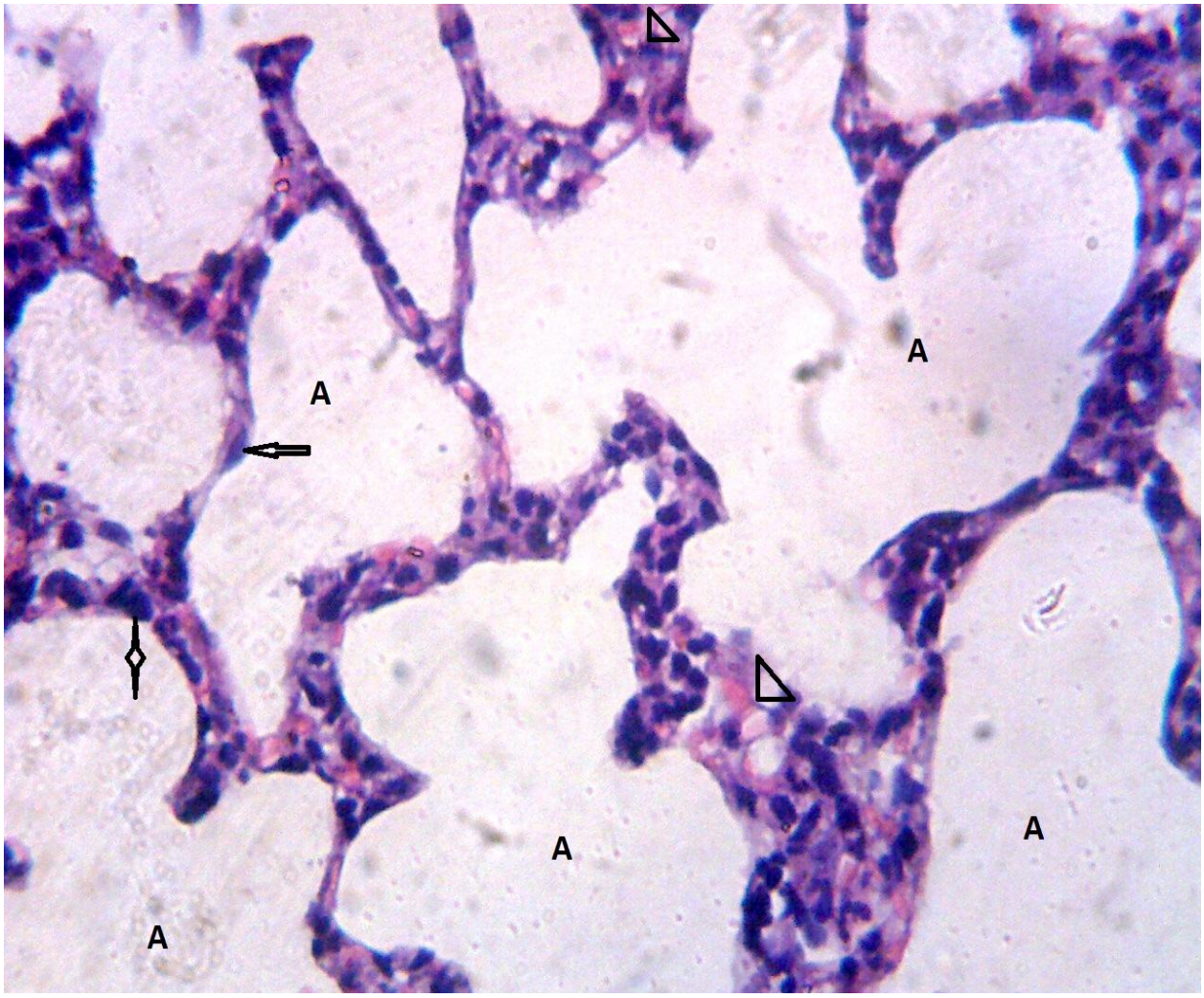
S/N	**Sample Location**	**Sample/location**	**Type of Enterotoxins**
1	Federal Medical Centre Asaba	6	Enterotoxin B Positive
2	General Hospital Sapele	7	Enterotoxin B Positive
3	University of Benin Teaching Hospital	9	Enterotoxin B Positive
4	Irrhua Specialist Hospital	7	Enterotoxin C Positive
5	DESUTH Oghara	8	Enterotoxin B Positive
6	Central Hospital Benin	5	Enterotoxin B Positive
7	Central Hospital Warri	4	Enterotoxin B Positive
8	Central Hospital Ughelli	5	Enterotoxin B Positive
9	Central Hospital Agbor	4	Enterotoxin B Positive
10	Central Hospital Sapele	4	Enterotoxin B Positive
11	Central Hospital Yenagoa	5	Enterotoxin B Positive
12	Central Hospital Ogwashi-ukwu	4	Enterotoxin B Positive
13	Federal Medical Centre Yenagoa	5	Enterotoxin B Positive
14	Central Hospital Sagbama	3	Enterotoxin B Positive
15	Stella Obasanjo Women and Children Hospital	3	Enterotoxin B Positive

4.4. Results of Histological pathology studies



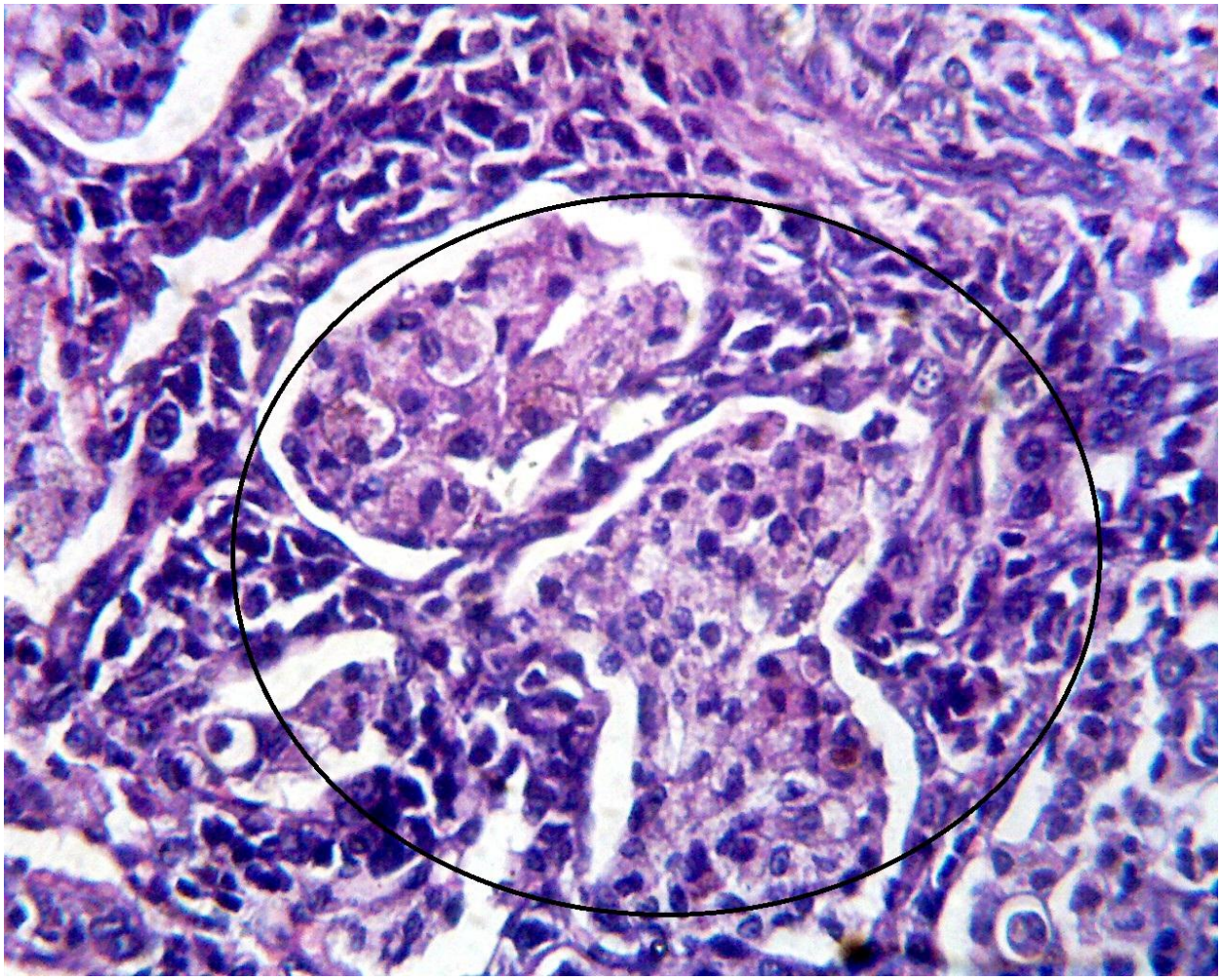
CONTROL 1 X400 HE TECHNIQUE

Figure 4: Micrograph shows the lung tissue consisting the alveoli (A) lined type I pnuemocytes (arrow), type ii pnuemocytes (Star), the blood capillaries lies within the Interstitium and are free from congestion and inflammatory cells



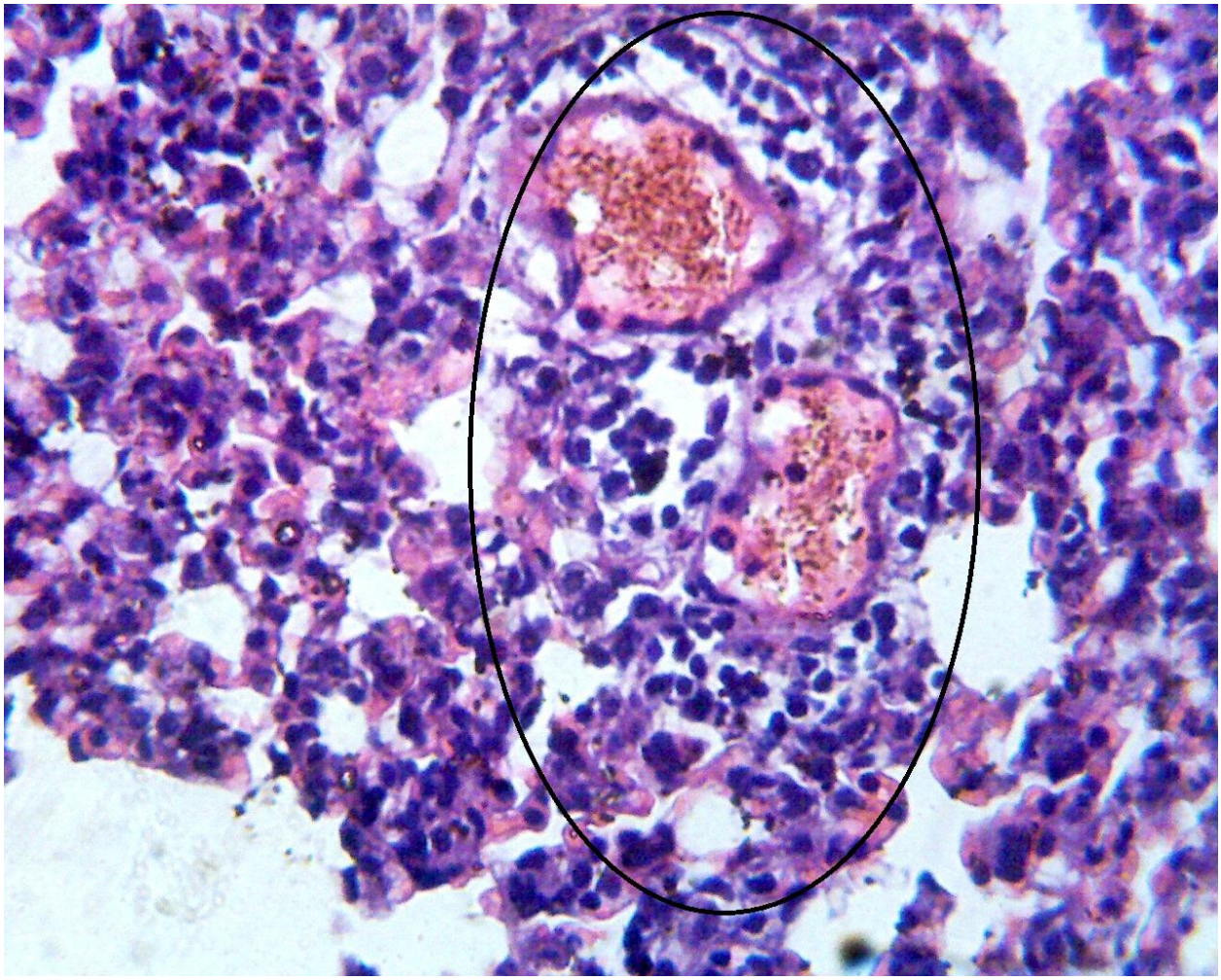
CONTROL 2 X400 HE TECHNIQUE

Figure 5: Micrograph shows the lung tissue consisting the alveoli (A)lined type I pnuemocytes (arrow), type ii pnuemocytes (Star), the blood capillaries lies within the Interstitium and are free from congestion and inflammatory cells



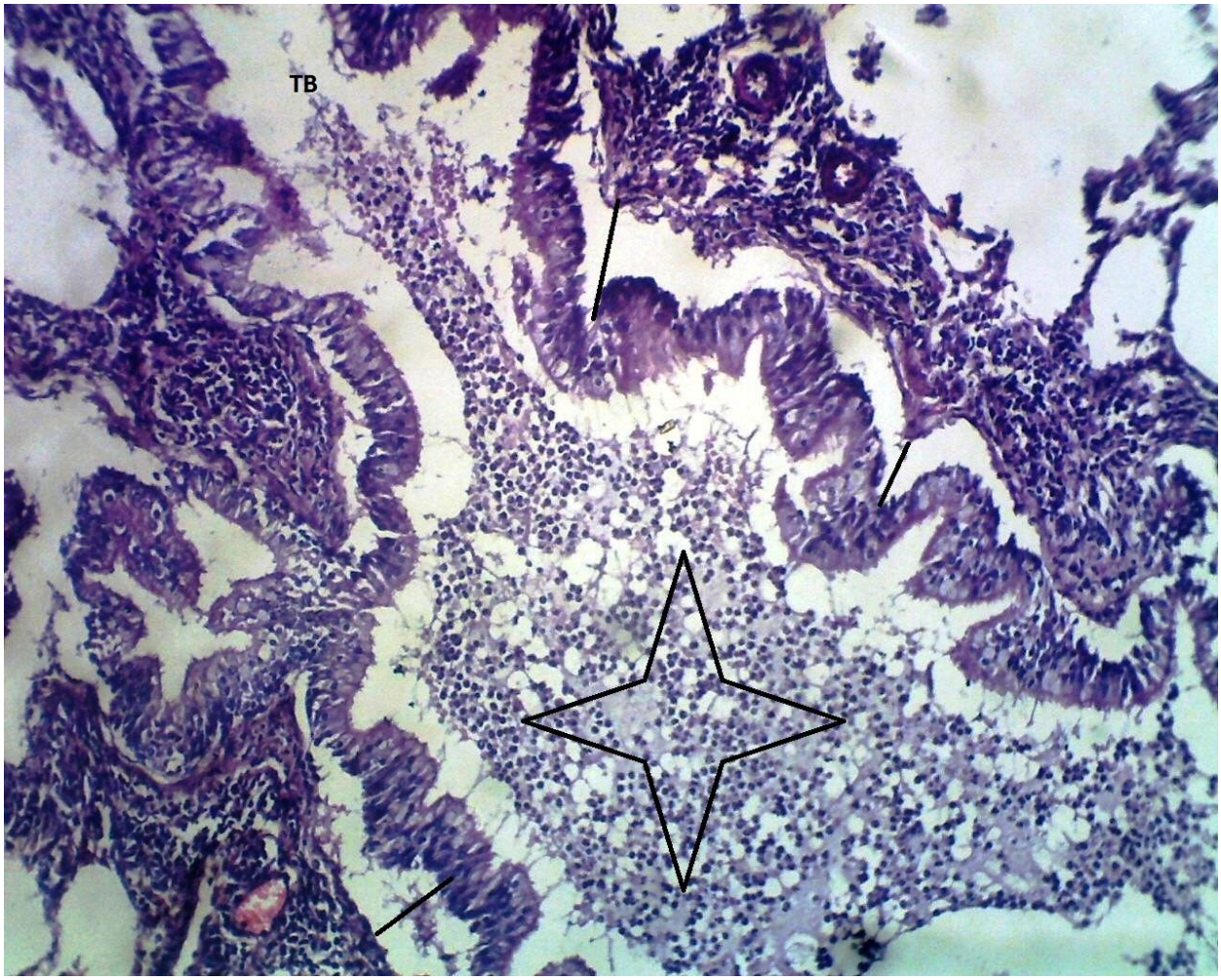
X400 HE TECHNIQUE

Figure 6: Micrograph shows marked infiltration of the alveoli with inflammatory cells (circle) and marked interstitial infiltrations with polymorphs caused by OGH 12



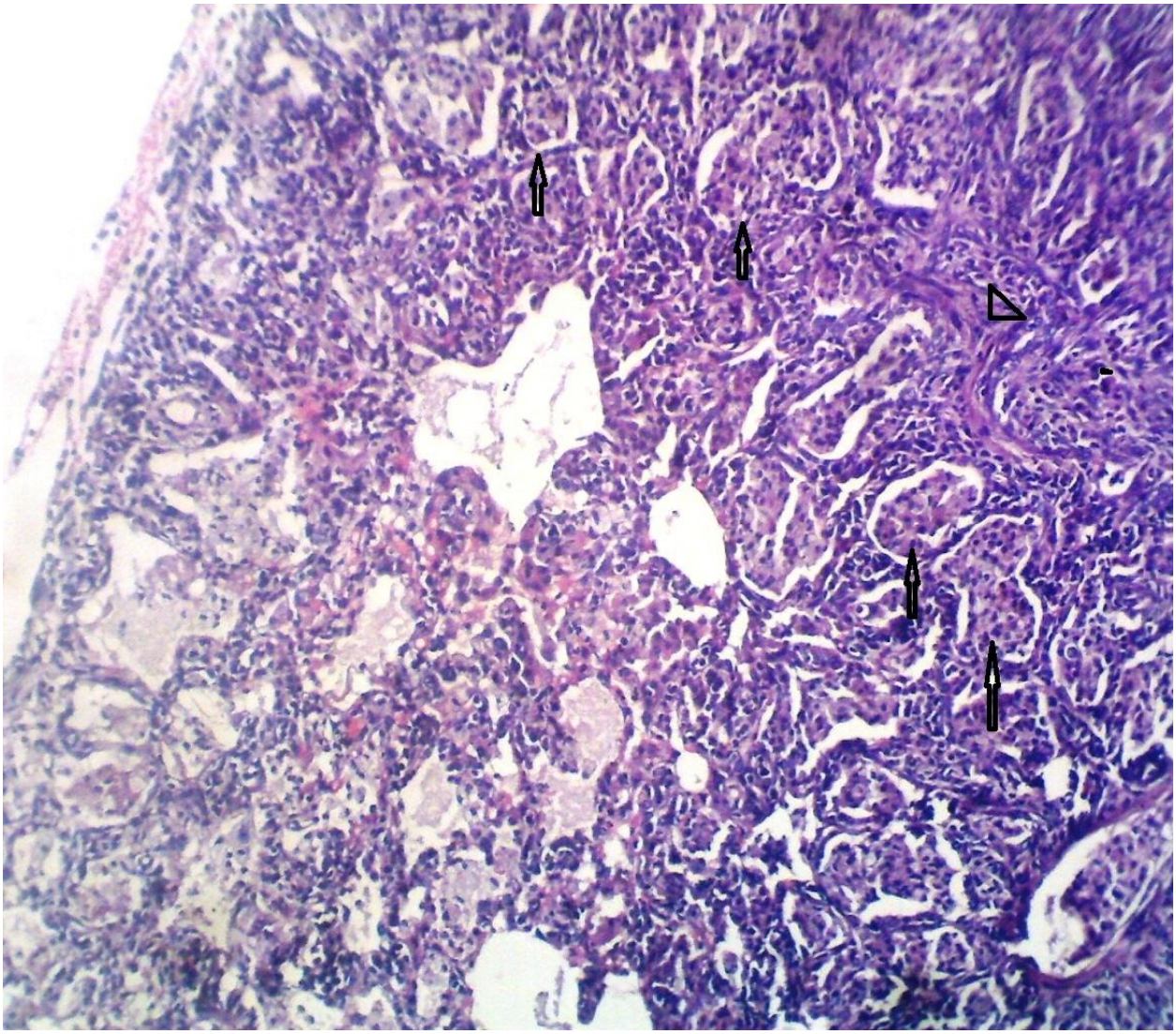
X400 HE TECHNIQUE

Figure 7: Photomicrograph shows marked perivascular and interstitial congestion and infiltration of inflammatory cells (Circle) caused by OGH 47



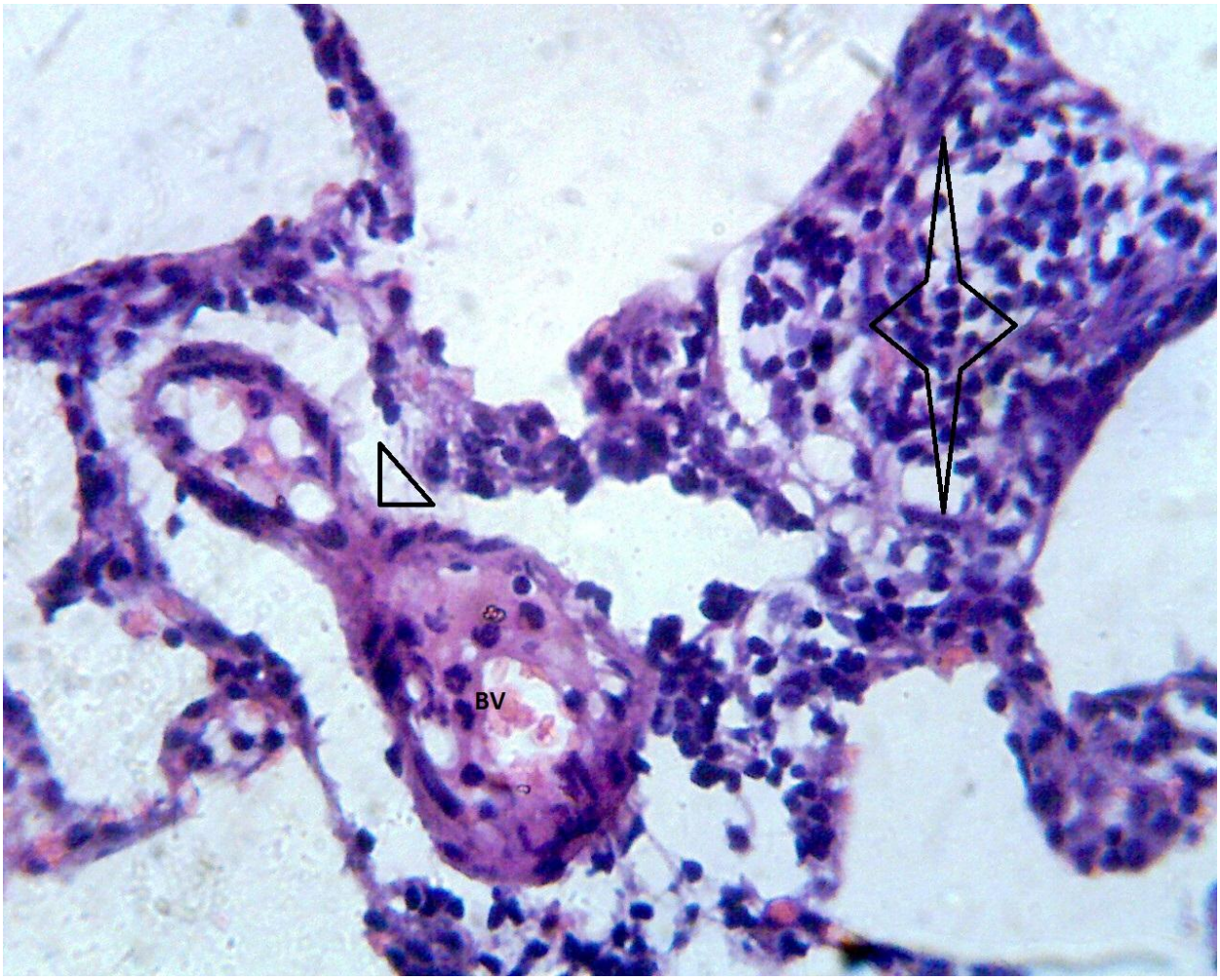
X400 HE TECHNIQUE

Figure 8: Photomicrograph shows marked infiltration of the terminal bronchiole with lymphocytes (Star) and sloughing of the epithelium caused by OGH 82



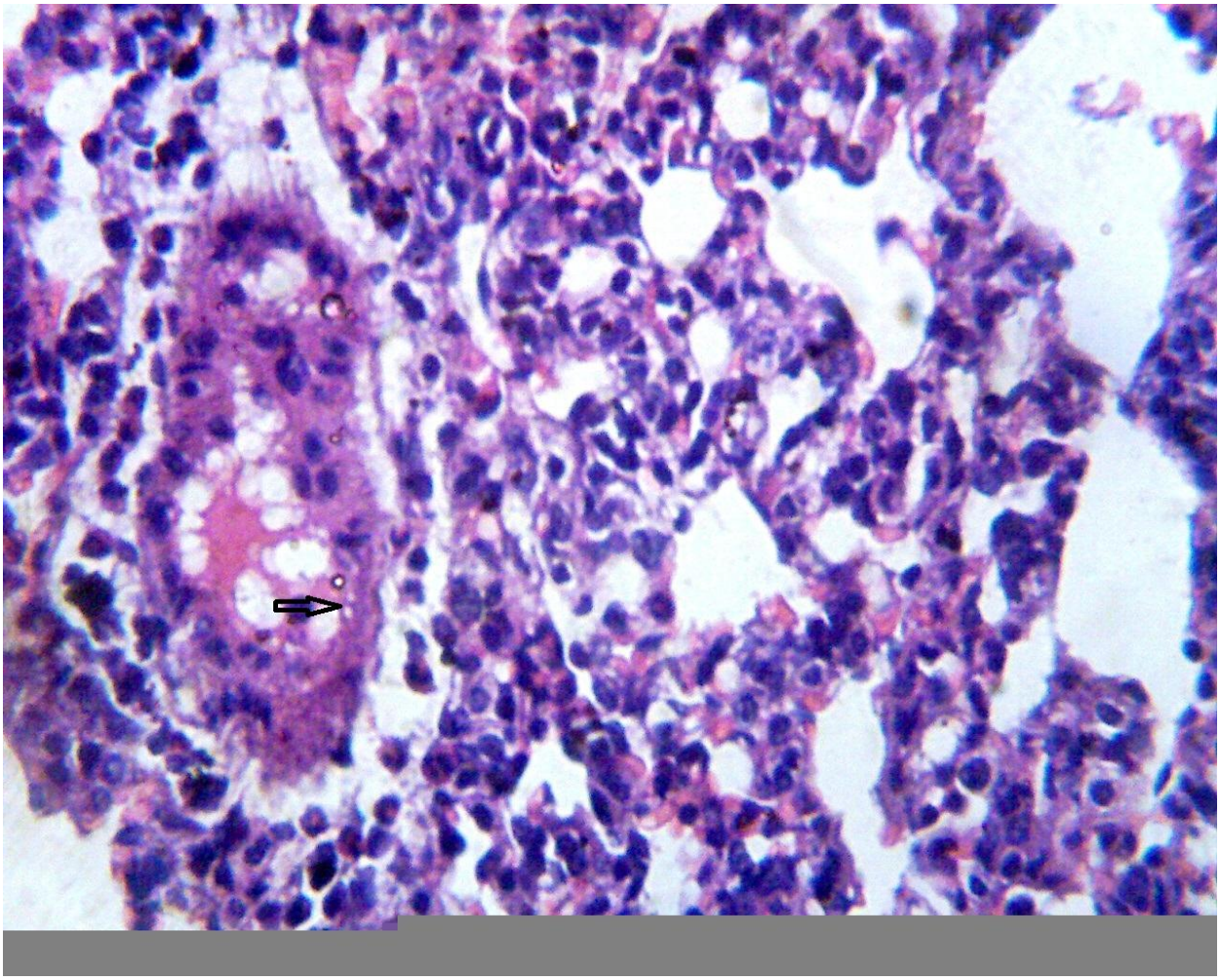
X400 H E TECHNIQUE

Figure 9: Section shows marked pnuemonitis (arrow) and fibrosis (Arrow head) caused by OGH 95



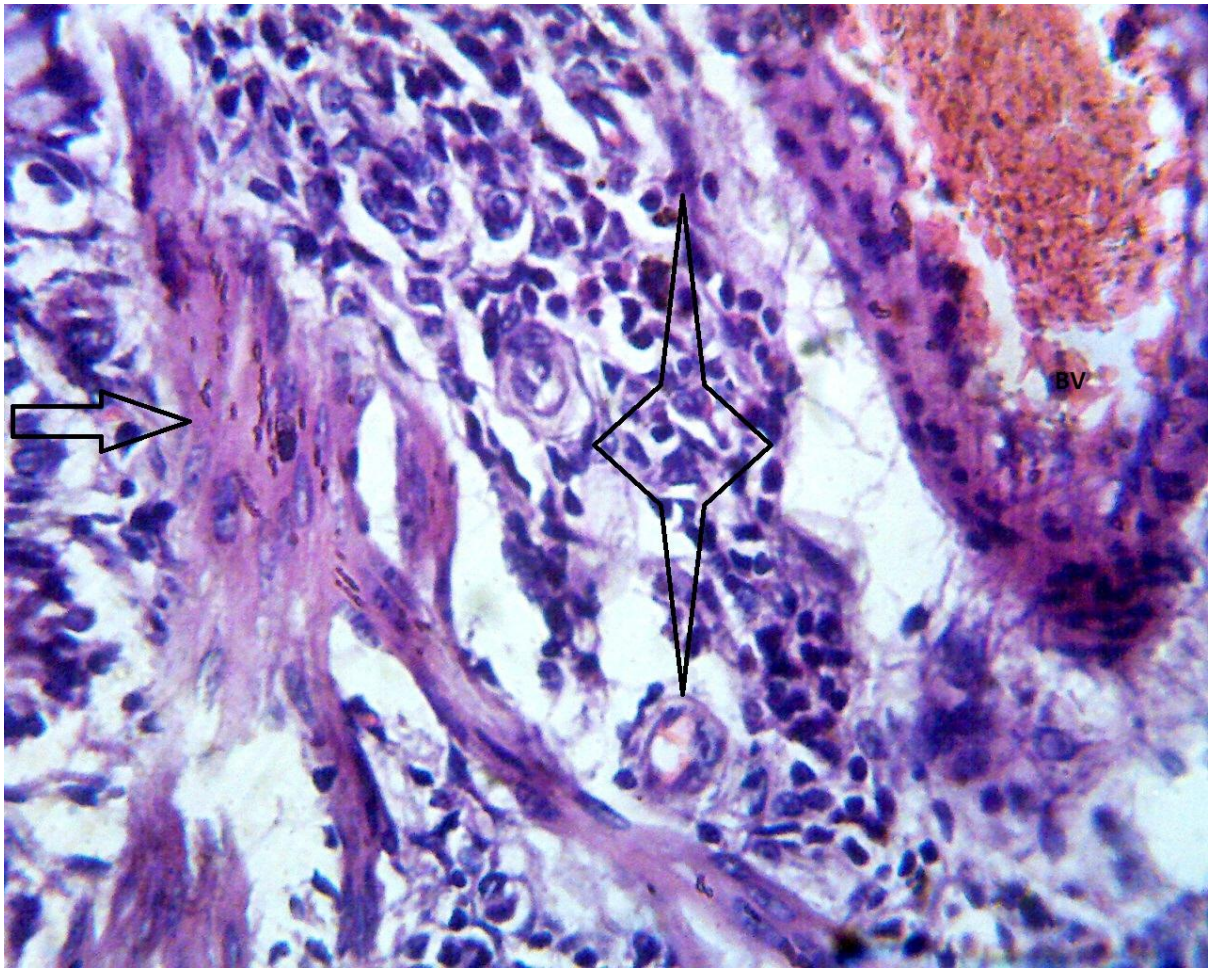
X400 HE TECHNIQUE

Figure 10: Photomicrograph shows marked interstitial pneumonia (Star) and stenosis of the blood vessel (Arrow head) caused by UBT 6



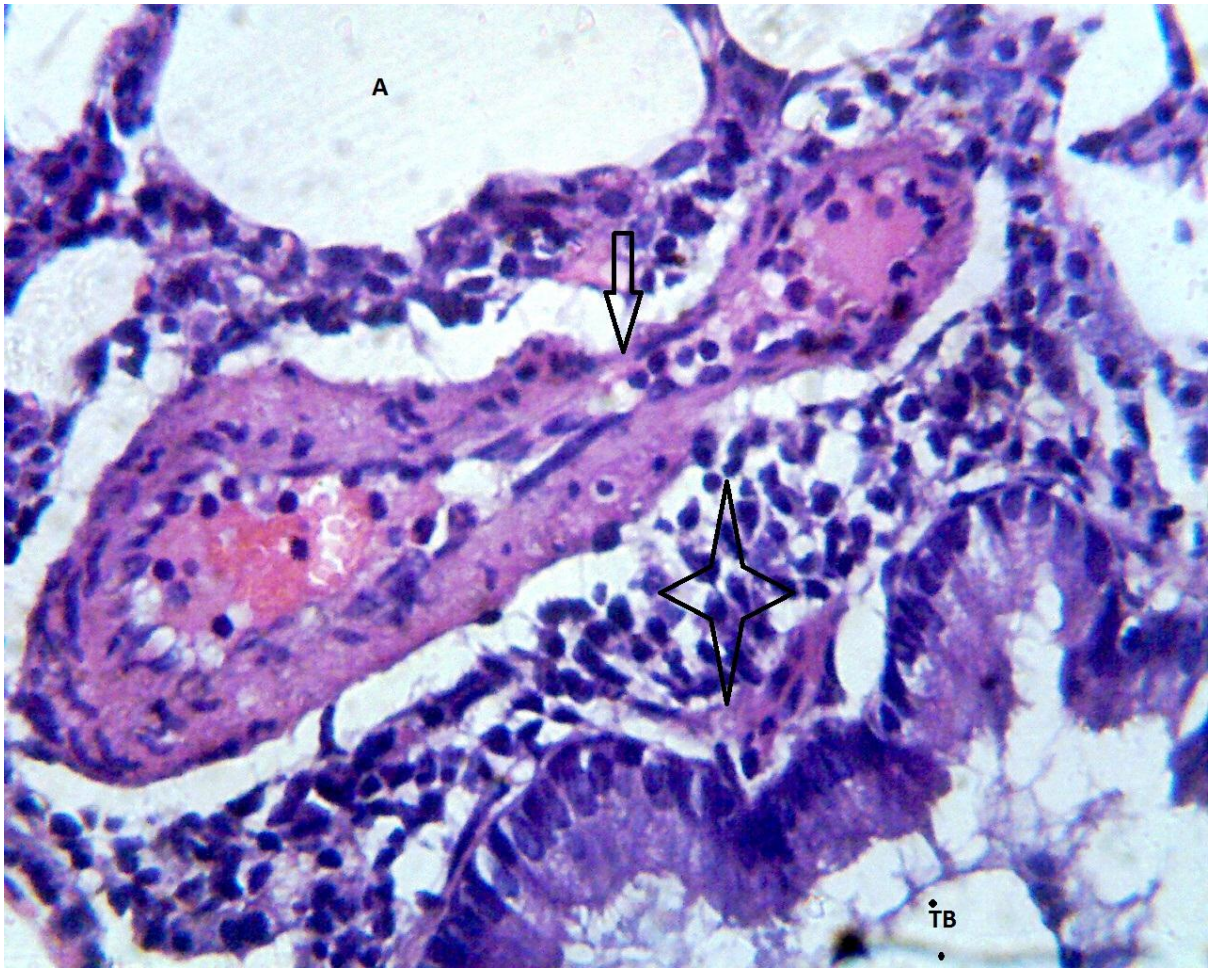
X400 HE TECHNIQUE

Figure 11: Photomicrograph shows marked interstitial pnuemonitis and intimal erosion (arrow) caused by UBT 36.



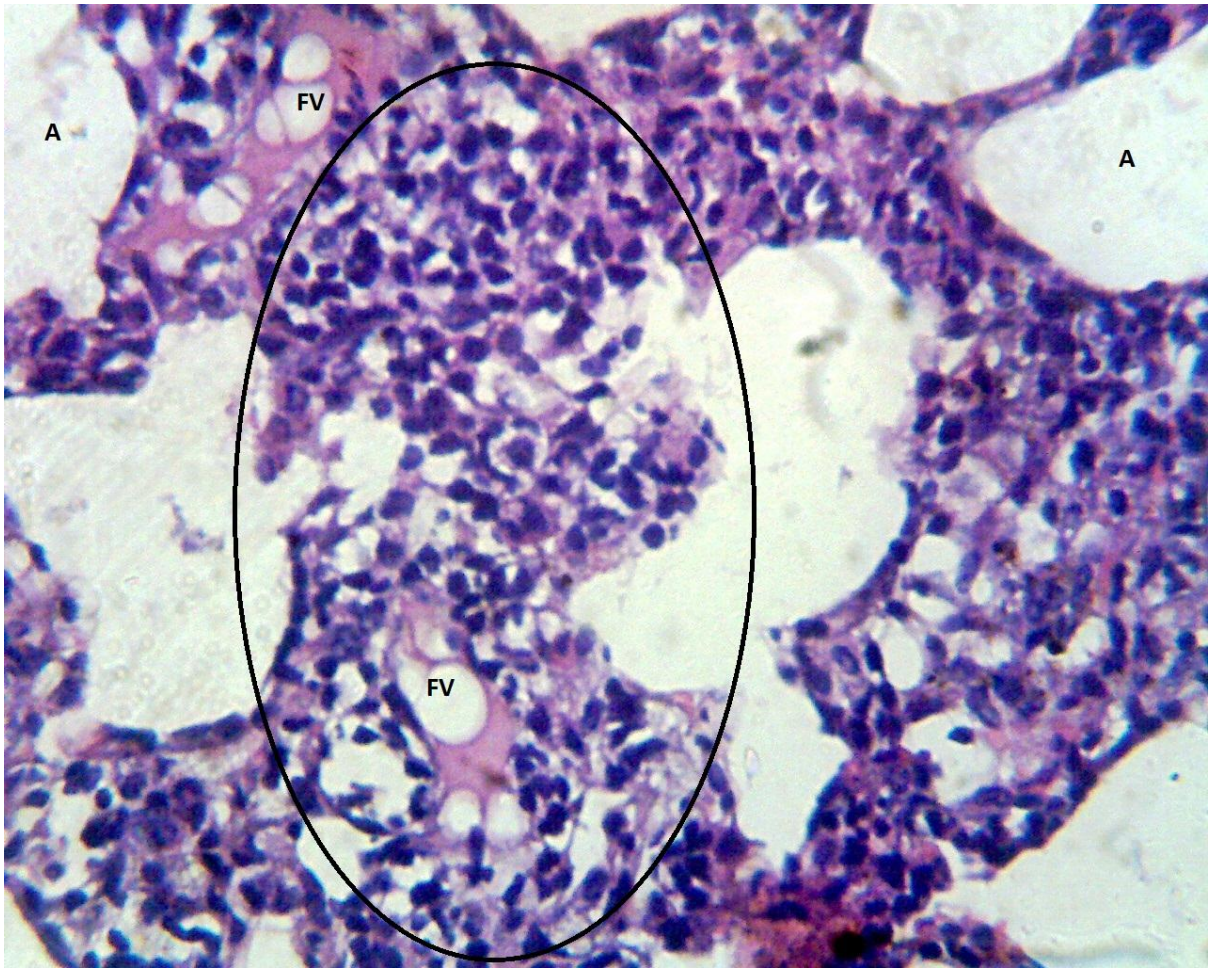
X400 HE TECHNIQUE

Figure 12: Photomicrograph shows marked infiltration of inflammatory cells (Star) and fibrosis (Arrow) caused by UBT 46.



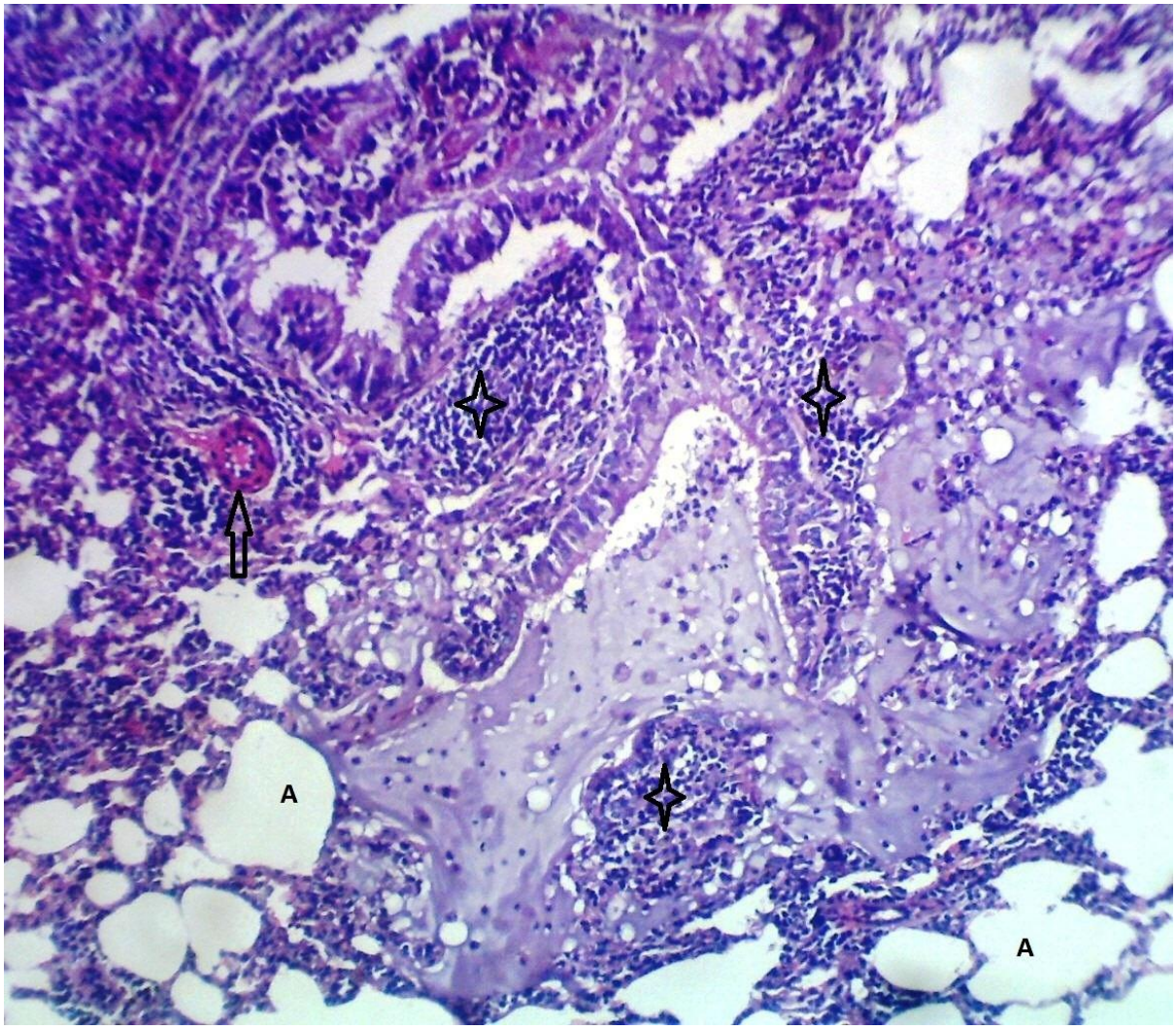
UBT 69 X400 HE TECHNIQUE

Figure 13: Photomicrograph shows perivascular infiltrations (Star) and stenosis of blood vessel (arrow). Terminal bronchiole (TB) and alveoli (A) are not remarkable caused by UBT 69



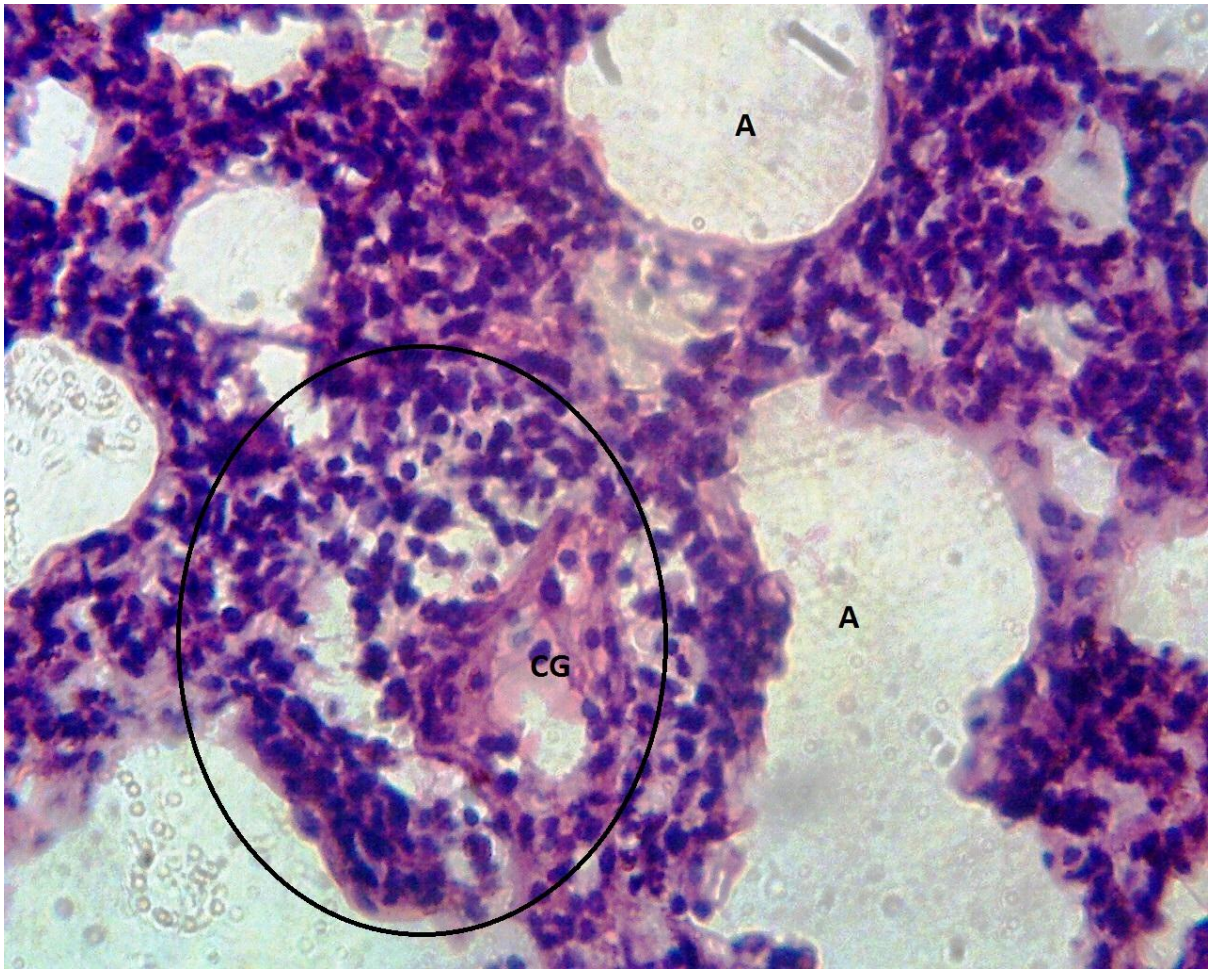
UBT 77 X400 HE TECHNIQUE

Figure 14: Photomicrograph shows interstitial pnuemonitis (circle) and fat vacuole within the capillaries (FV) caused by UBT 77.



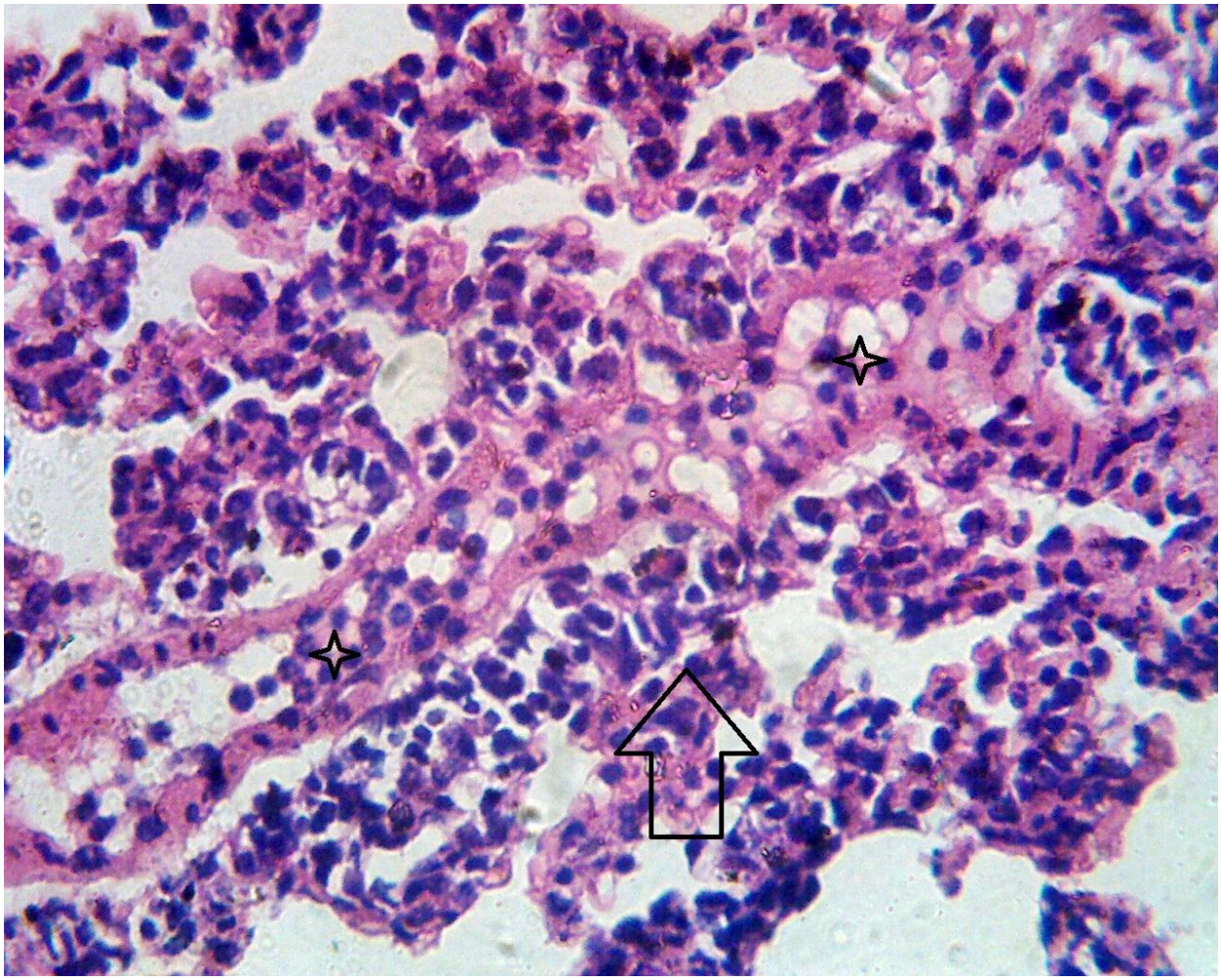
UBT 93 X400 HE TECHNIQUE

Figure 15: Section shows marked interstitial pneumonia (star) and thickening of blood vessel (BV) caused by UBT 93.



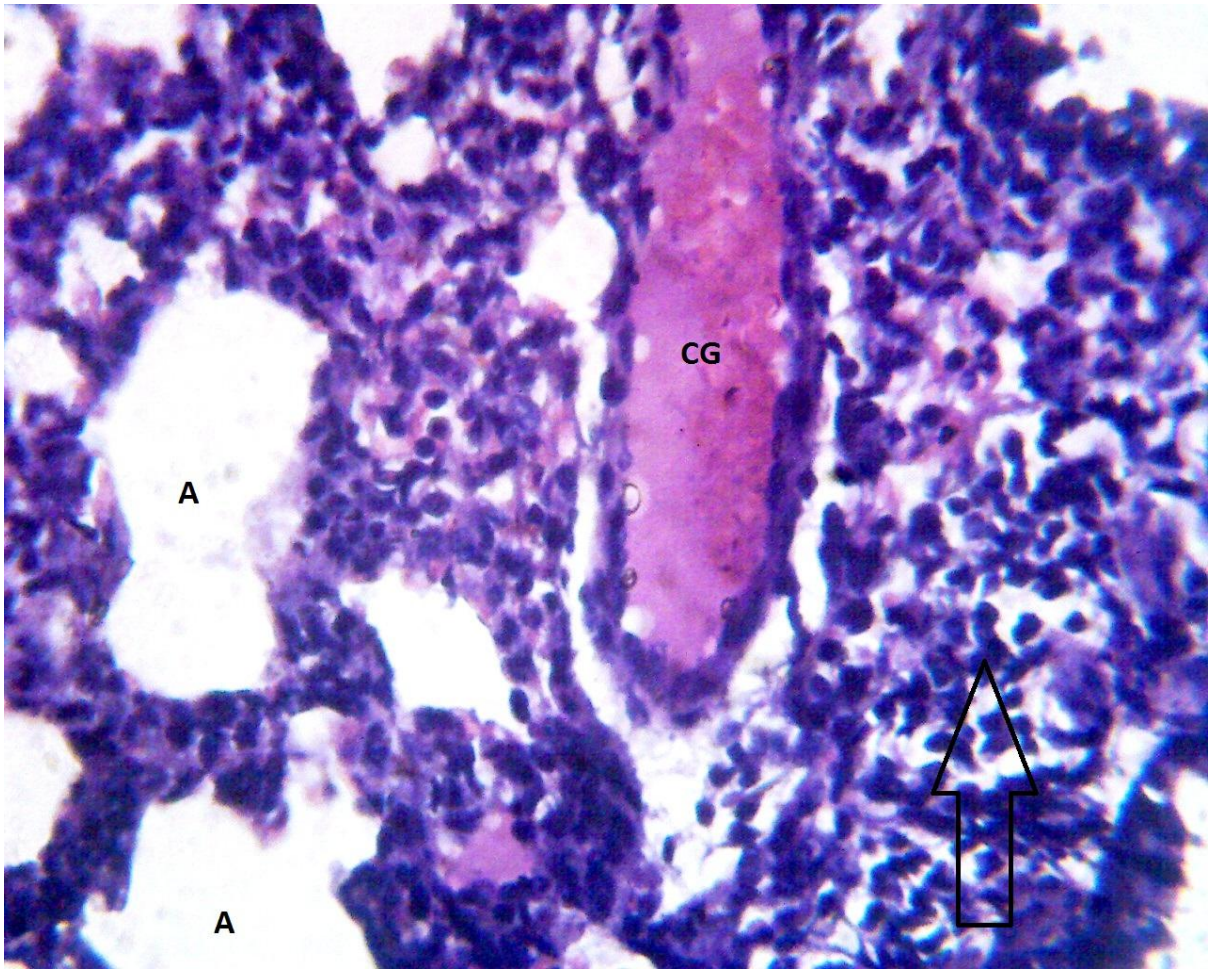
X400 HE TECHNIQUE

Figure 16: **Photomicrograph section of the lungs tissue with moderate perivascular infiltration by inflammatory cells (Circle) also seen is mild vascular congestion (CG) caused by CO 73.**



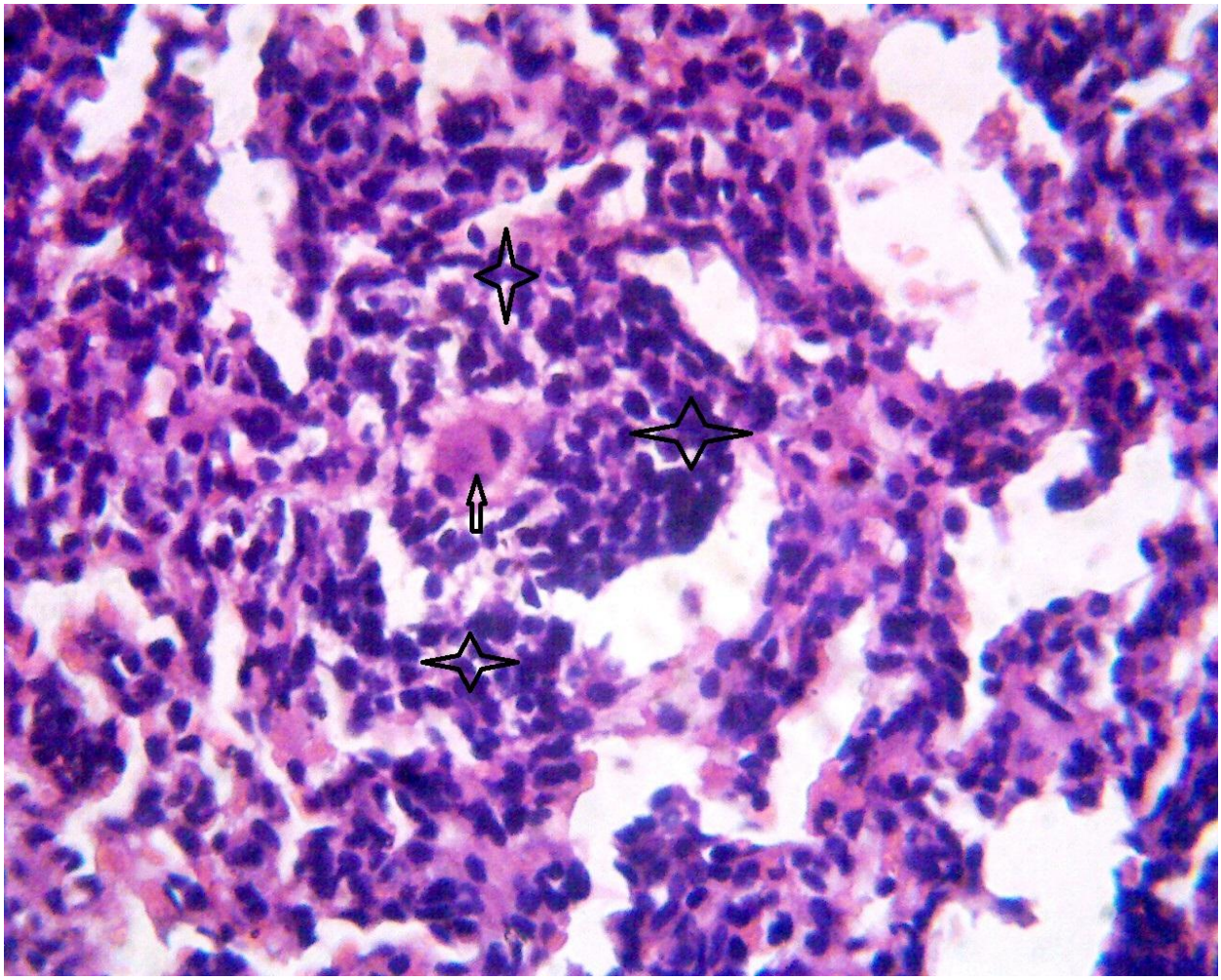
CS 29 X400 HE TECHNIQUE

Figure 17: Photomicrograph shows vascular (star) perivascular leucocytosis (Arrow) caused by CS 29.



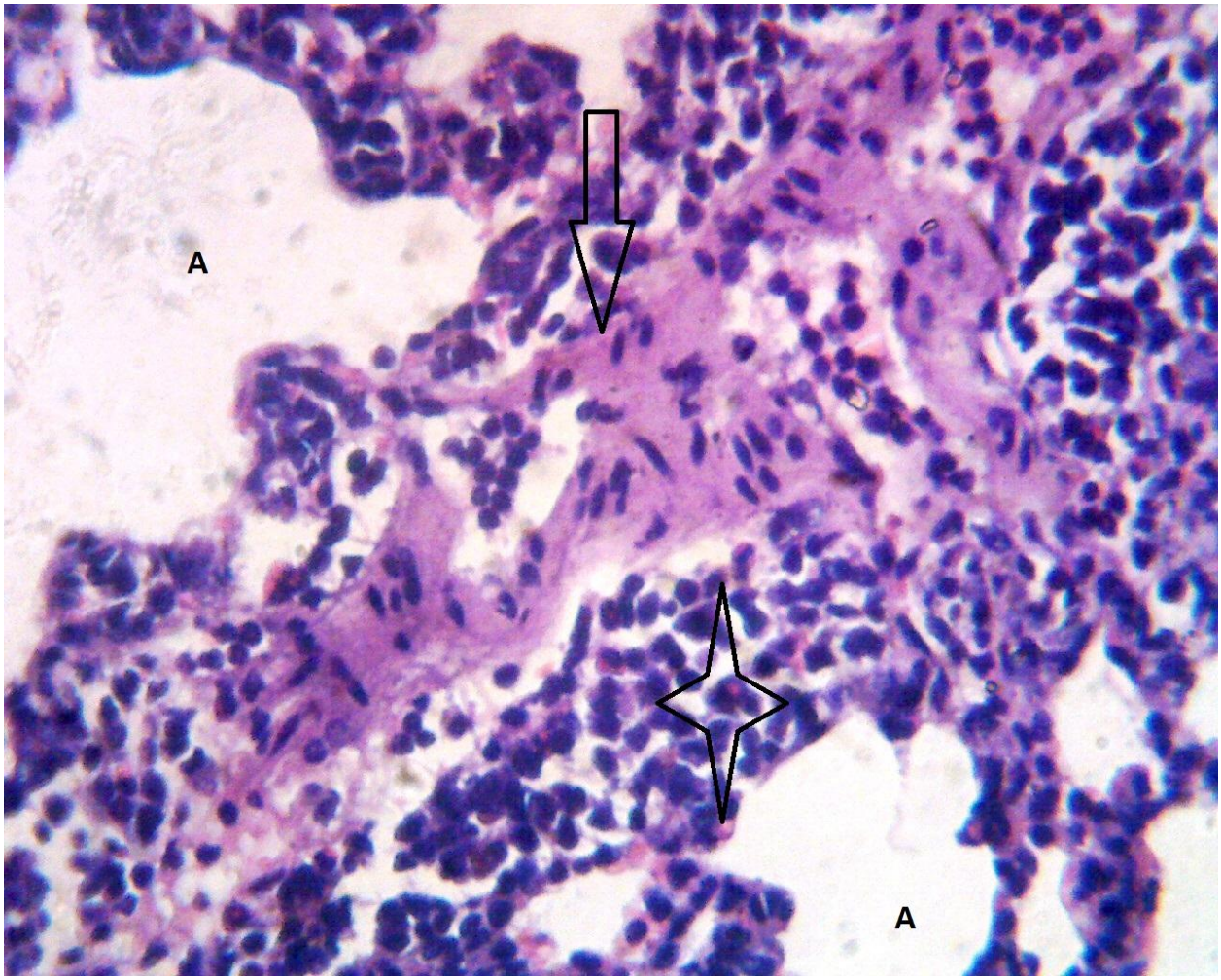
X400 HE TECHNIQUE

Figure 18: **Photomicrograph shows section of the lungs tissues with marked perivascular inflammatory cells infilterates and vascular** congestion caused by ASB 5.



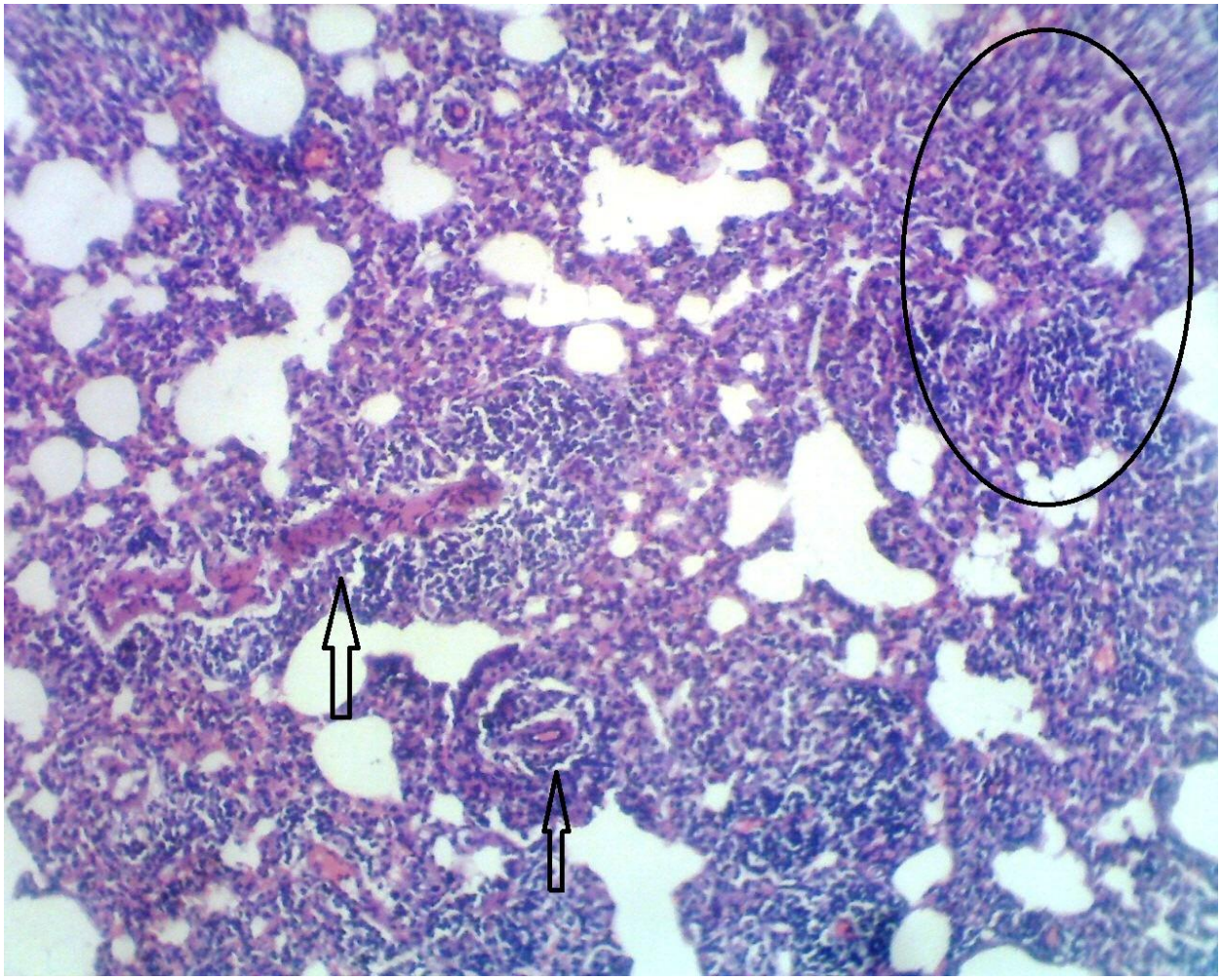
ASB 88 X400 HE TECHNIQUE

Figure 19: **Photomicrograph shows section of the lungs tissues marked interstitial, inflammatory cells infiltrates (star). Arrow shows histiocytes caused by ASB 88.**



CY 69 X400 HE TECHNIQUE

Figure 20: Photomicrograph shows moderate inflammatory cells infiltrates (Star) and atherosclerosis (Arrow) caused by CY 69.



X400 HE TECHNIQUE

Figure 21: Section shows perivascular (arrow) and interstitial (Circle) infiltration of inflammatory cells caused by CW 91.

Table 4.6: Values for the Haematogical Parameters

HAEMATOLOGICAL PROFILE

SAMPLE	WBC (mm3)	RBC (mm3)	Hgb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)	NEUTROPHIL (mm3)	LYMPHOCYTE (mm3)	PLATELET	MONOCYTE
CONTROL	10	5.9	16.1	50	80	27.1	36.1	70	24.2	450	5.8
ASB5	10.1	8.5	14.8	46	54	16.9	32	46	50	600	4
ASB19	10.1	17.9	14.9	45	72	22	34	36	64	339	0
ASB32	10.1	9	15.4	46	58	17.1	30.5	28	70	680	2
ASB44	9.6	8.9	15.9	48	56	17	32.6	50	50	500	0
ASB60	8.5	9.4	16.6	50	70	18	33.1	35	65	500	0
ASB85	9.6	8.4	14.9	46	57	17.1	31.1	48	52	450	0
SAP14	15	7.4	15.6	45	60	20.9	36.1	22	78	560	0
SAP41	25	4.74	11.6	34	74	24.4	33.1	18	82	675	0
SAP61	11	7	15.7	48	56	17.7	32	32	56	632	12
SAP70	21	5.1	11.4	35	79	24	33	28	72	600	0
SAP82	15	7.4	15.6	45	60	20.9	36.1	22	78	560	0
SAP83	26	4.75	11.4	34	74.1	24.4	32	29	71	560	0
SAP90	22	4.6	10.8	33	70	22	30	38	62	567	0
UBT6	11.78	8.78	15.4	47	53	17.5	33	28	63	643	9
UBT21	11.5	1.16	11.3	37	58	10.9	18.7	33	38	778	29
UBT35	12.88	8.57	13.9	44	51	16.2	31.89	31	56	594	11
UBT36	10.4	6.48	9.9	32	49	15.3	31.4	41	43	597	16
UBT46	11.4	6.77	12.4	39	58	18.2	31.6	23	64	856	13
UBT57	10.4	6.48	9.9	32	49	15.3	31.4	22	64	866	14
UBT69	5.06	7.68	13.5	43	56	17.6	31.4	32	56	531	12
UBT77	11.7	8.82	15.7	48	55	17.8	32.4	31	56	826	13
UBT93	11	7	15.7	48	56	17.7	32	32	56	632	12

OT2	19	7.5	15	44	58	19.9	35.6	21	74.5	350	5.5
OT14	18	7.2	15	45	58	19.8	35.2	45	55	510	0
OT45	18	7.4	15.9	44	60	21	35.3	26.5	68	450	5.5
OT53	16	7.3	14.8	42	56	20.1	36.1	30.5	66	550	3.5
OT68	17	7.7	16.4	46	62	22	36	41.1	50	500	8.9
OT87	15	7.4	15.6	45	60	20.9	36.1	22	78	560	0
OT93	16.7	7.3	16.2	44	59	19.6	34.1	28.2	68	320	2.8
OGH12	9.73	8.98	15.2	49	54	16.9	31.2	33	56	824	11
OGH13	9.31	8.54	14.7	46	53	17.2	32.2	27	63	567	10
OGH28	8.53	8.25	14.6	48	58	17.7	30.6	25	68	600	7
OGH47	9.58	8.11	14.7	47	58	18.1	31.2	25	61	492	13
OGH57	12.63	8.53	14.2	45	52	16.6	31	20	71	457	9
OGH66	8.53	8.25	14.6	48	58	17.7	30.6	25	68	558	7
OGH82	5.06	7.68	13.5	43	56	17.6	31.4	32	56	531	12
OGH95	8.53	7.65	13.6	44	52	17.8	30.7	24	68	601	8
CB4	7.4	8.7	14.6	44	58	17.2	30.7	40	60	456	0
CB27	11.2	8.5	15	47	62	16.9	31.2	40	60	856	0
CB55	9.4	9	15.9	47	56	17	33	50	48	500	2
CB88	8	8.6	15.6	48	60	17	33	32	60	400	8
CB91	8	9	16.5	50	62	18.1	33.1	35	65	400	0
CW9	8.24	6.6	11.7	38	57	17.7	30.9	22	70	601	8
CW47	14.76	7.9	13.5	42	54	7.1	31.9	33	57.5	680	9.5
CW49	11.73	7.73	13.3	42	54	17.2	31.6	20	66	566	14
CW91	8.24	6.6	11.7	38	57	17.7	30.9	26	58	514	16
CU14	7	7.33	15.1	43	58	20.7	35.5	15	71.2	213	13.8
CU31	7.58	8.34	15.1	43.5	52	18.1	34.7	36.6	56.2	466	7.1
CU42	15.8	8.8	17.1	48	55	19.4	35.6	27.4	64	641	8.5
CU70	13.7	9	16.6	46	51	18.4	35.9	14.3	78.9	560	6.8
CU82	16.8	9.26	17	47	51	18.3	36.1	12.5	77.5	597	10

CA20	12.9	7.54	15.4	43	57	20.4	36.1	10.5	75.3	608	14.2
CA43	11.39	8.89	17.1	48	54	19.2	35.4	21.1	68.7	684	10.2
CA58	7.9	7.31	15.1	43	60	20.6	34.3	31.8	58.3	459	9.9
CA79	10.2	8.5	16.9	47	55	19.9	36.3	20.9	64.4	721	14.6
CS29	12	7.8	16	45	61	23	34	24.9	65	450	10.1
CS41	19	8	16.8	48	62	21.1	36.2	27	68	491	5
CS53	17.6	7	14.8	42	55	18.1	34.8	18.2	72	600	9.8
CS80	17.8	7.5	15.5	45	58	19	35.1	27.5	66	491	6.5
CY8	9.2	9	15.1	47	70	20.2	33.1	28	72	519	0
CY31	8	8	15.4	47	74	20.3	33	27	73	512	0
CY44	9.6	10.1	17.2	52	73	18.6	32	45	55	700	0
CY69	7.5	8.3	15.9	48	57	16.9	32.1	25	75	640	0
CY79	7.2	8.1	15.9	46	55	16.9	32.1	30	70	630	0
CO5	9.6	9.6	17.3	51	72	18.2	31.6	60	40	300	0
CO22	7.6	9	17	50	80	22.1	34.1	55	40	498	5
CO59	8.9	8.6	15	46	69	18	31	32	78	740	0
CO73	18	8.6	15.1	44	58	19	35.1	20	77	450	3
YEN10	16	9	17.1	52	65	22.4	37	24.1	70	420	5.9
YEN41	15.6	7.6	15.6	48	62	24	37	30	65	290	5
YEN54	22	7.5	15.4	48	62	21	35	28.2	70	450	1.8
YEN70	23	8.9	15.8	47	60	20.7	36.1	24	70	300	6
YEN71	20	9	16.8	48	60	23.1	36.2	40.8	56.2	410	3
CSA24	15.9	8	16	46	58	19.9	34.2	15.8	74.9	649	9.3
CSA49	7.1	8.4	16	45	54	19	35	23	68	465	9
CSA65	7.1	8.45	16.4	46	55	19.5	35.4	23.8	69.9	653	6.3
SOH52	16	9.6	16.7	48	68	20.1	36.2	28	70	400	2
SOH85	15	8	15.8	44	58	20	34.6	32	59.9	500	9.1
SOH86	16	7.9	15	46	60	20	34	30.1	60	641	9.9

Normal Range	4.5-10x103	4.2-5.4	14-16.5	42-54	85-100	27-33pg	33.4-35.5	60-70	2

Table 4.7.Haematological parameters

HAEMATOLOGY PARAMETER	ABNORMAL LEVELS	% OF ABNORMAL LEVEL
WBC	40	51%
RBC	75	95%
HB	28	35%
PCV	7	9%
MCV	78	99%
MCH	79	100%
MCHC	38	48%
NEUTROPHIL	71	90%
LYMPHOCYTE	76	96%
PLATELET	69	69%
MONOCYTE	41	52%

KEY:

WBC
RBC
HB
PCV
MCV
MCH
MCHC
NEUTROPHIL
LYMPHOCYTE
PLATELET
MONOCYTE

Table 4.8: The values of the clinical chemistry parameters

CLINICAL CHEMISTRY PROFILE

SAMPLE	UREA (mg/dl)	CREATININE (mg/dl)	Na2+ (mmol/l)	K+ (mmol/l)	Cl- (mmol/l)
CONTROL	10.8	0.6	149	4.2	113
ASB5	11	0.5	133	4	100
ASB19	10.6	0.6	140	8	94
ASB32	10	0.5	130	5.4	97
ASB44	10	0.6	132	4.5	96
ASB60	11.8	0.6	135	6	94
ASB85	11	0.78	132	4.9	97
SAP14	10	0.5	134	6	97
SAP41	11	0.5	151	6.7	113
SAP61	11	0.5	136	5	92
SAP70	12	0.5	148	6.3	110
SAP82	10	0.5	134	6	97
SAP83	10	0.4	151	6.5	109
SAP90	11.8	0.5	150	5.4	111
UBT6	13	0.5	140	5.3	102
UBT21	12	0.4	136	4.6	96
UBT35	13	0.5	133	4.9	101
UBT36	11	0.5	133	5.2	101
UBT46	14	0.5	142	4.9	97
UBT57	16	0.5	134	5	94
UBT69	17	0.5	137	5.4	95
UBT77	16	0.5	134	5	94
UBT93	18	0.5	136	5	92
OT2	11	0.5	134	4.5	95
OT14	10	0.6	136	4.5	98
OT45	9	0.5	139	5.4	96
OT53	10	0.7	132	5.3	99
OT68	11	0.6	135	7	98
OT87	10	0.5	134	6	97
OT93	10	0.6	132	6.9	97
OGH12	12	0.5	140	4.5	98
OGH13	13	0.5	138	4.3	99
OGH28	17	0.5	135	4.5	96
OGH47	16	0.5	142	4.8	96
OGH57	12	0.5	136	4.3	98
OGH66	13	0.5	135	4.7	90
OGH82	14	0.5	135	4	98
OGH95	15	0.5	140	4.2	98
CB4	11	0.5	151	6.4	97
CB27	9	0.5	145	7	98
CB55	12	0.6	134	5.3	98
CB88	10	0.6	149	5.3	92

CB91	10	0.65	140	6.1	96
CW9	12	0.4	132	4.8	100
CW47	12	0.5	141	4.9	98
CW49	11	0.5	137	5.1	100
CW91	15	0.5	137	5	100
CU14	9	0.6	132	8.1	92
CU31	9	0.5	130	8.6	92
CU42	10	0.6	133	7	96
CU70	10	0.5	132	8.9	96
CU82	11	0.5	140	7.5	98
CA20	11	0.5	129	8	90
CA43	**10**	**0.5**	**134**	7.6	95
CA58	11	0.5	134	7.5	98
CA79	9	0.5	130	8	92
CS29	9	0.6	134	5.4	93
CS41	10	0.6	136	6	99
CS53	10	0.5	132	4.5	97
CS80	10	0.5	133	5.9	98
CY8	11	0.6	138	7	94
CY31	12	0.5	132	5.3	92
CY44	10.5	0.7	139	3.8	96
CY69	10	0.5	130	6.4	98
CY79	9	0.5	129	6.4	96
CO5	12	0.7	148	7	97
CO22	9	0.5	136	6.9	99
CO59	10.6	0.8	140	8	96
CO73	9	4	133	5.4	97
YEN10	10	0.6	137	6.5	95
YEN41	10	0.7	129	5.3	94
YEN54	12	0.6	133	6	101
YEN70	10	0.6	136	7	98
YEN71	10	0.6	140	4	97
CSA24	10	0.6	136	7.6	95
CSA49	10	0.5	138	8	97
CSA65	11	0.5	137	8	96
SOH52	9	0.5	134	6	100
SOH85	11	0.5	132	5.9	96
SOH86	11	0.5	140	6.5	95
Normal Range	7-20ml/dl	0.6 -1.m1/dl	135-145	3.5-5mEq/l	96-106mEq/l

Table 4.9: **CLINICAL CHEMISTRY PARAMETERS**

CHEMISTRY PARAMETER	ABNORMAL LEVELS	% OF ABNORMAL LEVEL
UREA	10	13%
CREATININ	56	71%
Na	38	48%
K	54	68%
CL	19	24%

KEY

Na – Sodium
K – Potassium
CL – Chloride

4.5. Molecular studies

Polymerase Chain reaction (PCR)

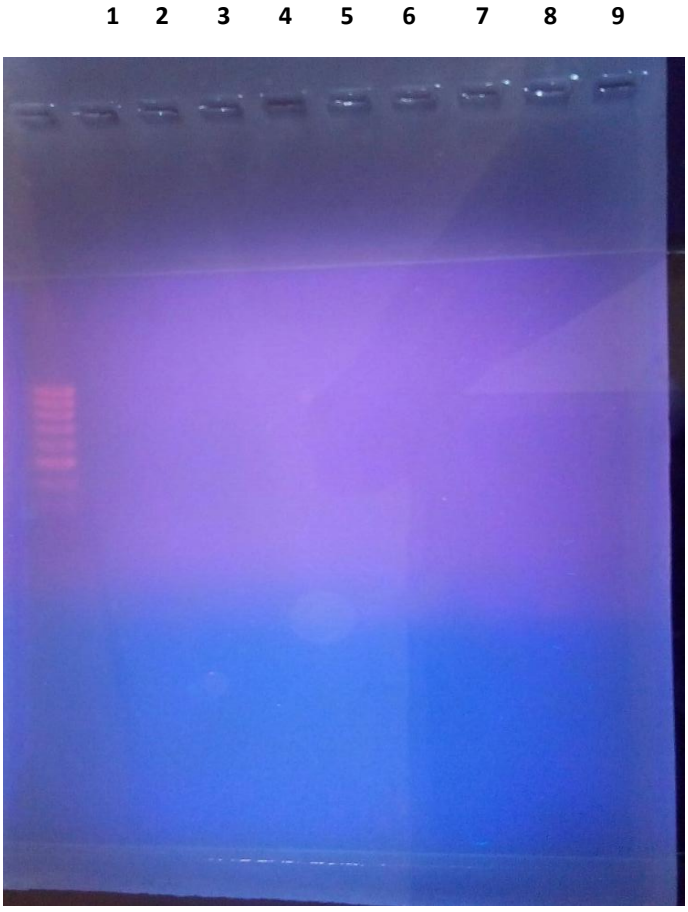


Figure 22: **Lane M; 1Kb DNA Ladder. Lanes 1 – 9 show no amplification at the expected band size for MEC A**

CHARPTER FIVE
DISSCUSSION, CONCLUSION, RECOMMEDATION AND CONTRIBUTION TO KNOWLEDGE

5.1. Disscussion

From the study, a total of one thousand five hundred (1,500) sputa samples obtained over a period of six (6) months (April – September 2015) from the different locations were screened for the presence of *S. aureus* causing pneumonia using Mannitol salt agar as a selective media. Of the 1,500 samples, only seventy- nine (79) were positive for both DNAse, coagulase and catalase test. Thus the prevalence of *S.aureus* associated with pneumonia is 5.3 % in the studied zones.

Gentamacin have the highest actvity profile amongst the antibiotic used in this study as shown in figure 4.1 and 4.2. However ofloxacin, clindamycin, tetracycline and rifampicin also recorded high activity. This was not so with oxacillin and erythromycin which have relatively low susceptibility profile.

Fig 4.3, shows the percentage sensitivity of the *S. aureus* isolates to the different antibiotics. The organisms were highly resistant to oxacillin compared to other antibiotics used. For the percentage susceptibility, the organisms were more susceptible to gentamycine followed by ofloxacin and vancomycine respectively.

From Table 1, there is no significant difference between RD and following; RD=VA, DA, OFX, E, TE at $P>0.05$, while there is significant difference between RD and OX, RD and CN at $P<0.001$, as well as $P>0.05$.There is difference between OX, VS, VA, CN, DA, OFX, E and TE while at $P<0.01$ for VA.There is significant difference between VA vs CN, DA, OFX at $P<0.001$ for CN and OFX, while $P<0.001$ for DA. There was no significant difference between VA vs E and TE at $P> 0.05$.There was a significant difference between CN vs DA, E and TE at varying degree at $P<0.01$ for DA while at $P<0.001$ for E and TE. Then, there was no significant difference between CN vs OFX.There was no significant difference between DA vs OFX, E and TE at $P>0.05$. There was significant difference between OFX vs E at

P<0.001 while there was no significant difference OFX vs TE at P>0.05. There was no significance difference between E and TE at P> 0.05.

The antimicrobial properties of herbs and spices had been described in several reports over the years (Gull *et al.*, 2012). Sagdic *et al.*, (2003) reported that the major determinant of their antimicrobial activities are the type and composition of plant extract, the temperature and pH value of the environment. According to Sasidharan *et al.,* (2011), the degree of inhibitory activity of the extract depends on the type of bioactive ingredient present in the extract such as saponins, flavoniods, alkaloids etc. This bioactive ingredient present is responsible for its activities though information about the exact mechanism of their antimicrobial action is not known.

From the susceptibility test carried out with the plants, all the ethanol extracts of the different plants showed antimicrobial activity against the strains of *staphylococcus aureus* while the aqueous extracts had no activity (Table 3.2e – 3.2f as shown in pg 139-141Appendix).

The inhibitory activity of garlic could be due to the action of the bioactive ingredient allicin which is known to cause immediate and total inhibition of RNA, although it partially inhibits DNA and protein synthesis of the bacteria. This implies that the primary target of allicin is RNA (Jan *et al.,* 2014). The inhibitory action of turmeric as reported by Niamsa and Sittiwet (2009) is as a result of curcumin the active ingredient while the active ingredient gingerol and shogaol have the inhibiting properties in the ginger plant, though the main mechanism of action by which this plant act is not well known.

Although Niamsa and Sittiwet (2009) reported that at higher concentration of 500g/l the aqueous extract of *C.longa* showed inhibitory activity against *Staphylococcus aureus* with an inhibiting zone diameter of 15.5mm suggesting that no inhibition in the present study might be due to lesser concentration used thus low potency of the aqueous extract of *C.longa*.

According to Usman and Osuji (2007) plant extracts whose inhibiting zone diameter is ≥10 are considered highly active. From table 4.3a, 4.3b and 4.3c, ethanol extract of turmeric at a concentration of 300mg/ml (most effective concentration) gave a zone of inhibition ranging from 4 -14mm while ethanol extracts of ginger and garlic ranged from 2-9mm and 3-6mm respectively thus ethanol extract of turmeric has the best activity against *Staphylococcus aureus* as compared to the mild activity of ginger and garlic extract against same organism.

As documented in this study result, the aqueous extract of the plants used had no activity at all concentration, thus suggesting that at concentration ≤300mg/ml the aqueous extracts had no activity against *S.aureus*. The findings of Onyeagba and his colleagues (2007) was in agreement with this study, in which aqueous crude extracts of garlic and ginger had no Invitro inhibition on the growth of various organism including *Staphylococcus aureus*. In contrast to this, Deresse (2011) reported that minimum inhibitory concentration of aqueous garlic extract was 15.00 – 60.00mg/ml. This might be due to the variation in garlic species in different country, difference in processing and the inoculums' densities.

From the result in table 4.3a, CA79 was more sensitive at a concentration of 300mg/ml. As documented in table 4.3b, the ethanol extract of garlic had no zone of inhibition against CY79, CU70, OGH82, SAP82, CW9, CB4, and CSA79 at all concentrations while ginger had none against SOH85, YEN54, CB4, and SAP82. SAP82 was resistant to all extract except ethanol extract of turmeric. Result from table 4.3b and 4.3c showed that CB4 was totally resistant to the ethanol extract of garlic and ginger respectively at all concentrations.

Comparatively, from table 4.3d, the zone of inhibition of oxacillin tested against the entire test organism was considerable higher than the zones of inhibition of all concentration of aqueous and ethanol extract of the plant used, thus oxacillin is more effective than both plants extract.

According to the result documented in Fig 4.4 below, the ethanol extract of turmeric with average MIC of 30.37mg/ml was most potent and had the best bacteriostatic activity against the test organisms compared to MIC of 394.92mg/ml and 274.22mg/ml of ginger and garlic of the same extracting solvent. This is in agreement with previous study that ethanol extract of turmeric though with MIC of 100ug/ml is most potent and bacteriostatic compared to 155mg/ml and 125mg/ml of ginger and garlic respectively (Virendra *et al.,* 2013). The low antibacterial activity of garlic might be due to the unstable nature of allicin; breaking down in 16 hours at 2^{o}C (Chowhury *et al*, 1991) thus reducing its antibacterial activity.

Biofilm thickness can range from a single cell layer to a substantial community encased by a viscous polymeric milieu. Biofilm quantification by crystal violet binding assay is based on the capacity of an organism to absorb light under UV-visible spectrophotometer. The absorbance value corresponds to the biofilm thickness i.e. the higher the absorbance, the thicker the biofilm formed and vice versa.

The Biofilm thickness (absorbance values) of clinical isolates of *Staphylococcus aureus* isolated from different location is shown in Table 4.4. The various samples of *Staphylococcus aureus* formed significant biofilm. Heath care institutions in Edo state (Central Hospital Benin, Irrhua Specialist Teaching Hospital, Stella Obasanjo Woman and Children Hospital and University of Benin Teaching Hospital) had relatively high percentage of biofilm thickness which may also be a reason for their resistnace to most of the antibiotics used in the study. Isolates from Delta State University Teaching Hospital Oghara and Central Hospital Sapele fell into this same category of high biofilm thickness and the reason could be simply because same patients access this same health facilities due to the proximity to Edo state.

The isolates from different location were screened for *Staphylococcus aureus* enterotoxins. Enterotoxins B was most prevalent(Table 4.5) and was found in the clinical isolates collected from Central Hospital Benin, University of Benin Teaching Hospital, Delta State University

Teaching Hospital, Federal Medical Centre Yenagoa, Stella Obasanjo Women and Children Hospital Benin, Central Hospital Yenagoa, Federal Medical Centre Asaba, General Hospital Sapele, Central Hospital Ughelli, General Hospital Sabongida, Central Hospital Ogwashi-ukwu, Central Hospital Agbor, Central Hospital Warri and Central Hospital Sagbama while Enterotoxin C was found to be positive in Irrhua Specialist Teaching Hospital.

Histological examinations of the lungs revealed inflammation, various degrees of congestion indicated by red patches, degeneration of lung epithelium and destruction of the alveoli cells unlike that of the control. Also from the result, it could be seen that the inflamed alveoli cells in some of the lungs are more obvious and severe than others, and this is as a result of the difference in virulence of the strains of the organism. Some strains are more virulent than the others. Also, the more virulent strains have more ability to develop antibiotic resistance. (Larry*et a*l., 2015).Based on information obtained from this study, it is of the opinion that these isolates CU82, CA 26 CSA 24, CU14, CY69, CA43, CW91, ASB5, ASB88, CS29, CO73, UBT6, UBT36, UBT46, UBT69, UBT77, UBT93,OGH47, OGH82, OGH95 and CA58 are more virulent than the others as more damage can be seen on the lungs micrograph presented (Fig 4-21) of lungs micrograph.Severity of the infection can become progressive if early therapeutic intervention is not carried out leading to several pulmonary abnormalities and complications. Early stage infection such as this case can lead to:

- Reduction in the total available surface area of the respiratory membrane
- Decreased ventilation-perfusion ratio and this 2 effect will cause hypoxemia (low blood oxygen), and hypercapnia (increased blood carbon dioxide).

When the concentration of oxygen in the alveoli decreases below normal (73mmHg Po_2). The adjacent blood vessels constricts (Guyton and Hall 2002) leading to vascular congestion as shown in the result. The infection also increases fluid filtration out of the pulmonary capillaries causing pulmonary interstitial fluid pressure to rise thus leading to rapid filling of

the pulmonary interstitial spaces and alveoli with large amounts of free fluid resulting in pulmonary edema which could be associated with vascular dilatation and hemorrhage due to damage caused on the pulmonary vessels and excessive extra vascular accumulation of fluid.

From the haematological results displayed in table 4.6 and 4.7 below, the percentage of indices not within the normal range was highest in MCH (100%) > MCV (99%) > Lymphocyte counts (96%) > RBC counts (95%) > Neutrophil count (90%) > Platelet count (87%) > Monocyte count (52%) > WBC (51%) > MCHC (48%) > HB (35%) > PCV (9%). While for clinical chemistry results, the parameters not within acceptable normal range was highest in creatinine (71%) > K (68%) > Na (48%) > CL (24%) > Urea (12.70%) (Table 4.8 and 4.9).

Haematological parameters are very useful determinant in the evaluation of the health status of an individual. Singh *et al*., (2013) stated that, the presence of an infection can easily be detected via blood examination. The results obtained reveals that *S. aureus* is capable of affecting the haematological parameters of the animals that were infected. The result showed that there was pronounced leukocytosis. The blood differential counts reveals increased numbers of neutrophils (neutrophilia) which is as a result of the toxin (enterotoxin) released by the organisms (*S. aureus*) and this is in line with the study done by Kwon *et al* 2008.

The RBC count was affected by the isolates; the result obtained showed that only a few infected rats had its RBC maintained within the normal ranges. Quite a large number of infected rats could be said to have an elevated red blood cell count (Erthrocytosis). Erthrocytosis is as a result of increased RBC production (McMullin and Claire, 2013).This increase could be triggered by Erythropoietin (EPO). The isolated organism is capable of inducing several pathogenic conditions in host, one of such is its capability of cause pneumonia. *Staphylococcus aureus* associated with pneumonia involves the inflammation of the air sac of the lungs causing the air sac to be filled with fluid and WBC thus reducing the

oxygen exchange ability of the lungs. The inability of the lungs to perform its function cause the RBC to transport poorly oxygenated blood to tissue causing the tissues to become hypoxic. As a compensatory mechanism by the kidney, EPO is secreted stimulating the increasing production of RBC.

On exposure to *Staphylococcus aureus*, slight changes in the haemoglobin was noted in the animals. Studies have shown that *S. aureus*in quest for survival, compete with host for iron nutrient has the tendency to release toxins which destroys the RBC to haemoglobin content and it's capable of extracting its needed iron from haemoglobin (Pischany *et al.,* 2010), and this can result inanaemia. Only a few of the animals could be said to be anaemic.

MCV, MCH and MCHC describe the morphological classification of anaemia (Desforges, 2016). MCV and MCH values obtained were below the normal ranges; such result can be interpreted as RBC of the animals being small in size (microcytic) and have decreased haemoglobin content (Hypochromic). It could be implied from this study that, although there was an increased production of RBC. The PCV and MCHC values obtained were within the normal ranges as compared with the control rat.An increase WBC in count indicates the presence of an infection. *S. aureus* caused a slight increase in WBC count of the animal. The presence of foreign bodies in the body triggers the host immune system to destroy these invaders. Likewise in this study, the host immune system was able to sense the presence of the invading organism and in response to that more WBC was produced to enable the host fight against the invasive *S. aureus.*Studies have shown that there is an overall increase in leukocyte count which may constitutes an increased monocyte and neutrophil this is due to increased proliferation, maturation and release of mature and immature of both cells (Sayed *et al.,* 2002). In contrast, results obtained reveals neutrophil count below the normal ranges of 45-70/mm^3. An explainable reason for this could be either due to decreased production of neutrophil or increased destruction of neutrophil on exposure to the isolates. On the other

hand, monocyte count obtained from each rats varied with the hospital source of isolated organism injected into them. Isolates from Federal Medical Centre Asaba, Central Hospital Sapele (SAP) and Federal Medical Centre Yenegoa that was introduced into the rats, decreased their monocyte counts and that of Central Hospital Agbor, Central Hospital Warri and University of Benin Teaching Hospital resulted in an increased monocyte count while monocyte results of samples from the other locations remained within the normal range.

Selah *et al.,* (2014) reported reduction in the lymphocyte count when *Staphylococcus aureus* was introduced into the animals. This present study shows that lymphocyte count was markedly increased. This increase may be due to underlying infections not necessary caused by exposure to *Staphylococcus aureus.*The platelet count was greatly increased above normal. During an infection, the immune cells like monocyte, T-Lymphocytes and B-Lymphocyte stimulates the secretion of cytokines which are proteins responsible for immune functions also mediate an increase in platelet production. Excessive blood clotting activity results from thrombocytosis (increased platelet count).

For the control, the values obtained for Urea, Creatinine, Sodium, Potassium and Chloride were 14mg/dl, 0.47mg/dl, 136mmol/l, 4.8mmol/l and 98mmol/l respectively.With respect to changes that occur in serum electrolytes following the infection of the wister rats with *S. aureus* isolates from clinically diagnosed pneumonia patients, the results of this study shows that there were significant reduction in serum concentration of sodium and chloride while serum values of potassium were significantly increased, this agrees with the work of Gabor *et al.,* (2000).

High serum potassium levels usually occurs in respiratory diseases especially if acidosis is present because H^+ ions accumulated in the extracellular fluids (ECF) is exchanging with potassium in the intracellular (ICF) leading to hyperkalemia (Kaneko *et al.,* 1997).

From the results above, it could be inferred that the level of potassium was relatively high (Hyperkalemia) compared to the normal range of 3.5 to 5.0mg/dl. Hyperkalemia is an abnormal increase in serum potassium >5.5 milliequivalents per liter, hence any level greater than 6mEq can be life-threatening, depending on the clinical setting (Rastergar *et al*., 2001; Grim *et al*., 1980). Normal blood levels of potassium are critical for maintaining normal heart electrical rhythm. High blood potassium levels can lead to abnormal heart rhythm.

Isolates from Central Hospital Ughelli (CU) had the highest mean value for potassium (8.02+0.779), that from Central Hospital Okwe (CO) for Urea (15.00 + 1.445), Central Hospital Yenagua (YEN) for creatinine (1.7 +2.404), Central Hospital Benin (CB) for sodium (143.8+6.906) and Central Hospital Sapele (CS) for Chloride (104.142+8.4936). It was the isolates that caused the observed changes in the parameters.

Sodium levels drastically reduced (Hyponatremia) with the exception of SAP41, SAP83 and CB4. Increased sodium in the blood occurs whenever there is excess sodium in relation to water. Hypernatremia could be caused by kidney disease, too little water intake and loss of water due to diarrhea and vomiting.

Usually patients with pneumonia are at high risk of experiencing low sodium levels since the principal organ of effect is the lung with continuous production of cough with sputum. Pneumonia also affects the gastrointestinal tract (GIT) causing nausea, vomiting and diarrhea. Due to loss of fluids, it is worthy to note here that the removal of sodium is faster. Any disease or condition that causes a fall in the glomerular filtration (GFR) will increase plasma creatinine. In previous literatures increase plasma creatinine has been implicated in renal tuberculosis, so there is every possibility that *Staphylococcus aureus* that is capable of causing pneumonia is also capable of causing renal damage. Considering the findings obtained from the study done by Monica in 2001, 85% of the blood sample showed an

increased level of plasma creatinine. Any condition associated with protein breakdown such as pneumonia can increases both plasma creatinine and urea levelsMonica (2004).

Sodium is the main extracellular cation.Plasma sodium level is a major factor in the control of water homeostasis and extracellular fluid volume. In most lungs infection, there is a corresponding increase in sodium level resulting in hypovolemia.

From the results obtained as indicated in Table 4.8, 54 samples showed decreased level of creatinine with a range of 0.4-0.5 as opposed the normal range of 0.6 -1.1. For urea, *Staphylococcus aureus*associated pneumonia had no significant effect. This may be due to the level of infection, the duration of infection as well as the virulence of the organism on the pneumonia patient.

No amplification of MecA was observed using both primers (Figure 22).The test was then repeated using another set of primer that is mecA-F ACG AGT AGA TGC TCA ATA TAA mecA-R CTT AGT TCT TTA GAG ATT GA SIZE: 293 bp. Still no amplification was observed. Previous study in Nigeria reported the complete absence of five major SCCmec types and mecA genes as well as the gene product of PBP2a in isolates which were phenotypically MRSA suggesting a probability of hyper production of β- lactamase as a cause of the phenomenon(Olayinka,2009). Recently Ba and colleagues, (2014) also mentioned specific alterations in different amino acids present in protein binding proteins cascade (PBPs 1, 2, and 3) which may be the basis of resistance. These alterations were found to include three amino acid substitutions which were identical and were present in PBPs 1, 2, and 3. These findings provided clear evidence that there are mechanisms other than the presence of mecA gene responsible for beta-lactam resistance of MRSA and that molecular methods alone are not enough for confirmed characterization of MRSA isolates, a point that should be under consideration by regional and reference laboratories(Berger-Bächi, 1999).

5.2. Conclusions

This research placed emphasis on *Staphylococcus aureus* resistance to antibiotics and *Staphylococcus aureus* causing diseases including pneumonia.

From the study, the following conclusions could be drawn based on our findings;

- The overall prevalence of *Staphylococcus aureus*associated with pneumonia in the South-South is low.
- The biofilm forming capacity of *Staphylococcus aureus* in the South-South is high.
- Enterotoxin B is the most prevalent *Staphylococcus aureus* enterotoxins in the South-South.
- *Staphylococcus aureus* incured high resistance to antibiotics in the South-South.
- Increase resistance of *Staphylococcus aureus* to antibiotics is basically due to its biofilm forming capacity.
- The ability of *Staphylococcus aureus* to precipitate different diseases is due to its enterotoxins.
- There were alteration in the body's electrolyte level of Wistar rats causing an increase in potassium and decreased sodium and chloride levels. There was slight changes in the urea level with a remarkable effect on the creatinine level.
- There were alteration in the haematological parameters of the Whister rats revealing microcytic hypochromic anaemia, leucocytosis and an elevated red blood cell count.
- Ethanol extract of turmeric has better efficacy and potency than both ethanol extract of ginger and garlic.
- The study data suggest that the ethanol extract of the plants have mild antibacterial activity and low potency therefore combination therapy with orthodox medicine for treatment of *Staphylococcus aureus*associated pneumonia is advisable.

- One of the isolates(SAP82) was resistant to all extract except ethanol extract of turmeric and another isolate (CB4) was totally resistant to the ethanol extract of garlic at all concentrations.

5.3. Recommendation

From this work, it can be said that the haematological system should not be overlooked when assessing patients with pneumonia induced by Staphylococcus aureus. Which is to say a thorough clinical evaluation and panel of laboratory test that relates to the organ system should be carried out to prevent empiric ways of treatment as rapid identification and treatment of heamatological dysfunction thereby decreasing morbidity and mortality and leading to improved survival. This could actually provide a framework for prompt diagnosis and rational drug therapy.

5.4. Contribution to knowledge

- This study as shown that not only *Streptococcus pneumonia* is capable of causing pneumonia infection but *S. aureus* is also implicated in pneumonia incidence.
- It also shows that there are marked changes in both heamatological and clinical chemistry parameters of subjects suffering from pneumonia associated with *Staphylococcus aureus*.
- This research work also brings to light that in the study zone, enterotoxin C was only observed in Edo State.
- The *Staphylococcus aureus*isolates are capable of causing damage to lungs tissue.

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APPENDIX

TABLE 4.3.1: Thickness of Biofilm of the Clinical Isolates.

S/N	**Sample location**	**Code**	**Biofilm thickness (absorbance)**
	Central Hospital Benin		
1		CB4	0.415
2		CB27	0.360
3		CB88	0.360
4		CB91	0.224
	Irrhua Specialist Hospital		
5		OT2	0.194
6		OT14	0.376
7		OT45	0.548
8		OT53	0.255
9		OT93	0.183
10		OT87	0.343
11		OT68	0.423
	University of Benin Teaching Hospital		
12		UBT6	0.216
13		UBT21	0.289
14		UBT69	0.267
15		UBT46	0.392

16		UBT77	0.276
17		UBT77®	0.328
18		UBT36	0.213
19		UBT93	0.300
20		UBT35	0.229
	Delta State University Teaching Hospital Oghara		
21		OGH66	0.429
22		OGH95	0.420
23		OGH13	0.379
24		OGH28	0.426
25		OGH82	0.343
26		OGH57	0.317
27		OGH12	0.238
28		OGH47	0.193
	Federal Medical Centre Yenagoa		
29		YEN70	0.395
30		YEN41	0.453
31		YEN54	0.313
32		YEN10	0.254
33		YEN71	0.231
	Stella Obasanjo Women and Children Hospital Benin		
34		SOH52	0.443
35		SOH85	0.289
36		SOH86	0.334
37		SOH75	0.366

	Central Hospital Yenagoa		
38		CY44	0.271
39		CY8	0.213
40		CY79	0.209
41		CY31	0.193
42		CY69	0.421
	Federal Medical Centre Asaba		
43		ASB60	0.135
44		ASB85	0.266
45		ASB44	0.308
46		ASB32	0.289
47		ASB5	0.239
48		ASB19	0.257
	General Hospital Sapele		
49		SAP90	0.195
50		SAP82	0.342
51		SAP61	0.256
52		SAP14	0.328
53		SAP41	0.319
54		SAP83	0.382
55		SAP70	0.291
	Central Hospital Ughelli		
56		CU31	0.200
57		CU82	0.270
58		CU14	0.232
59		CU70	0.196

60		CU42	0.264
	Central Hospital Sapele		
61		CS41	0.360
62		CS29	0.361
63		CS53	0.224
64		CS80	0.415
	Central Hospital Ogwashi-ukwu		
65		CO73	0.272
66		CO5	0.366
67			
68		CO22	0.256
		CO59	0.303
	Central Hospital Agbor		
69		CA79	0.229
70		CA43	0.286
71		CA58	0.139
72		CA20	0.197
	Central Hospital Warri		
73		CW49	0.274
74		CW9	0.267
75		CW47	0.107
76		CW91	0.199
	Central Hospital Sagbama		
77		CSA65	0.267
78		CSA24	0.263
79		CSA49	0.267

Table 1. ***Percentage sensitivity of organisms to antibiotic***

	RD	OX	VA	CN	DA	OFX	E	TE
RESISTANCE	54.30%	72%	44%	1%	32%	19%	50.60%	58%
INTERMIDATE	15.20%	8%	O	1%	19%	10%	31.60%	32%
SUSCEPTIBLE	30.40%	20%	56%	98%	49%	71%	17.70%	10%

HAEMATOLOGY

SAMPLE	WBC (mm3)	RBC (mm3)	Hgb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC(%)	NEUTROPHIL (mm3)	LYMPHOCYTE (mm3)	PLATELET	MONOCYTE
CONTROL	20	8.9	17.1	50	69	27	36.1	24.2	70	500	5.8
ASB5	10.1	8.5	14.8	46	54	16.9	32	46	50	600	4
ASB19	10.1	17.9	14.9	45	72	22	34	36	64	339	0
ASB32	10.1	9	15.4	46	58	17.1	30.5	28	70	680	2
ASB44	9.6	8.9	15.9	48	56	17	32.6	50	50	500	0
ASB60	8.5	9.4	16.6	50	70	18	33.1	35	65	500	0
ASB85	9.6	8.4	14.9	46	57	17.1	31.1	48	52	450	0
SAP14	15	7.4	15.6	45	60	20.9	36.1	22	78	560	0
SAP41	25	4.74	11.6	34	74	24.4	33.1	18	82	675	0
SAP61	11	7	15.7	48	56	17.7	32	32	56	632	12
SAP70	21	5.1	11.4	35	79	24	33	28	72	600	0
SAP82	15	7.4	15.6	45	60	20.9	36.1	22	78	560	0
SAP83	26	4.75	11.4	34	74.1	24.4	32	29	71	560	0
SAP90	22	4.6	10.8	33	70	22	30	38	62	567	0
UBT6	11.78	8.78	15.4	47	53	17.5	33	28	63	643	9
UBT21	1.5	1.16	1.3	7	58	10.9	18.7	33	38	78	29
UBT35	12.88	8.57	13.9	44	51	16.2	31.89	31	56	594	11
UBT36	10.4	6.48	9.9	32	49	15.3	31.4	41	43	597	16
UBT46	11.4	6.77	12.4	39	58	18.2	31.6	23	64	856	13
UBT57	10.4	6.48	9.9	32	49	15.3	31.4	22	64	866	14
UBT69	5.06	7.68	13.5	43	56	17.6	31.4	32	56	531	12
UBT77	11.7	8.82	15.7	48	55	17.8	32.4	31	56	826	13
UBT93	11	7	15.7	48	56	17.7	32	32	56	632	12
OT2	19	7.5	15	44	58	19.9	35.6	21	74.5	350	5.5
OT14	18	7.2	15	45	58	19.8	35.2	45	55	510	0

OT45	18	7.4	15.9	44	60	21	35.3	26.5	68	450	5.5
OT53	16	7.3	14.8	42	56	20.1	36.1	30.5	66	550	3.5
OT68	17	7.7	16.4	46	62	22	36	41.1	50	500	8.9
OT87	15	7.4	15.6	45	60	20.9	36.1	22	78	560	0
OT93	16.7	7.3	16.2	44	59	19.6	34.1	28.2	68	320	2.8
OGH12	9.73	8.98	15.2	49	54	16.9	31.2	33	56	824	11
OGH13	9.31	8.54	14.7	46	53	17.2	32.2	27	63	567	10
OGH28	8.53	8.25	14.6	48	58	17.7	30.6	25	68	600	7
OGH47	9.58	8.11	14.7	47	58	18.1	31.2	25	61	492	13
OGH57	12.63	8.53	14.2	45	52	16.6	31	20	71	457	9
OGH66	8.53	8.25	14.6	48	58	17.7	30.6	25	68	558	7
OGH82	5.06	7.68	13.5	43	56	17.6	31.4	32	56	531	12
OGH95	8.53	7.65	13.6	44	52	17.8	30.7	24	68	601	8
CB4	7.4	8.7	14.6	44	58	17.2	30.7	40	60	456	0
CB27	11.2	8.5	15	47	62	16.9	31.2	40	60	856	0
CB55	9.4	9	159	47	56	17	33	50	48	500	2
CB88	8	8.6	15.6	48	60	17	33	32	60	400	8
CB91	8	9	16.5	50	62	18.1	33.1	35	65	400	0
CW9	8.24	6.6	11.7	38	57	17.7	30.9	22	70	601	8
CW47	14.76	7.9	13.5	42	54	7.1	31.9	33	57.5	680	9.5
CW49	11.73	7.73	13.3	42	54	17.2	31.6	20	66	566	14
CW91	8.24	6.6	11.7	38	57	17.7	30.9	26	58	514	16
CU14	7	7.33	15.1	43	58	20.7	35.5	15	71.2	213	13.8
CU31	7.58	8.34	15.1	43.5	52	18.1	34.7	36.6	56.2	466	7.1
CU42	15.8	8.8	17.1	48	55	19.4	35.6	27.4	64	641	8.5
CU70	13.7	9	16.6	46	51	18.4	35.9	14.3	78.9	560	6.8
CU82	16.8	9.26	17	47	51	18.3	36.1	12.5	77.5	597	10
CA20	12.9	7.54	15.4	43	57	20.4	36.1	10.5	75.3	608	14.2
CA43	11.39	8.89	17.1	48	54	19.2	35.4	21.1	68.7	684	10.2

CA58	7.9	7.31	15.1	43	60	20.6	34.3	31.8	58.3	459	9.9
CA79	10.2	8.5	16.9	47	55	19.9	36.3	20.9	64.4	721	14.6
CS29	12	7.8	16	45	61	23	34	24.9	65	450	10.1
CS41	19	8	16.8	48	62	21.1	36.2	27	68	491	5
CS53	17.6	7	14.8	42	55	18.1	34.8	18.2	72	600	9.8
CS80	17.8	7.5	15.5	45	58	19	35.1	27.5	66	491	6.5
CY8	9.2	9	15.1	47	70	20.2	33.1	28	72	519	0
CY31	8	8	15.4	47	74	20.3	33	27	73	512	0
CY44	9.6	10.1	17.2	52	73	18.6	32	45	55	700	0
CY69	7.5	8.3	15.9	48	57	16.9	32.1	25	75	640	0
CY79	7.2	8.1	15.9	46	55	16.9	32.1	30	70	630	0
CO5	9.6	9.6	17.3	51	72	18.2	31.6	60	40	300	0
CO22	7.6	9	17	50	80	22.1	34.1	55	40	498	5
CO59	8.9	8.6	15	46	69	18	31	32	78	740	0
CO73	18	8.6	15.1	44	58	19	35.1	20	77	450	3
YEN10	16	9	17.1	52	65	22.4	37	24.1	70	420	5.9
YEN41	15.6	7.6	15.6	48	62	24	37	30	65	290	5
YEN54	22	7.5	15.4	48	62	21	35	28.2	70	450	1.8
YEN70	23	8.9	15.8	47	60	20.7	36.1	24	70	300	6
YEN71	20	9	16.8	48	60	23.1	36.2	40.8	56.2	410	3
CSA24	15.9	8	16	46	58	19.9	34.2	15.8	74.9	649	9.3
CSA49	7.1	8.4	16	45	54	19	35	23	68	65	9
CSA65	7.1	8.45	16.4	46	55	19.5	35.4	23.8	69.9	653	6.3
SOH52	16	9.6	16.7	48	68	20.1	36.2	28	70	400	2
SOH85	15	8	15.8	44	58	20	34.6	32	59.9	500	9.1
SOH86	16	7.9	15	46	60	20	34	30.1	60	641	9.9

CLINICAL CHEMISTRY

SAMPLE	UREA (mg/dl)	CREATININE (mg/dl)	Na2+ (mmol/l)	K+ (mmol/l)	Cl- (mmol/l)
CONTROL	10.8	0.5	149	6.2	113
ASB5	11	0.5	133	4	100
ASB19	10.6	0.6	140	8	94
ASB32	10	0.5	130	5.4	97
ASB44	10	0.6	132	4.5	96
ASB60	11.8	0.6	135	6	94
ASB85	11	0.78	132	4.9	97
SAP14	10	0.5	134	6	97
SAP41	11	0.5	151	6.7	113
SAP61	11	0.5	136	5	92
SAP70	12	0.5	148	6.3	110
SAP82	10	0.5	134	6	97
SAP83	10	0.4	151	6.5	109
SAP90	11.8	0.5	150	5.4	111
UBT6	13	0.5	140	5.3	102
UBT21	12	0.4	136	4.6	96
UBT35	13	0.5	133	4.9	101
UBT36	11	0.5	133	5.2	101
UBT46	14	0.5	142	4.9	97
UBT57	16	0.5	134	5	94
UBT69	17	0.5	137	5.4	95
UBT77	16	0.5	134	5	94
UBT93	18	0.5	136	5	92
OT2	11	0.5	134	4.5	95
OT14	10	0.6	136	4.5	98
OT45	9	0.5	139	5.4	96
OT53	10	0.7	132	5.3	99
OT68	11	0.6	135	7	98
OT87	10	0.5	134	6	97
OT93	10	0.6	132	6.9	97
OGH12	12	0.5	140	4.5	98
OGH13	13	0.5	138	4.3	99
OGH28	17	0.5	135	4.5	96
OGH47	16	0.5	142	4.8	96
OGH57	12	0.5	136	4.3	98
OGH66	13	0.5	135	4.7	90
OGH82	14	0.5	135	4	98
OGH95	15	0.5	140	4.2	98
CB4	11	0.5	151	6.4	97
CB27	9	0.5	145	7	98
CB55	12	0.6	134	5.3	98

CB88	10	0.6	149	5.3	92
CB91	10	0.65	140	6.1	96
CW9	12	0.4	132	4.8	100
CW47	12	0.5	141	4.9	98
CW49	11	0.5	137	5.1	100
CW91	15	0.5	137	5	100
CU14	9	0.6	132	8.1	92
CU31	9	0.5	130	8.6	92
CU42	10	0.6	133	7	96
CU70	10	0.5	132	8.9	96
CU82	11	0.5	140	7.5	98
CA20	11	0.5	129	8	90
CA43	**10**	**0.5**	**134**	7.6	95
CA58	11	0.5	134	7.5	98
CA79	9	0.5	130	8	92
CS29	9	0.6	134	5.4	93
CS41	10	0.6	136	6	99
CS53	10	0.5	132	4.5	97
CS80	10	0.5	133	5.9	98
CY8	11	0.6	138	7	94
CY31	12	0.5	132	5.3	92
CY44	10.5	0.7	139	3.8	96
CY69	10	0.5	130	6.4	98
CY79	9	0.5	129	6.4	96
CO5	12	0.7	148	7	97
CO22	9	0.5	136	69	99
CO59	10.6	0.8	140	8	96
CO73	9	4	133	5.4	97
YEN10	10	0.6	137	6.5	95
YEN41	10	0.7	129	5.3	94
YEN54	12	6	133	6	101
YEN70	10	0.6	136	7	98
YEN71	10	0.6	140	4	97
CSA24	10	0.6	136	7.6	95
CSA49	10	0.5	138	8	97
CSA65	11	0.5	137	8	96
SOH52	9	0.5	134	6	100
SOH85	11	0.5	132	5.9	96
SOH86	11	0.5	140	6.5	95

One-way Analysis of Variance (ANOVA)

The P value is < 0.0001, considered extremely significant.
Variation among column means is significantly greater than expected by chance.

Tukey-Kramer Multiple Comparisons Test

If the value of q is greater than 4.286 then the P value is less than 0.05.

Comparison	Mean Difference	q	P value
RD vs OX	9.498	9.346	*** P<0.001
RD vs VA	3.823	3.762	ns P>0.05
RD vs CN	-7.536	7.415	*** P<0.001
RD vs DA	-1.890	1.860	ns P>0.05
RD vs OFX	-3.911	3.849	ns P>0.05
RD vs E	2.363	2.325	ns P>0.05
RD vs TE	-0.2574	0.2533	ns P>0.05
OX vs VA	-5.675	5.584	** P<0.01
OX vs CN	-17.034	16.761	*** P<0.001
OX vs DA	-11.388	11.206	*** P<0.001
OX vs OFX	-13.409	13.195	*** P<0.001
OX vs E	-7.135	7.021	*** P<0.001
OX vs TE	-9.755	9.599	*** P<0.001
VA vs CN	-11.359	11.177	*** P<0.001
VA vs DA	-5.713	5.622	** P<0.01

VA vs OFX	-7.734	7.610	***	P<0.001
VA vs E	-1.460	1.437	ns	P>0.05
VA vs TE	-4.080	4.015	ns	P>0.05
CN vs DA	5.646	5.555	**	P<0.01
CN vs OFX	3.624	3.566	ns	P>0.05
CN vs E	9.899	9.740	***	P<0.001
CN vs TE	7.278	7.162	***	P<0.001
DA vs OFX	-2.021	1.989	ns	P>0.05
DA vs E	4.253	4.185	ns	P>0.05
DA vs TE	1.633	1.607	ns	P>0.05
OFX vs E	6.274	6.174	***	P<0.001
OFX vs TE	3.654	3.595	ns	P>0.05
E vs TE	-2.620	2.578	ns	P>0.05

Difference	Mean Difference	95% Confidence Interval From	To
RD - OX	9.498	5.142	13.854
RD - VA	3.823	-0.5330	8.179
RD - CN	-7.536	-11.892	-3.180
RD - DA	-1.890	-6.246	2.465
RD - OFX	-3.911	-8.267	0.4444
RD - E	2.363	-1.993	6.719
RD - TE	-0.2574	-4.613	4.098
OX - VA	-5.675	-10.031	-1.319
OX - CN	-17.034	-21.390	-12.678
OX - DA	-11.388	-15.744	-7.032
OX - OFX	-13.409	-17.765	-9.054
OX - E	-7.135	-11.491	-2.779
OX - TE	-9.755	-14.111	-5.400
VA - CN	-11.359	-15.714	-7.003
VA - DA	-5.713	-10.069	-1.357
VA - OFX	-7.734	-12.090	-3.378

VA - OFX	-7.734	-12.090	-3.378
VA - E	-1.460	-5.816	2.896
VA - TE	-4.080	-8.436	0.2756
CN - DA	5.646	1.290	10.001
CN - OFX	3.624	-0.7313	7.980
CN - E	9.899	5.543	14.254
CN - TE	7.278	2.923	11.634
DA - OFX	-2.021	-6.377	2.335
DA - E	4.253	-0.1026	8.609
DA - TE	1.633	-2.723	5.989
OFX - E	6.274	1.919	10.630
OFX - TE	3.654	-0.7017	8.010
E - TE	-2.620	-6.976	1.735

Assumption test: Are the standard deviations of the groups equal?

ANOVA assumes that the data are sampled from populations with identical SDs. This assumption is tested using the method of Bartlett.

Bartlett statistic (corrected) = 54.320
The P value is < 0.0001.
Bartlett's test suggests that the differences among the SDs is extremely significant.
Since ANOVA assumes populations with equal SDs, you should consider transforming your data (reciprocal or log) or selecting a nonparametric test.

Assumption test: Are the data sampled from Gaussian distributions?

ANOVA assumes that the data are sampled from populations that follow Gaussian distributions. This assumption is tested using the method Kolmogorov and Smirnov:

Group	KS	P Value	Passed normality test?
RD	0.1273	0.0029	No
OX	0.4059	<0.0001	No
VA	0.2559	<0.0001	No
CN	0.09368	0.0832	Yes
DA	0.2109	<0.0001	No
OFX	0.1915	<0.0001	No
E	0.1819	<0.0001	No
TE	0.1574	<0.0001	No

At least one column failed the normality test with P<0.05. Consider using a nonparametric test or transforming the data (i.e. converting to logarithms or reciprocals).

Intermediate calculations. ANOVA table

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments (between columns)	7	14662	2094.5
Residuals (within columns)	624	50913	81.592
Total	631	65575	

F = 25.670 =(MStreatment/MSresidual)

Summary of Data

Group	Number of Points	Mean	Standard Deviation	Standard Error of Mean	Median
RD	79	15.110	10.507	1.182	14.000
OX	79	5.612	8.412	0.9465	0.000
VA	79	11.287	8.750	0.9844	15.333
CN	79	22.646	4.684	0.5270	23.333
DA	79	17.000	10.126	1.139	20.333
OFX	79	19.021	8.906	1.002	22.000
E	79	12.747	9.795	1.102	13.000
TE	79	15.367	9.761	1.098	18.667

Group	Minimum	Maximum	95% Confidence Interval From	To
RD	0.000	34.667	12.753	17.467
OX	0.000	29.333	3.725	7.499
VA	0.000	23.333	9.324	13.250
CN	10.000	32.000	21.595	23.696
DA	0.000	30.000	14.728	19.272
OFX	0.000	32.333	17.023	21.019
E	0.000	30.667	10.549	14.944
TE	0.000	34.000	13.177	17.557

* * *

Col. title	RD	OX	VA	CN	DA	OFX	E	TE
Mean	15.1097046406	5.6118143458	11.286919831	22.6455696208	17.0000000003	19.0210970465	12.7468354433	15.3670886076
SEM	1.182	0.9465	0.9844	0.5270	1.139	1.002	1.102	1.098
Sample size (N)	79	79	79	79	79	79	79	79
SD	10.507	8.412	8.750	4.684	10.126	8.906	9.795	9.761
Lower 95% conf. limit	12.753	3.725	9.324	21.595	14.728	17.023	10.549	13.177
Upper 95% conf. limit	17.467	7.499	13.250	23.696	19.272	21.019	14.944	17.557
Minimum	0.000	0.000	0.000	10.000	0.000	0.000	0.000	0.000
Median (50th percentile)	14.000	0.000	15.333	23.333	20.333	22.000	13.000	18.667
Maximum	34.667	29.333	23.333	32.000	30.000	32.333	30.667	34.000
Normality test KS	0.1273	0.4059	0.2559	0.09368	0.2109	0.1915	0.1819	0.1574
Normality test P value	0.0029	<0.0001	<0.0001	0.0832	<0.0001	<0.0001	<0.0001	<0.0001
Passed normality test?	No	No	No	Yes	No	No	No	No

TUMERIC MIC (mg/ml)										ZONE OF INHIBITION					
code	600	300	150	75	37.5	18.75	9.375	4.187	2.344	300	150	75	37.5	18.75	9.375
CA79	-	-	-	-	-	-	-	+	+	14	9	9	7	6	
SOH85		-	-	-	+	+	+	+	+	+	6	6	5	5	4
CSA49	-	-	-	-	-	-	-	-	+	9	7	7.5	7	3	
ABS60	-	-	-	-	-	-	-	-	+	7	5.5	6	5	4	
CO22	-	-	-	-	-	-	-	+	+	8	6.5	5	4	4	
YEN54		-	-	-	-	+	+	+	+	+	7	6	6.5	4	4
SAP41	-	-	-	-	-	-	-	-	+	9	8	6	5	5	
CB4	-	-	-	-	-	-	-	-	+	9.4	6.5	6.5	6	5	
CW9	-	-	-	-	-	-	-	-	+	9.5	9.5	5	5.5	4	
SAP82	-	-	-	-	-	-	-	-	+	9	8	8	7.5	6	
OGH82		-	-	-		-	-	-	+	+	8	7	7	6	4
CU70	-	-	-	-	-	-	-	+	+	14	10	8	6	6	
UBT35		-	-	-	-	-	-	-	+	+	9	7	6	4	5
CS41	-	-	-	+	+	+	+	+	+	6	5	5	4	3	
OT87	-	-	-	-		-	+	+	+	4	4	3	3	2	
CY79	-	-	-	-		-	+	+	+	10	9	8	8	7	

3.5.2. Mic and zone of inhibition of ethanol extract of Garlic

GARLIC MIC (mg/ml)										ZONE OF INHIBITION					
Code	600	300	150	75	37.5	18.75	9.375	4.187	2.344	300	150	75	37.5	18.75	
CA79	-	-	+	+	+	+	+	+	+	3	5	N	N	N	
SOH85		+	+	+	+	+	+	+	+	+	5	3	2	N	N
CSA49	+	+	+	+	+	+	+	+	+	N	N	N	N	N	
ABS60	+	+	+	+	+	+	+	+	+	6	4	4	3	N	
CO22	-	-	-	+	+	+	+	+	+	6	N	N	N	N	
YEN54		-	-	+	+	+	+	+	+	+	N	N	N	N	N
SAP41	-	-	-	+	+	+	+	+	+	5	3	4	4	2	
CB4	-	-	-	-	+	+	+	+	+	N	N	N	N	N	
CW9	-	-	-	-	-	+	+	+	+	N	N	N	N	N	
SAP82	-	-	-	-	-	+	+	+	+	N	N	N	N	N	
OGH82		-	+	+	+	+	+	+	+	+	-	-	-	-	-
CU70	-	+	+	+	+	+	+	+	+	-	-	-	-	-	
UBT35		-	-	-		+	+	+	+	+	6	4	5	4	3
CS41	-	-	+	+	+	+	+	+	+	5	5	4	4	3	
OT87	-	-	-	+	+	+	+	+	+	4	4	5	3	3	
CY79	-	-	+	+	+	+	+	+	+	-	-	-	-	-	

										ZONE OF INHIBITION					
GINGER MIC (mg/ml)															
code	600	300	150	75	37.5	18.75	9.375	4.187	2.344	300	150	75	37.5	18.75	
CA79	+	+	+	+	+	+	+	+	+	2	4	5	6	5	
SOH85		+	+	+	+	+	+	+	+	+	N	N	N	N	N
CSA49	+	+	+	+	+	+	+	+	+	3	3	4	4	3	
ABS60	+	+	+	+	+	+	+	+	+	3	4	5	8	7.5	
CO22	+	+	+	+	+	+	+	+	+	4	3	4	2	2	
YEN54		+	+	+	+	+	+	+	+	+	N	N	N	N	N
SAP41	+	+	+	+	+	+	+	+	+	6	5	3	2	3	
CB4	+	+	+	+	+	+	+	+	+	N	N	N	N	N	
CW9	+	+	+	+	+	+	+	+	+	4	4	3	4	3	
SAP82	+	+	+	+	+	+	+	+	+	N	N	N	N	N	
OGH82		-	-	+	+	+	+	+	+	+	6	6	5	5	4
CU70	-	-	+	+	+	+	+	+	+	7	6	4	5	3	
UBT35		-	+	+	+	+	+	+	+	+	0	6	5	4	5
CS41	-	-	+	+	+	+	+	+	+	4	7	8	6	6	
OT87	-	-	+	+	+	+	+	+	+	0	4	4	6	6	
CY79	-	+	+	+	+	+	+	+	+	5	4	6	3	4	

. **Minimum Inhibitory Concentration of the Different Antibiotics for Mic**

		MIC																			
S/N	**SAMPLES**	ERYTHROMYCINE					TETRACYCLIN					CIPROFLOXACINE					OXACILINE				
		30	15	7.5	3.75	1.875	10	5	2.5	1.25	0.625	10	5	2.5	1.25	0.625	1	0.5	0.25	0.125	0.063
1	ASB5	-	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	+	+	+
2	ASB19	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+	+	+
3	ASB32	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	+	+	+
4	ASB44	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	+	+	+	+	+
5	ASB60	-	-	-	+	+	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+
6	ASB85	+	+	+	+	+	-	-	+	+	+	-	-	-	-	-	+	+	+	+	+
7	SAP14	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
8	SAP41	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
9	SAP61	-	-	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+
10	SAP70	-	-	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
11	SAP82	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+
12	SAP83	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+	+	+
13	SAP90	-	-	-	+	+	-	-	-	-	+	-	-	-	+	+	+	+	+	+	+
14	UBT6	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
15	UBT21	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+

16	**UBT35**	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17	UBT36	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
18	UBT46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
19	UBT57	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+
20	UBT69	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
21	UBT77	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
22	UBT93	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	OT2	-	+	+	+	+	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+
24	OT14	-	+	+	+	+	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+
25	OT45	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	+
26	OT53	-	+	+	+	+	-	-	-	-	+	-	+	+	+	+	-	+	+	+	+
27	OT68	-	-	+	+	+	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+
28	OT87	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+
29	OT93	-	-	-	-	-	-	+	+	+	+	-	-	-	+	+	-	+	+	+	+
30	OGH12	-	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
31	OGH13	-	+	+	+	+	-	-	-	-	-	-	-	+	+	+	-	+	+	+	+
32	OGH28	-	-	-	+	+	-	+	+	+	+	-	-	-	-	+	+	+	+	+	+
33	OGH47	+	+	+	+	+	-	+	+	+	+	-	-	-	+	+	+	+	+	+	+
34	OGH57	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
35	OGH66	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
36	OGH82	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

37	OGH95	-	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
38	CB4	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
39	CB27	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
40	CB55	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
41	CB88	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
42	CB91	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
43	CW9	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
44	CW47	-	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+	+	+	+
45	CW49	-	-	-	+	+	-	-	+	+	+	-	-	-	-	-	+	+	+	+	+
46	CW91	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
47	CU14	-	-	-	+	+	-	+	+	+	+	-	-	-	-	-	+	+	+	+	+
48	CU31	-	-	-	+	+	-	-	-	+	+	-	-	-	-	-	+	+	+	+	+
49	CU42	-	-	-	+	+	-	+	+	+	+	-	-	-	-	-	+	+	+	+	+
50	CU70	-	-	-	-	-	-	+	+	+	+	--	-	-	-	-	+	+	+	+	+
51	CU82	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
52	CA20	-	+	+	+	+	-	-	+	+	+	-	-	-	-	-	+	+	+	+	+
53	**CA43**	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
54	CA58	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	+	+	+	+	+
55	CA79	-	-	+	+	+	-	-	-	-	+	-	-	-	-	-	+	+	+	+	+
56	CS29	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	+	+	+	+	+
57	CS41	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+

58	CS53	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+
59	CS80	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	+	+	+
60	CY8	-	-	-	+	+	-	-	-	+	+	-	-	-	-	-	+	+	+	+	+
61	CY31	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	+	+	+	+	+
62	CY44	-	+	+	+	+	-	+	+	+	+	-	-	-	-	-	+	+	+	+	+
63	CY69	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	+	+	+	+	+
64	CY79	-	-	+	+	+	-	+	+	+	+	-	-	-	-	-	+	+	+	+	+
65	CO5	-	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
66	CO22	-	+	+	+	+	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+
67	CO59	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
68	CO73	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
69	YEN10	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
70	YEN41	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
71	YEN54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
72	YEN70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
73	YEN71	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
74	CSA24	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	+	+	+	+	+
75	CSA49	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	+	+	+	+	+
76	CSA65	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
77	SOH52	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	+
78	SOH85	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	+

79	SOH86	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+	+

INHIBITING ZONE DIAMETER OF EXTRACTS

TABLE 3.2a: Zone of Inhibition of *Curcuma longa*Ethanol Extract against the Test Organism

	Concentration(mg/ml**)**				
SAMPLE	**300**	**150**	**75**	**37.5**	**18.75**
CA79	14	9	9	7	6
SOH85	6	6	5	5	4
CSA49	9	7	8	7	3
ASB60	7	6	6	5	4
CO22	8	7	5	4	4
YEN54	7	6	7	4	4
SAP41	9	8	6	5	5
CB4	10	7	7	6	5
CW9	10	10	5	6	4
SAP82	9	8	8	8	6
OGH82	8	7	7	6	4
CU70	14	10	8	6	6
UBT35	9	7	6	4	5
CS41	6	5	5	4	3
OT87	4	4	3	3	2
CY79	10	9	8	8	7

This shows that there was inhibition of growth of all strains of staphylococcus aureus at all concentrations.

TABLE 3.2b: Zone of Inhibition of Alliumsativum Ethanol Extract against the Test Organism

	Concentration(mg/ml)				
SAMPLE	**300**	**150**	**75**	**37.5**	**18.75**
CA79	3	5	Nz	nz	nz
SOH85	5	3	2	nz	nz
CSA49	nz	Nz	Nz	Nz	nz
ASB60	6	4	4	3	nz
CO22	6	Nz	Nz	Nz	nz
YEN54	nz	Nz	Nz	Nz	nz
SAP41	5	3	4	4	2
CB4	nz	Nz	Nz	Nz	nz
CW9	nz	Nz	Nz	Nz	nz
SAP82	nz	Nz	Nz	Nz	nz
OGH82	nz	Nz	nz	Nz	nz
CU70	nz	Nz	nz	Nz	nz
UBT35	6	4	5	2	3
CS41	4	5	5	4	4
OT87	5	4	4	5	3
CY79	nz	Nz	nz	Nz	nz

Key:

nz no zone of inhibition

TABLE 3.2c: Zone of Inhibition of *Zingiber officinale* Ethanol Extract against the Test Organism

	Concentration(mg/ml**)**				
SAMPLE	**300**	**150**	**75**	**37.5**	**18.75**
CA79	2	4	5	6	5
SOH85	nz	Nz	nz	Nz	nz
CSA49	3	3	4	4	3
ASB60	3	4	5	8	8
CO22	4	3	4	2	2
YEN54	nz	Nz	nz	Nz	nz
SAP41	6	5	3	2	3
CB4	nz	Nz	nz	nz	nz
CW9	4	4	3	4	3
SAP82	nz	Nz	nz	nz	nz
OGH82	9	6	6	5	5
CU70	8	7	6	4	5
UBT35	nz	Nz	6	5	4
CS41	6	4	7	8	6
OT87	nz	Nz	4	4	6
CY79	nz	5	4	6	3

Key:.

nz no zone of inhibition

Table 3.2d: Zone of inhibition of oxacillin as control

	ZONE OF INHIBITION(mm)			
SAMPLE	**1ST**	**2ND**	**3RD**	**AVERAGE ±SD**
CA79	10	9	8	9 ± 1
SOH85	11	11	10	10.7 ± 0.58
CSA49	0	0	0	0
ASB60	27	25	26	26 ± 1
CO22	12	12	12	12 ± 0
YEN54	32	33	32	32.3 ± 0.58
SAP41	16	16	15	15.7 ± 0.58
CB4	10	10	10	10 ± 0
CW9	12	12	13	12.3 ± 0.58
SAP82	8	8	8	8 ± 0
OGH82	32	33	31	32 ± 1
CU70	12	12	13	12.3 ± 0.58
UBT35	0	0	0	0
CS41	0	0	0	0
OT87	19	18	18	18.3 ± O.58
CY79	8	8	8	8 ± 0

This shows the result of oxacillin used as a control for the research, done in triplicate for comparism with the zone of inhibition of the extracts used.

Table 3.2e: Zone of Inhibition of Curcumalonga aqueous extract against the Test Organism

	Concentration(mg/ml)				
SAMPLE	**300**	**150**	**75**	**37.5**	**18.75**
CA79	nz	Nz	nz	nz	nz
SOH85	nz	Nz	nz	nz	nz
CSA49	nz	Nz	nz	nz	nz
ASB60	nz	Nz	nz	nz	nz
CO22	nz	Nz	nz	nz	nz
YEN54	nz	Nz	nz	nz	nz
SAP41	nz	Nz	nz	nz	nz
CB4	nz	Nz	nz	nz	nz
CW9	nz	Nz	nz	nz	nz
SAP82	nz	Nz	nz	nz	nz
OGH82	nz	Nz	nz	nz	nz
CU70	nz	Nz	nz	nz	nz
UBT35	nz	Nz	nz	nz	nz
CS41	nz	Nz	nz	nz	nz
OT87	nz	Nz	nz	nz	nz
CY79	nz	Nz	nz	nz	nz

This shows there was no zone of inhibition when aqueous extract of curcuma longa was used against the test organism.

Key:

nz no zone of inhibition

Table 3.2f: Zone of Inhibition of <u>Alliumsativum</u> aqueous extract against the Test Organism

	Concentration(mg/ml)				
SAMPLE	**300**	**150**	**75**	**37.5**	**18.75**
CA79	nz	Nz	nz	nz	nz
SOH85	nz	Nz	nz	nz	nz
CSA49	nz	Nz	nz	nz	nz
ASB60	nz	Nz	nz	nz	nz
CO22	nz	Nz	nz	nz	nz
YEN54	nz	Nz	nz	nz	nz
SAP41	nz	Nz	nz	nz	nz
CB4	nz	Nz	nz	nz	nz
CW9	nz	Nz	nz	nz	nz
SAP82	nz	Nz	nz	nz	nz
OGH82	nz	Nz	nz	nz	nz
CU70	nz	Nz	nz	nz	nz
UBT35	nz	Nz	nz	nz	nz
CS41	nz	Nz	nz	nz	nz
OT87	nz	Nz	nz	nz	nz
CY79	nz	Nz	nz	nz	nz

From table 3.5 presented above there was no inhibiting zone on the test organism when tested against aqueous extract of allium sativum.

Key:

nz no zone of inhibition

Table 3.2g: Zone of inhibition of <u>Zingiberofficinale</u> aqueous extract against the test organism.

	Concentration(mg/ml)				
SAMPLE	**300**	**150**	**75**	**37.5**	**18.75**
CA79	nz	Nz	nz	nz	nz

SOH85	nz	Nz	nz	nz	nz
CSA49	nz	Nz	nz	nz	nz
ASB60	nz	Nz	nz	nz	nz
CO22	nz	Nz	nz	nz	nz
YEN54	nz	Nz	nz	nz	nz
SAP41	nz	Nz	nz	nz	nz
CB4	nz	Nz	nz	nz	nz
CW9	nz	Nz	nz	nz	nz
SAP82	nz	Nz	nz	nz	nz
OGH82	nz	Nz	nz	nz	nz
CU70	nz	Nz	nz	nz	nz
UBT35	nz	Nz	nz	nz	nz
CS41	nz	Nz	nz	nz	nz
OT87	nz	Nz	nz	nz	nz
CY79	nz	Nz	nz	nz	nz

From table 3.6 presented above there was no inhibiting zone on the test organism when tested against aqueous extract of zingiber officinale.

Key:.

nz no zone of inhibition.

3.3 ANTIMICROBIAL SENSITIVITY TEST RESULT

Table 3.3a: Minimum inhibition concentration of Curcumalonga ethanol extract against the Test Organism.

		MINIMUM INHIBITORY CONCENTRATION (mg/ml)								
s/n	**Code**	**600**	**300**	**150**	**75**	**37.5**	**18.75**	**9.375**	**4.688**	**2.344**
1	CA79	-	-	-	-	-	-	-	+	+
2	SOH85	-	-	-	+	+	+	+	+	+
3	CSA49	-	-	-	-	-	-	-	-	+
4	ASB60	-	-	-	-	-	-	-	-	+
5	CO22	-	-	-	-	-	-	-	+	+
6	YEN54	-	-	-	-	+	+	+	+	+
7	SAP41	-	-	-	-	-	-	-	-	+
8	CB4	-	-	-	-	-	-	-	-	+
9	CW9	-	-	-	-	-	-	-	-	+
10	SAP82	-	-	-	-	-	-	-	-	+
11	OGH82	-	-	-	-	-	-	-	+	+
12	CU70	-	-	-	-	-	-	-	+	+
13	UBT35	-	-	-	-	-	-	-	+	+
14	CS41	-	-	-	+	+	+	+	+	+
15	OT87	-	-	-	-	-	-	+	+	+
16	CY79	-	-	-	-	-	-	+	+	+

Key; + Growth

_ No growth

Table 3.3b Minimum inhibition concentration of Alliumsativum ethanol extract against the Test Organism

		CONCENTRATION (mg/ml)								
s/n	**Code**	**600**	**300**	**150**	**75**	**37.5**	**18.75**	**9.375**	**4.688**	**2.344**
1	CA79	-	-	-	-	+	+	+	+	+
2	SOH85	-	-	-	+	+	+	+	+	+
3	CSA49	-	+	+	+	+	+	+	+	+
4	ASB60	-	-	-	-	+	+	+	+	+
5	CO22	-	-	+	+	+	+	+	+	+
6	YEN54	-	+	+	+	+	+	+	+	+
7	SAP41	-	-	-	-	-	+	+	+	+
8	CB4	+	+	+	+	+	+	+	+	+
9	CW9	-	-	+	+	+	+	+	+	+
10	SAP82	-	+	+	+	+	+	+	+	+
11	OGH82	-	+	+	+	+	+	+	+	+
12	CU70	-	+	+	+	+	+	+	+	+
13	UBT35	-	-	-	-	+	+	+	+	+
14	CS41	-	-	-	+	+	+	+	+	+
15	OT87	-	-	-	-	+	+	+	+	+
16	CY79	-	-	-	+	+	+	+	+	+

Key;

+ Growth

_ No growth

Table 3.3c: Minimum inhibition concentration of Zingiberofficinale ethanol extract against the Test Organism.

		MINIMUM INHIBITORY CONCENTRATION (mg/ml)								
s/n	**Code**	**600**	**300**	**150**	**75**	**37.5**	**18.75**	**9.375**	**4.688**	**2.344**
1	CA79	-	+	+	+	+	+	+	+	+
2	SOH85	-	+	+	+	+	+	+	+	+
3	CSA49	-	-	+	+	+	+	+	+	+
4	ASB60	-	-	-	-	-	-	+	+	+
5	CO22	-	-	+	+	+	+	+	+	+
6	YEN54	-	+	+	+	+	+	+	+	+
7	SAP41	-	-	-	+	+	+	+	+	+
8	CB4	-	+	+	+	+	+	+	+	+
9	CW9	-	-	-	+	+	+	+	+	+
10	SAP82	-	+	+	+	+	+	+	+	+
11	OGH82	-	-	+	+	+	+	+	+	+
12	CU70	-	-	+	+	+	+	+	+	+
13	UBT35	-	+	+	+	+	+	+	+	+
14	CS41	-	-	+	+	+	+	+	+	+
15	OT87	-	-	+	+	+	+	+	+	+
16	CY79	-	+	+	+	+	+	+	+	+

Key;

+ Growth

_ No growth

Table 3.3d: Minimum inhibition concentration of Curcumalonga aqueous extract against the Test Organism.

		CONCENTRATION (mg/ml)								
s/n	**Code**	**600**	**300**	**150**	**75**	**37.5**	**18.75**	**9.375**	**4.688**	**2.344**
1	CA79	+	+	+	+	+	+	+	+	+
2	SOH85	+	+	+	+	+	+	+	+	+
3	CSA49	+	+	+	+	+	+	+	+	+
4	ASB60	+	+	+	+	+	+	+	+	+
5	CO22	+	+	+	+	+	+	+	+	+
6	YEN54	+	+	+	+	+	+	+	+	+
7	SAP41	+	+	+	+	+	+	+	+	+
8	CB4	+	+	+	+	+	+	+	+	+
9	CW9	+	+	+	+	+	+	+	+	+
10	SAP82	+	+	+	+	+	+	+	+	+
11	OGH82	+	+	+	+	+	+	+	+	+
12	CU70	+	+	+	+	+	+	+	+	+
13	UBT35	+	+	+	+	+	+	+	+	+
14	CS41	+	+	+	+	+	+	+	+	+
15	OT87	+	+	+	+	+	+	+	+	+
16	CY79	+	+	+	+	+	+	+	+	+

Key;

+ Growth

_ No growth

Table 3.3e: Minimum inhibition concentration of Alliumsativum aqueous extract against the Test Organism.

		CONCENTRATION (mg/ml)								
s/n	**Code**	**600**	**300**	**150**	**75**	**37.5**	**18.75**	**9.375**	**4.688**	**2.344**
1	CA79	+	+	+	+	+	+	+	+	+
2	SOH85	+	+	+	+	+	+	+	+	+
3	CSA49	+	+	+	+	+	+	+	+	+
4	ASB60	+	+	+	+	+	+	+	+	+
5	CO22	+	+	+	+	+	+	+	+	+
6	YEN54	+	+	+	+	+	+	+	+	+
7	SAP41	+	+	+	+	+	+	+	+	+
8	CB4	+	+	+	+	+	+	+	+	+
9	CW9	+	+	+	+	+	+	+	+	+
10	SAP82	+	+	+	+	+	+	+	+	+
11	OGH82	+	+	+	+	+	+	+	+	+
12	CU70	+	+	+	+	+	+	+	+	+
13	UBT35	+	+	+	+	+	+	+	+	+
14	CS41	+	+	+	+	+	+	+	+	+
15	OT87	+	+	+	+	+	+	+	+	+
16	CY79	+	+	+	+	+	+	+	+	+

Key;

+ Growth

_ No growth

Table 3.3f: Minimum inhibition concentration of Zingiberofficinale aqueous extract against the Test Organism.

		CONCENTRATION (mg/ml)								
s/n	**Code**	**600**	**300**	**150**	**75**	**37.5**	**18.75**	**9.375**	**4.688**	**2.344**
1	CA79	+	+	+	+	+	+	+	+	+
2	SOH85	+	+	+	+	+	+	+	+	+
3	CSA49	+	+	+	+	+	+	+	+	+
4	ASB60	+	+	+	+	+	+	+	+	+
5	CO22	+	+	+	+	+	+	+	+	+
6	YEN54	+	+	+	+	+	+	+	+	+
7	SAP41	+	+	+	+	+	+	+	+	+
8	CB4	+	+	+	+	+	+	+	+	+
9	CW9	+	+	+	+	+	+	+	+	+
10	SAP82	+	+	+	+	+	+	+	+	+
11	OGH82	+	+	+	+	+	+	+	+	+
12	CU70	+	+	+	+	+	+	+	+	+
13	UBT35	+	+	+	+	+	+	+	+	+
14	CS41	+	+	+	+	+	+	+	+	+
15	OT87	+	+	+	+	+	+	+	+	+
16	CY79	+	+	+	+	+	+	+	+	+

Key;

+ Growth

_ No growth

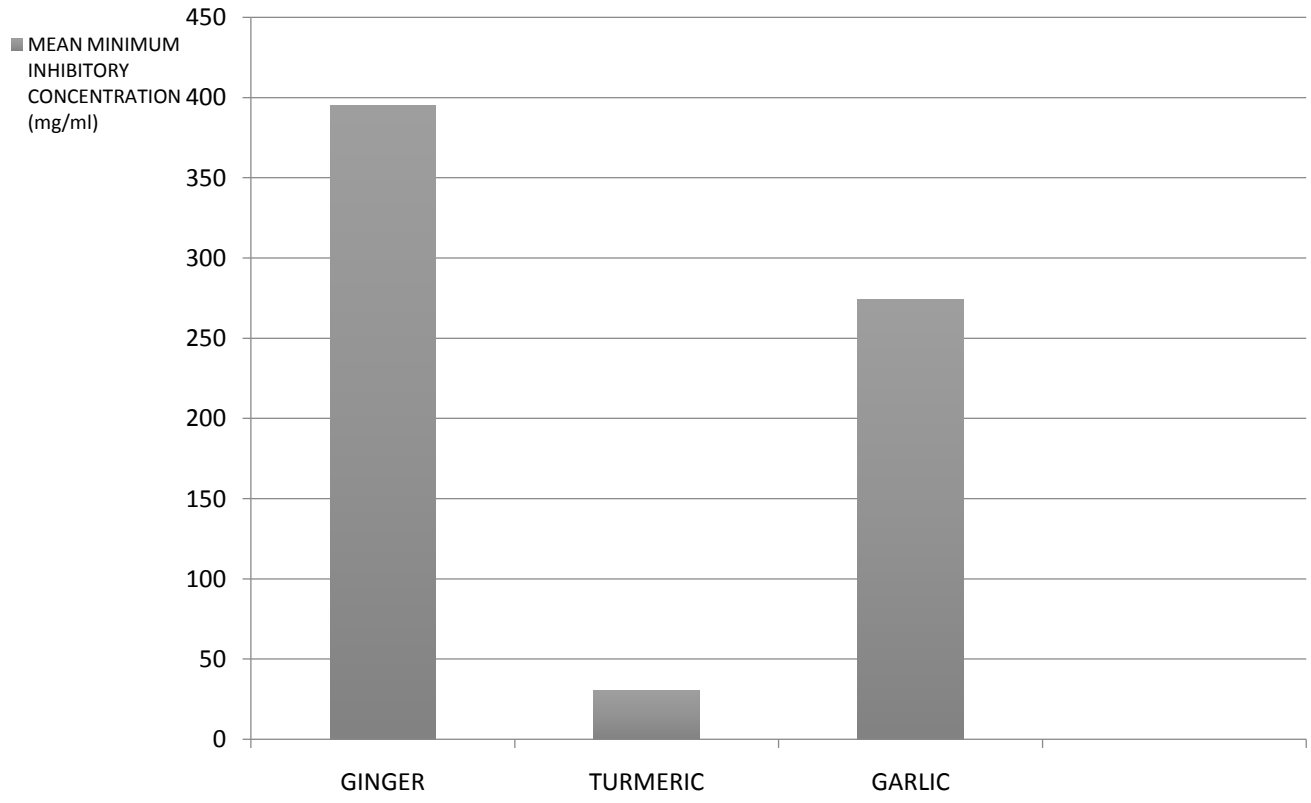


Fig 1: Bar chart showing the difference in the mean minimum inhibition concentration of the extracts.

TABLE 4. 3.1: Thickness of Biofilm of the Clinical Isolates.

S/N	**Sample location**	**Code**	**Biofilm thickness (absorbance)**
	Central Hospital Benin		
1		CB4	0.415
2		CB27	0.360
3		CB88	0.360
4		CB91	0.224
	Irrhua Specialist Hospital		
5		OT2	0.194
6		OT14	0.376
7		OT45	0.548
8		OT53	0.255
9		OT93	0.183
10		OT87	0.343
11		OT68	0.423
	University of Benin Teaching Hospital		
12		UBT6	0.216
13		UBT21	0.289
14		UBT69	0.267
15		UBT46	0.392
16		UBT77	0.276
17		UBT77®	0.328
18		UBT36	0.213
19		UBT93	0.300
20		UBT35	0.229
	Delta State University Teaching Hospital Oghara		
21		OGH66	0.429
22		OGH95	0.420

23		OGH13	0.379
24		OGH28	0.426
25		OGH82	0.343
26		OGH57	0.317
27		OGH12	0.238
28		OGH47	0.193
	Federal Medical Centre Yenagoa		
29		YEN70	0.395
30		YEN41	0.453
31		YEN54	0.313
32		YEN10	0.254
33		YEN71	0.231
	Stella Obasanjo Women and Children Hospital Benin		
34		SOH52	0.443
35		SOH85	0.289
36		SOH86	0.334
37		SOH75	0.366
	Central Hospital Yenagoa		
38		CY44	0.271
39		CY8	0.213
40		CY79	0.209
41		CY31	0.193
42		CY69	0.421
	Federal Medical Centre Asaba		
43		ASB60	0.135
44		ASB85	0.266
45		ASB44	0.308

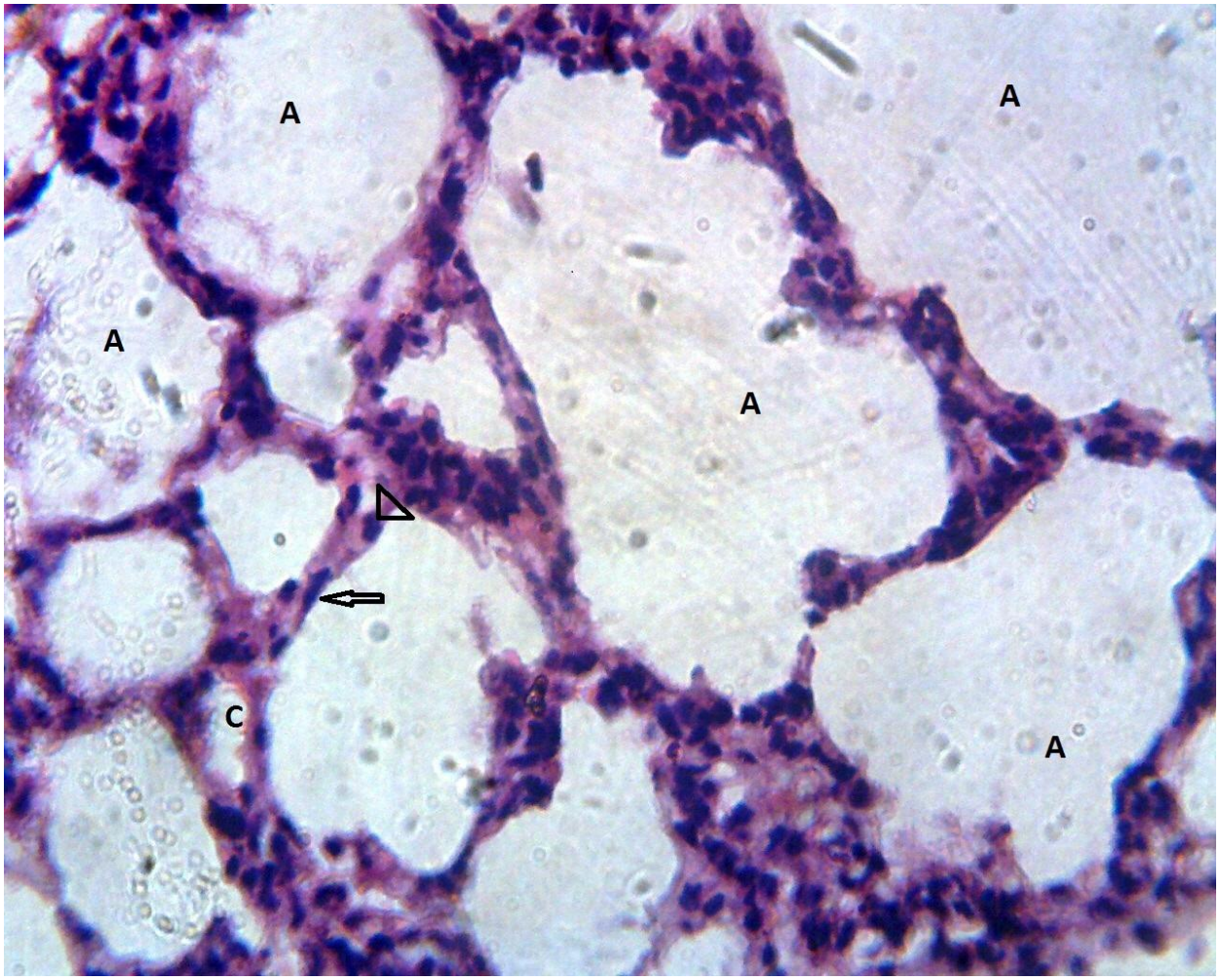
46		ASB32	0.289
47		ASB5	0.239
48		ASB19	0.257
	General Hospital Sapele		
49		SAP90	0.195
50		SAP82	0.342
51		SAP61	0.256
52		SAP14	0.328
53		SAP41	0.319
54		SAP83	0.382
55		SAP70	0.291
	Central Hospital Ughelli		
56		CU31	0.200
57		CU82	0.270
58		CU14	0.232
59		CU70	0.196
60		CU42	0.264
	Central Hospital Sapele		
61		CS41	0.360
62		CS29	0.361
63		CS53	0.224
64		CS80	0.415
	Central Hospital Ogwashi-ukwu		
65		CO73	0.272
66		CO5	0.366
		CO22	0.256
67		CO59	0.303
68			

	Central Hospital Agbor		
69		CA79	0.229
70		CA43	0.286
71		CA58	0.139
72		CA20	0.197
	Central Hospital Warri		
73		CW49	0.274
74		CW9	0.267
75		CW47	0.107
76		CW91	0.199
	Central Hospital Sagbama		
77		CSA65	0.267
78		CSA24	0.263
79		CSA49	0.267

TABLE 5.3.2: Prevalence of Biofilm formed by *Staphylococcus aureus*in different location.

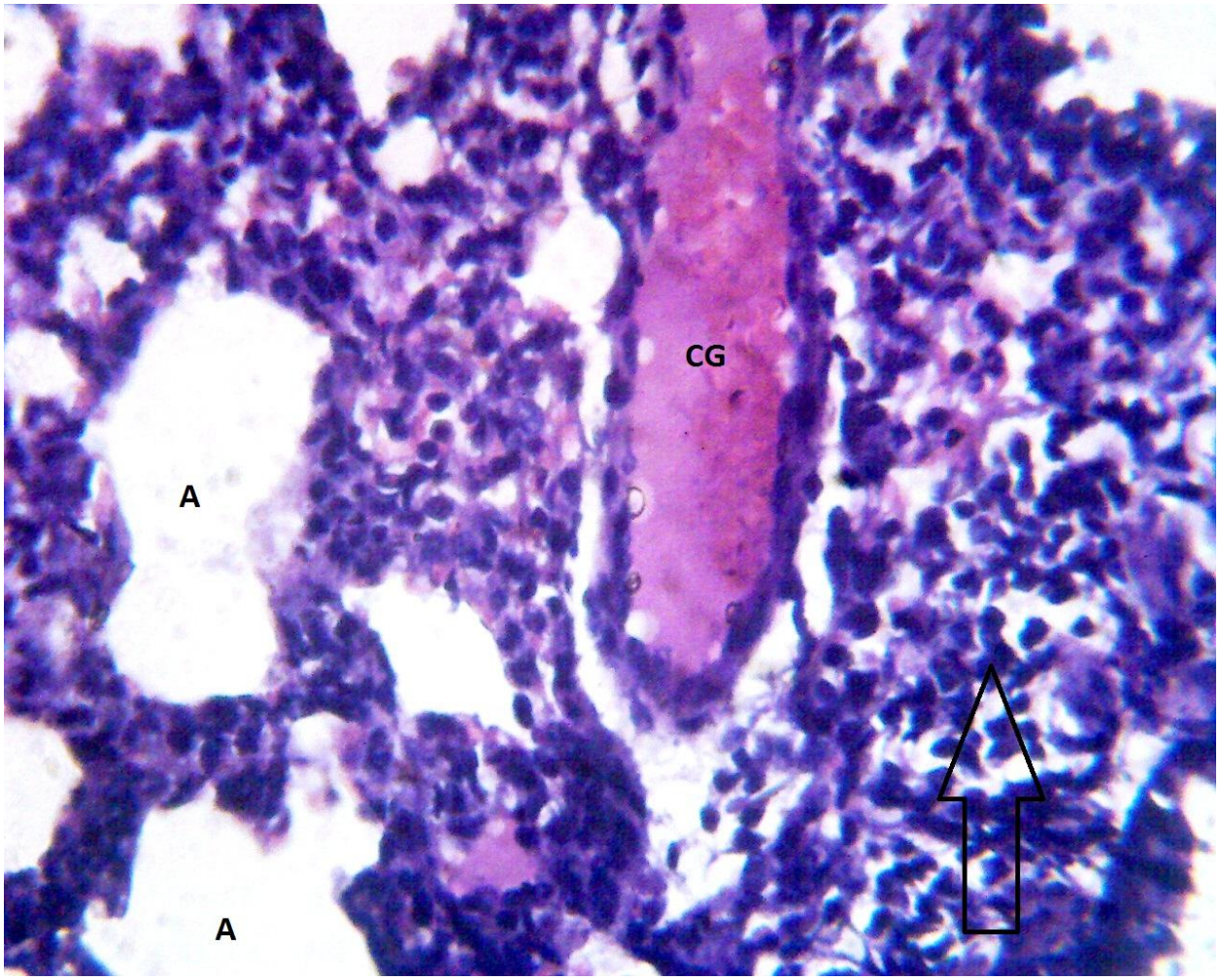
S/N	Sample Location	Total Thickness of Biofilm at each Location	Mean Thickness	Standard Deviation (S.D)	Percentage (%) Thickness
1	Central Hospital Benin	1.359	0.340	0.08	7.81
2	Irrhua Specialist Teaching Hospital	2.322	0.331	0.1	7.60
3	University of Benin Teaching Hospital	2.510	0.279	0.05	6.41
4	Delta State University Teaching Hospital	2.745	0.343	0.08	7.90
5	Federal Medical Centre Yenagoa	1.646	0.329	0.09	7.56
6	Stella Obasanjo Women and Children Hospital	1.432	0.358	0.06	8.22

7	Central Hospital Yenagoa	1.307	0.261	0.09	6.0
8	Federal Medical Centre Asaba	1.494	0.249	0.06	5.72
9	General Hospital Sapele	2.11	0.301	0.06	6.91
10	Central Hospital Ughelli	1.162	0.232	0.03	5.33
11	Central Hospital Sapele	1.360	0.340	0.08	7.81
12	Central Hospital Ogwashi-ukwu	1.197	0.299	0.04	6.91
13	Central Hospital Agbor	0.851	0.212	0.06	4.91
14	Central Hospital Warri	0.847	0.211	0.07	4.9
15	Central Hospital Sagbama	0.797	0.265	0.02	6.0



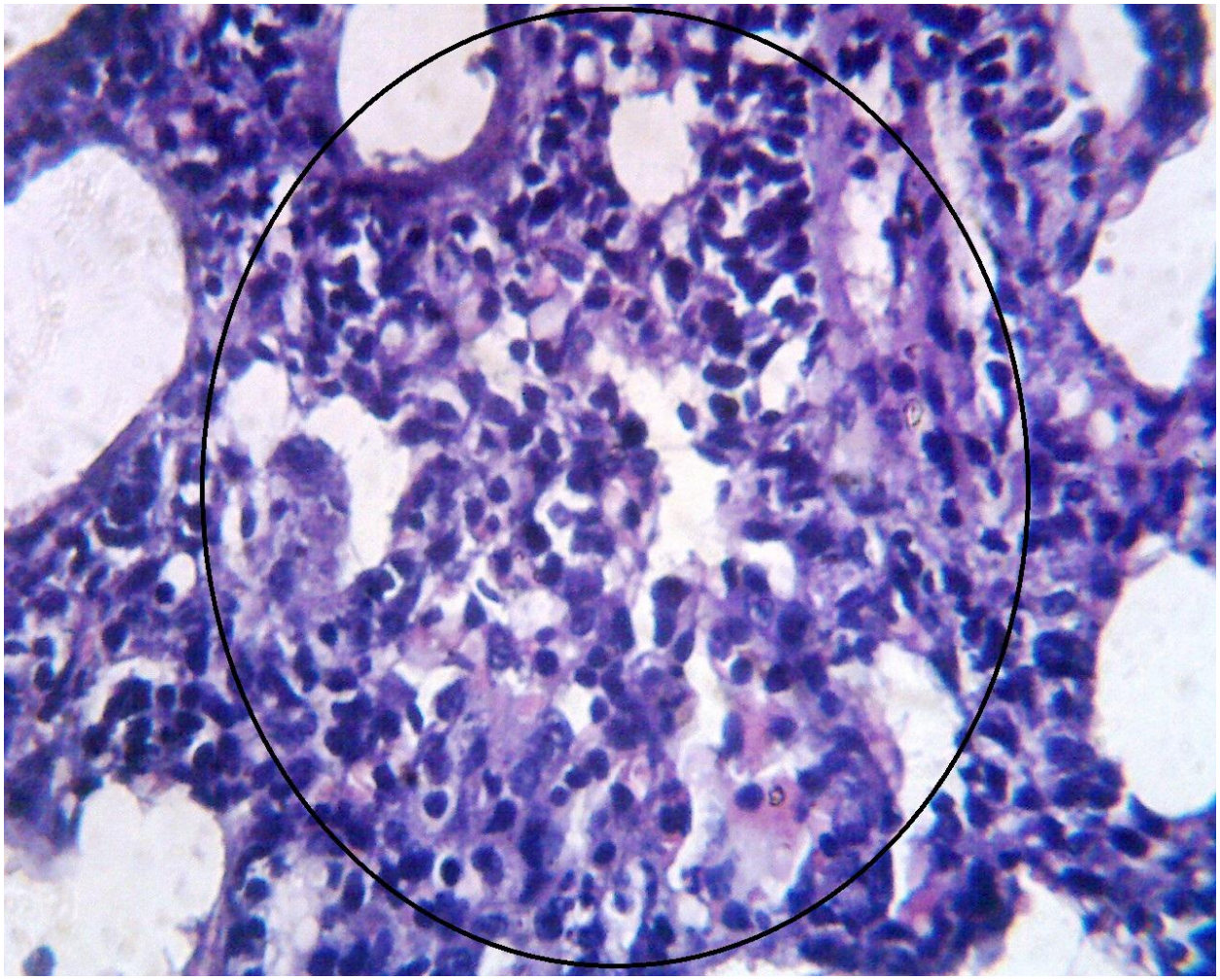
CONTROL X400 HE TECHNIQUE

Photomicrograph shows section of the lung tissue consisting the alveoli (A)lined type I pnuemocytes (arrow), type ii pnuemocytes (arrow head), the blood capillaries (C) lies within the Interstitium. Section free from congestion and inflammatory cells.



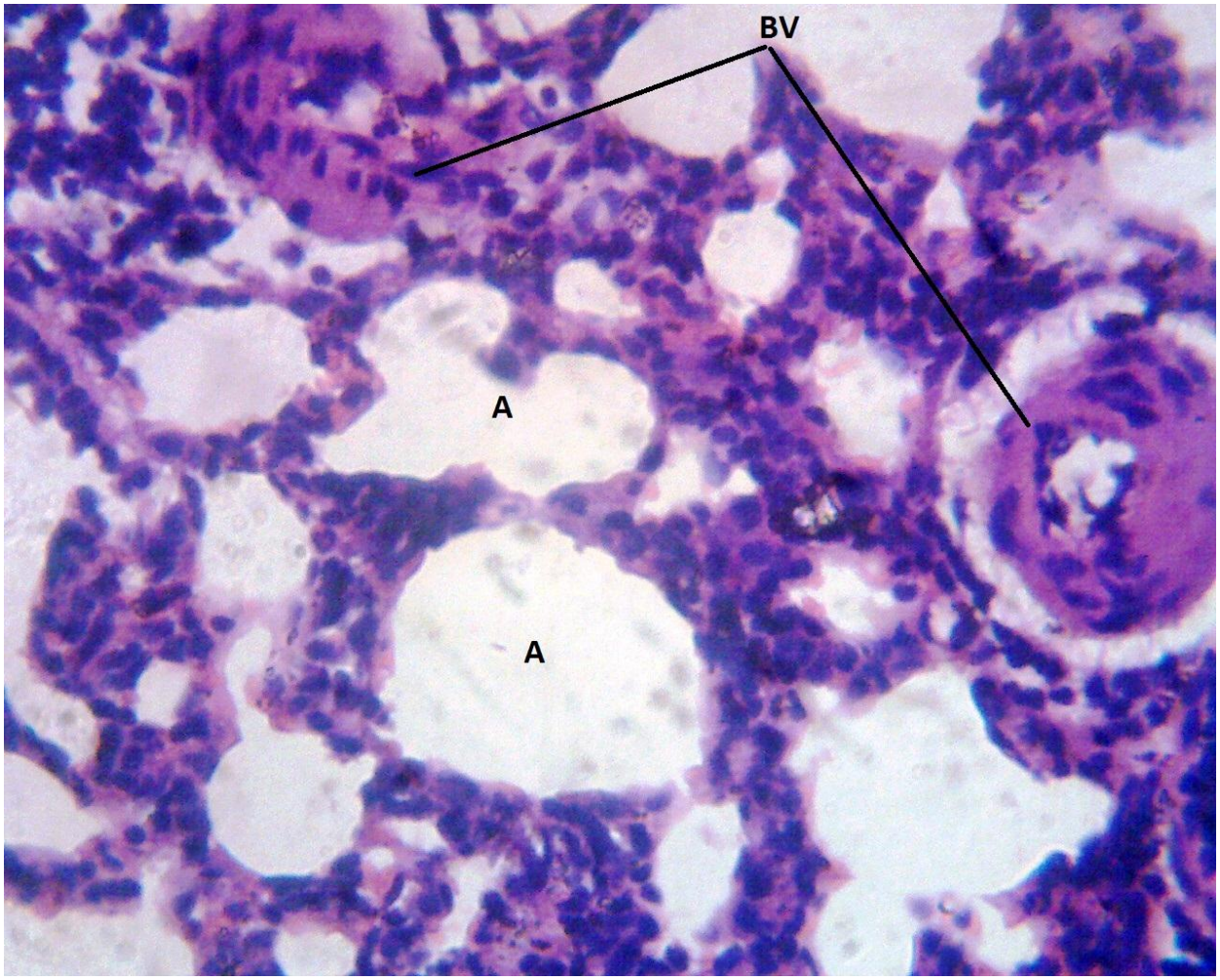
ASB 5 X400 HE TECHNIQUE

Photomicrograph shows section of the lungs tissues with marked perivascular inflammatory cells infilterates and vascular congestion.



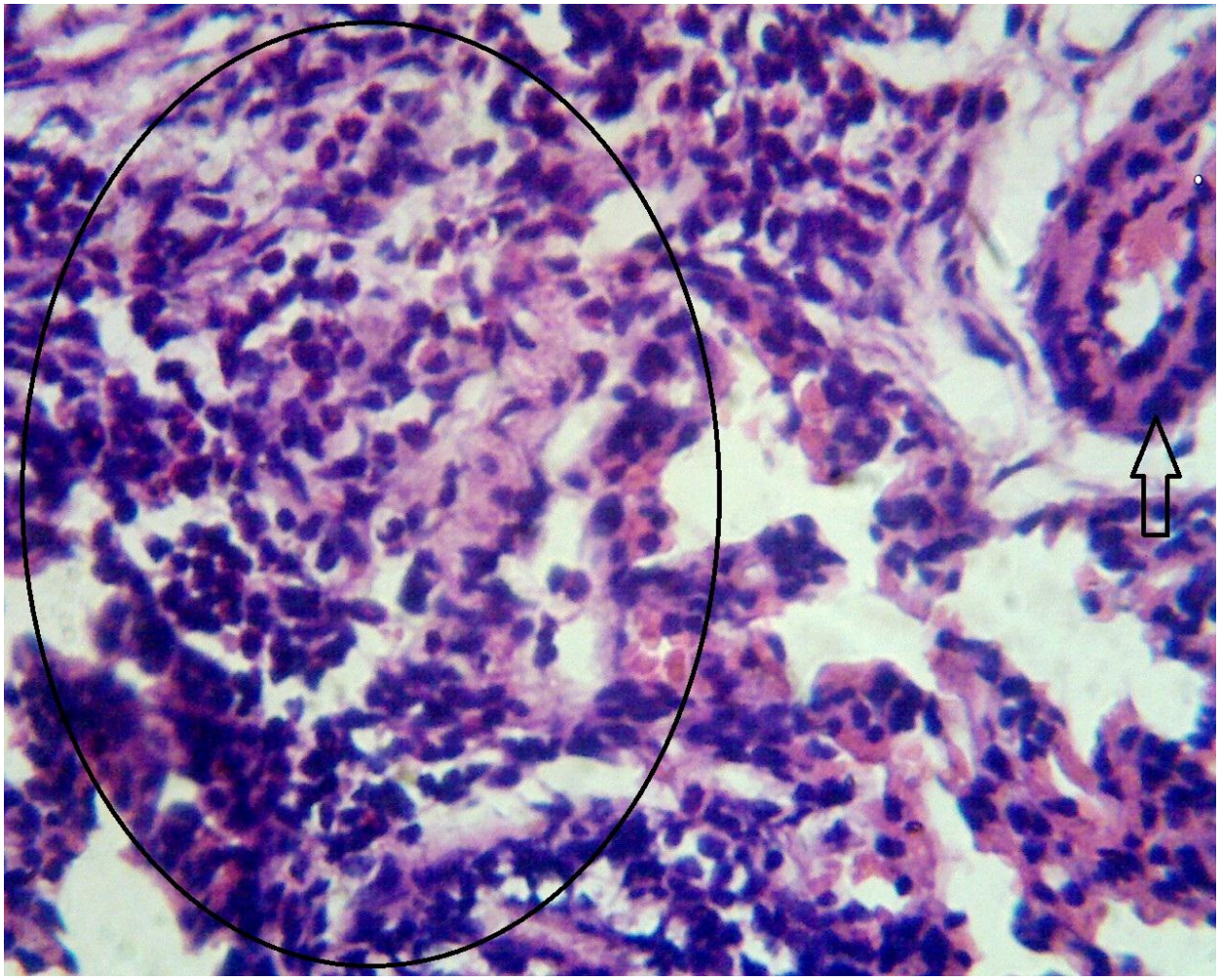
ASB 19 **X400** **HE TECHNIQUE**

Photomicrograph shows section of the lungs tissues marked interstitial inflammatory cells infiltrates. (Circle).



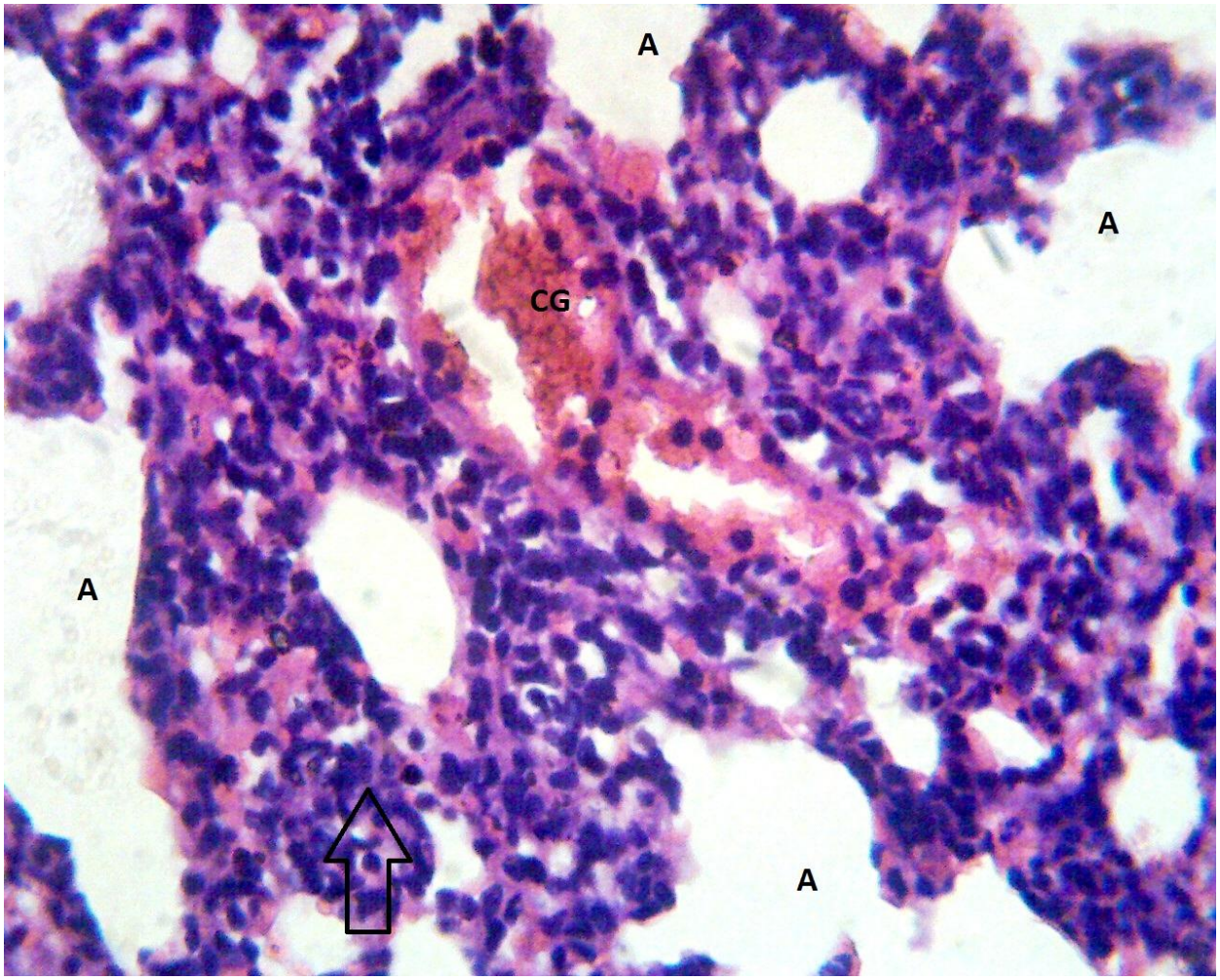
ASB 32 **X400** **HE TECHNIQUE**

Photomicrograph shows asymmetrical medial hypertrophy and perivascular inflammatory cells infilterates.



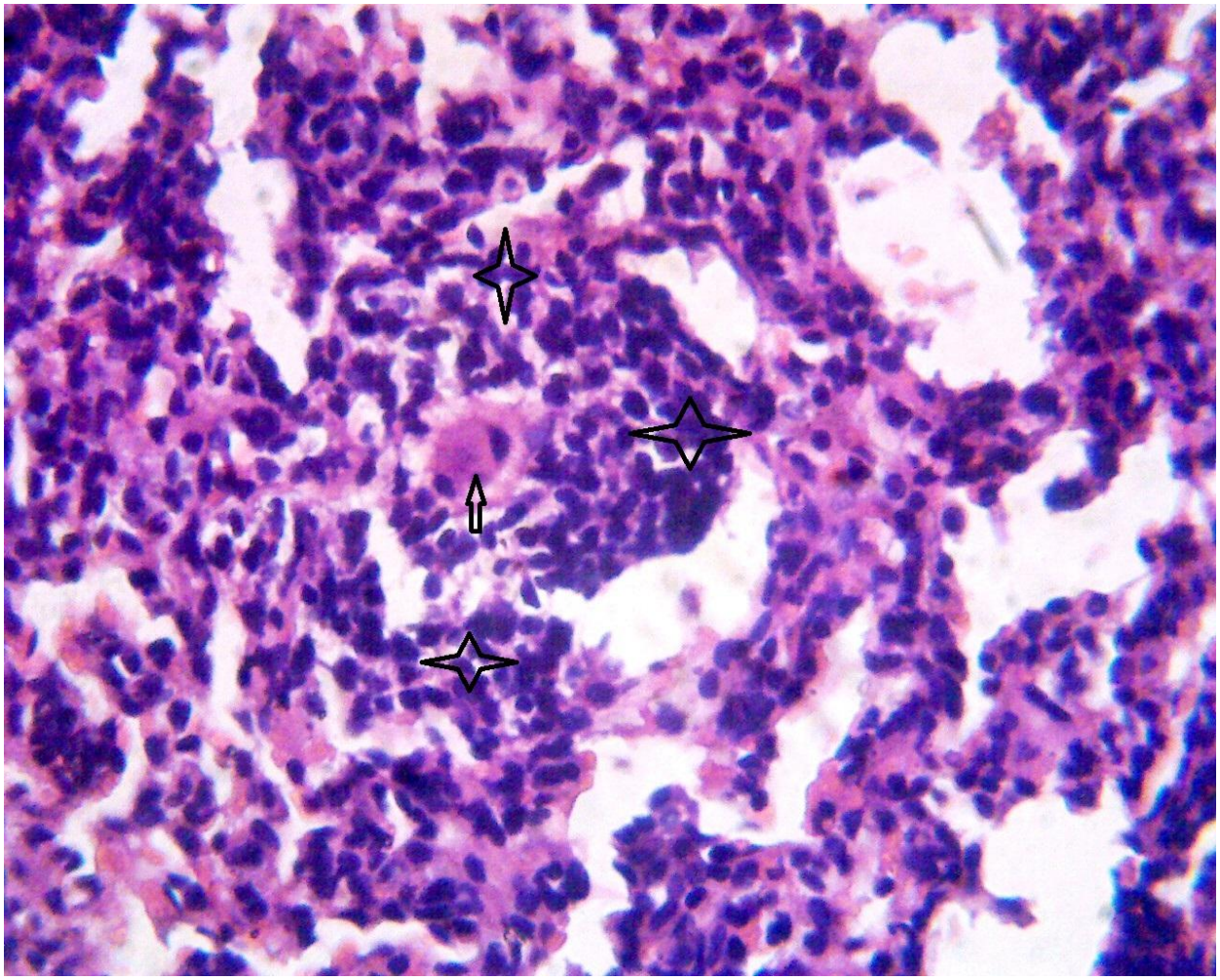
ASB 44 **X400** **HE TECHNIQUE**

Photomicrograph shows section of the lungs tissues marked interstitial, perivascular inflammatory cells infiltrates (Circle) and interstitial congestion.



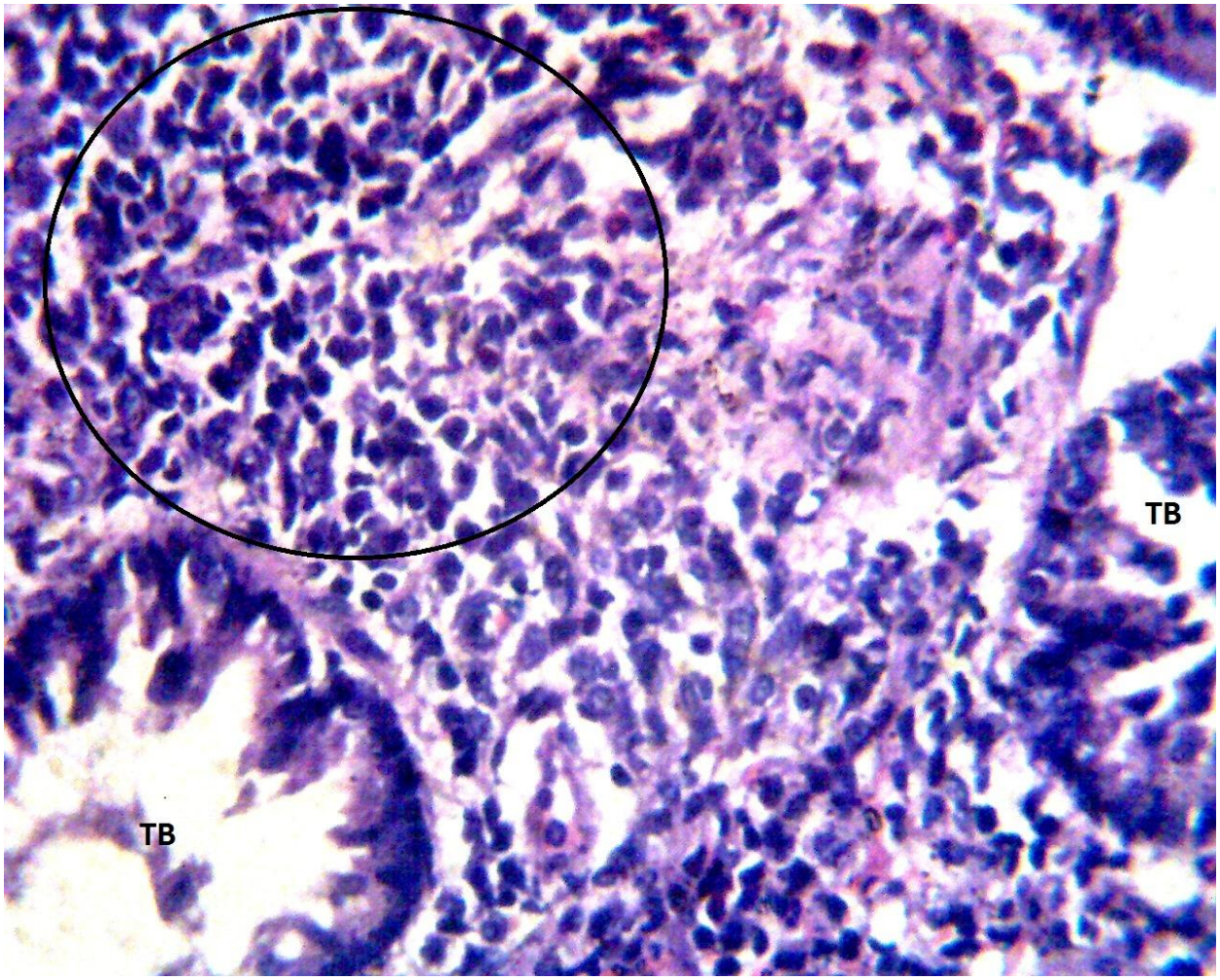
ASB 60 **X400** **HE TECHNIQUE**

Photomicrograph shows section of the lungs tissues marked interstitial, perivascular inflammatory cells infiltrates (arrow) and vascular congestion.



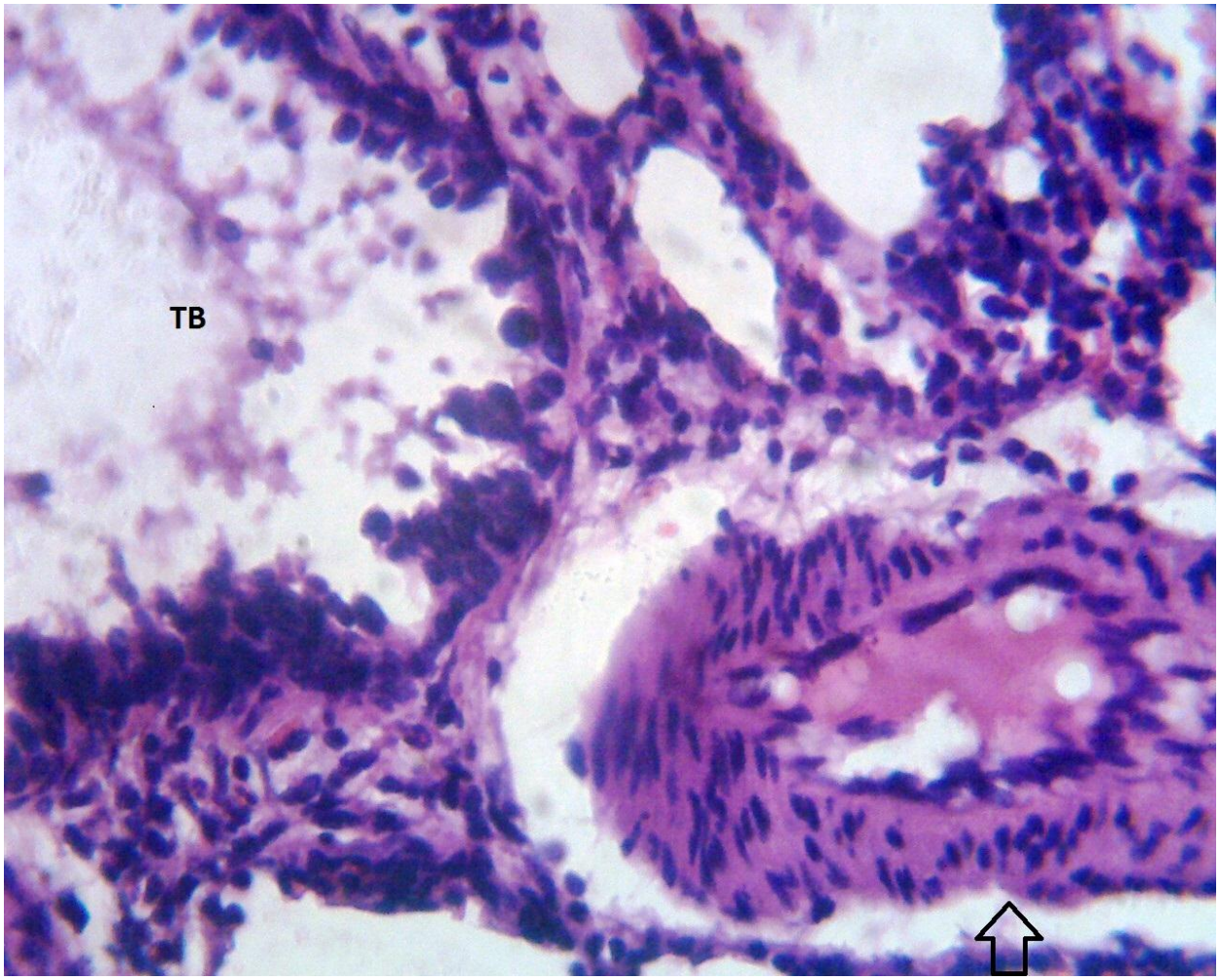
ASB 88 **X400** **HE TECHNIQUE**

Photomicrograph shows section of the lungs tissues marked interstitial, inflammatory cells infiltrates (star). Arrow shows histiocytes.



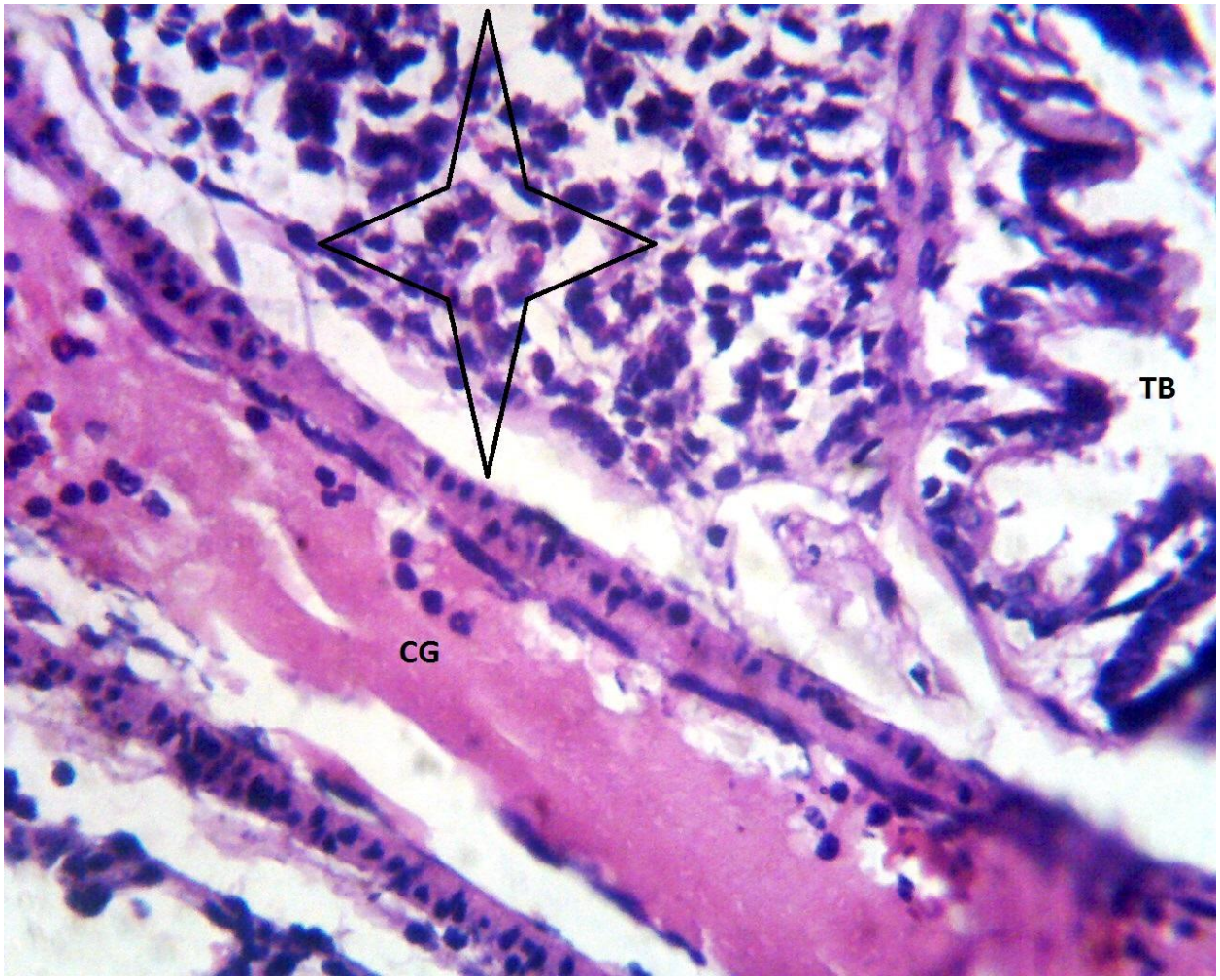
CA 58 X400 HE TECHNIQUE

Section shows marked interstitial inflammatory cells infiltrate (circle) and degeneration of the bronchiole epithelium (TB).



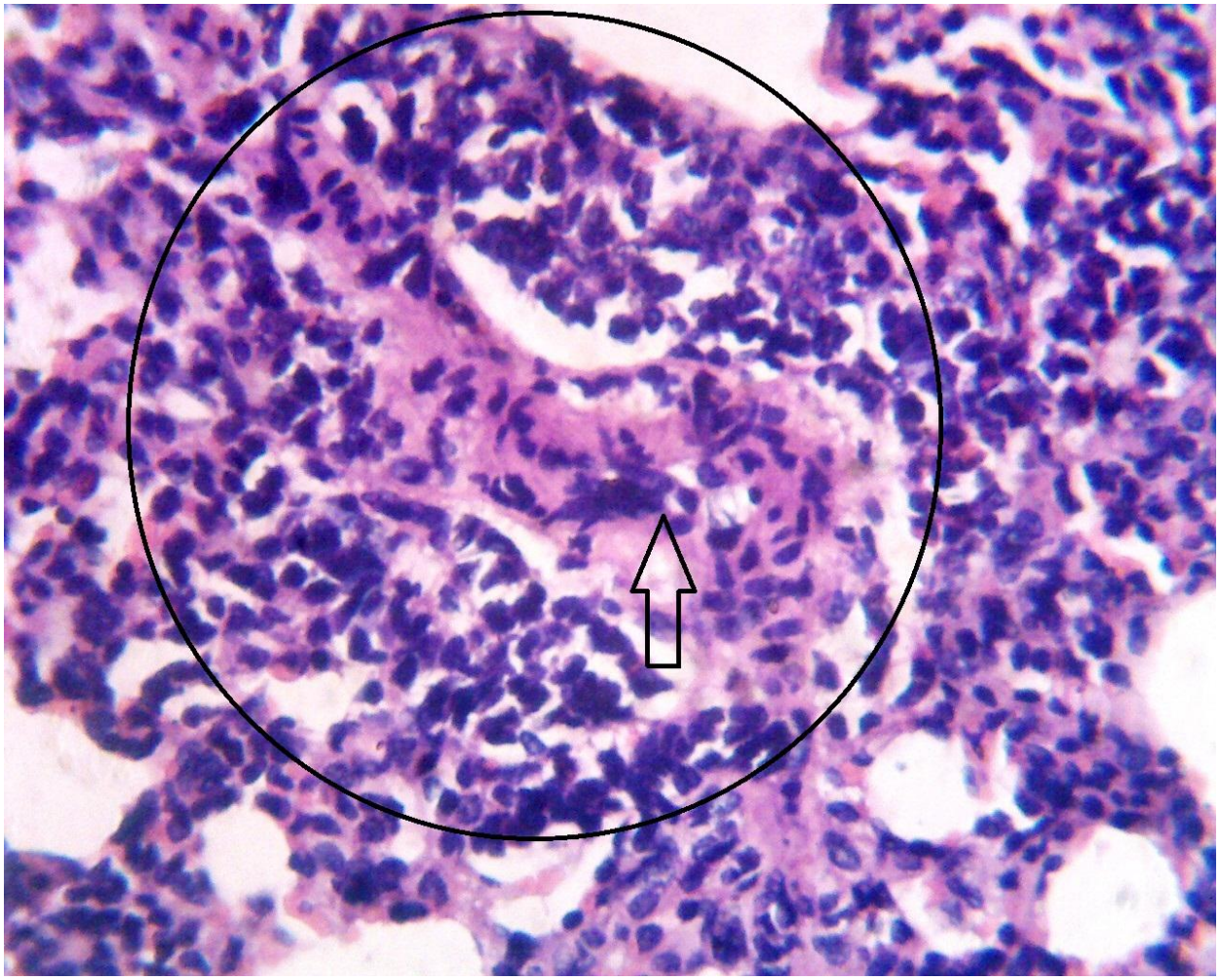
CB 4 **X400** **HE TECHNIQUE**

Section shows marked vascular congestion and epithelia degeneration.



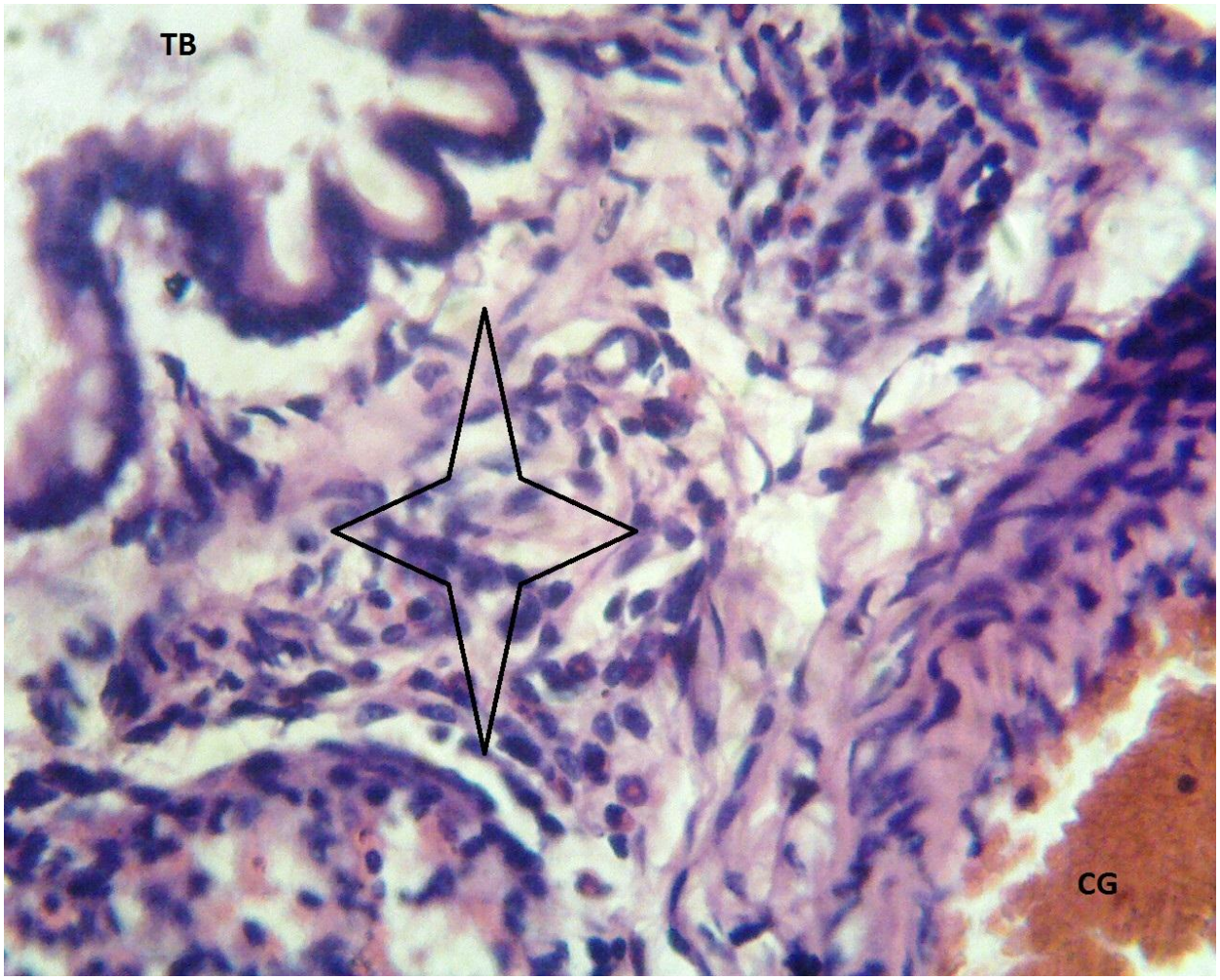
CB 27 X400 HE TECHNIQUE

Photomicrograph shows marked perivascular and interstitial inflammatory cells infiltrates (star) and vascular congestion.



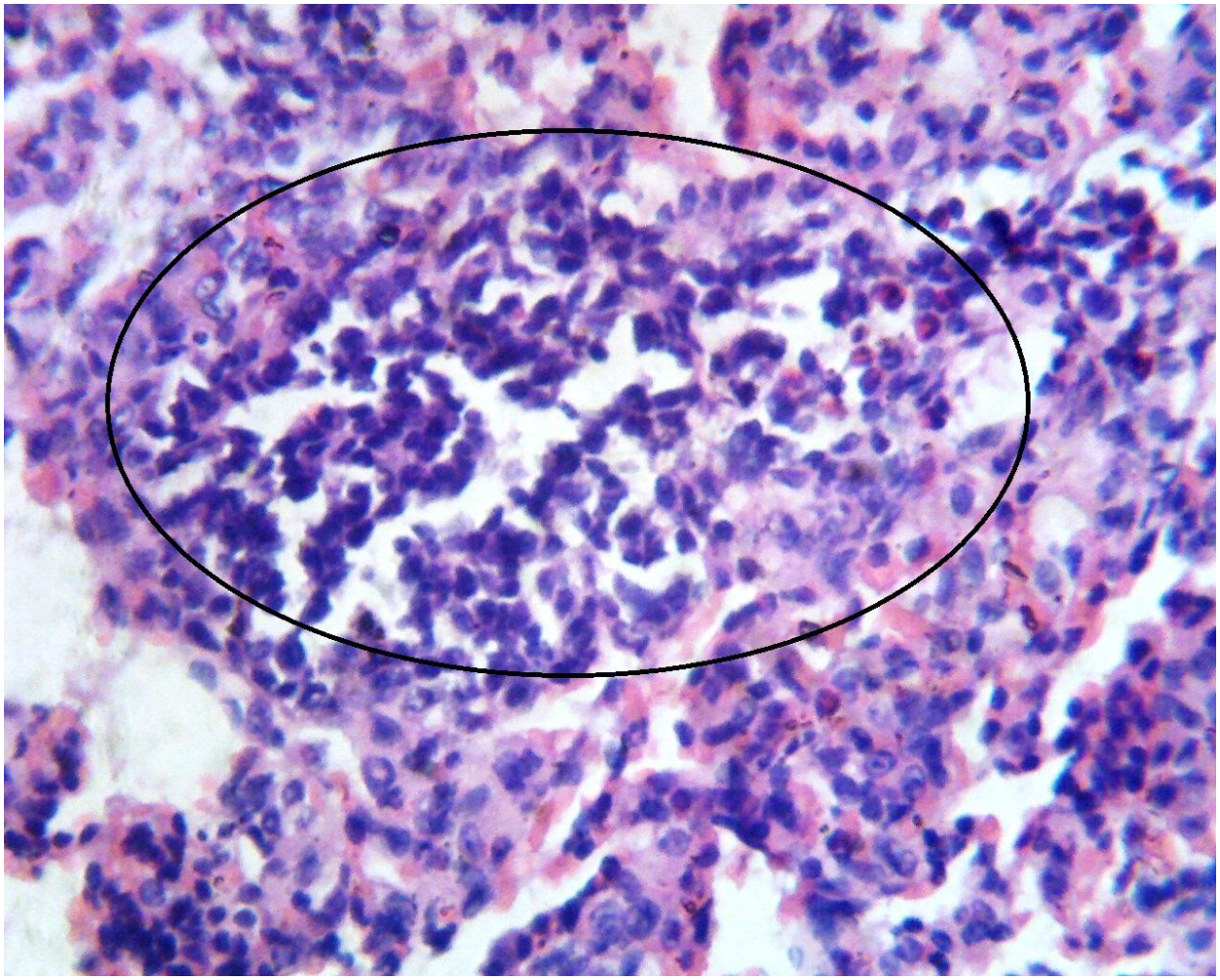
CB 56 X400 HE TECHNIQUE

Section shows marked interstitial inflammatory cells infiltrate (circle) and medial hypertrophy (arrow).



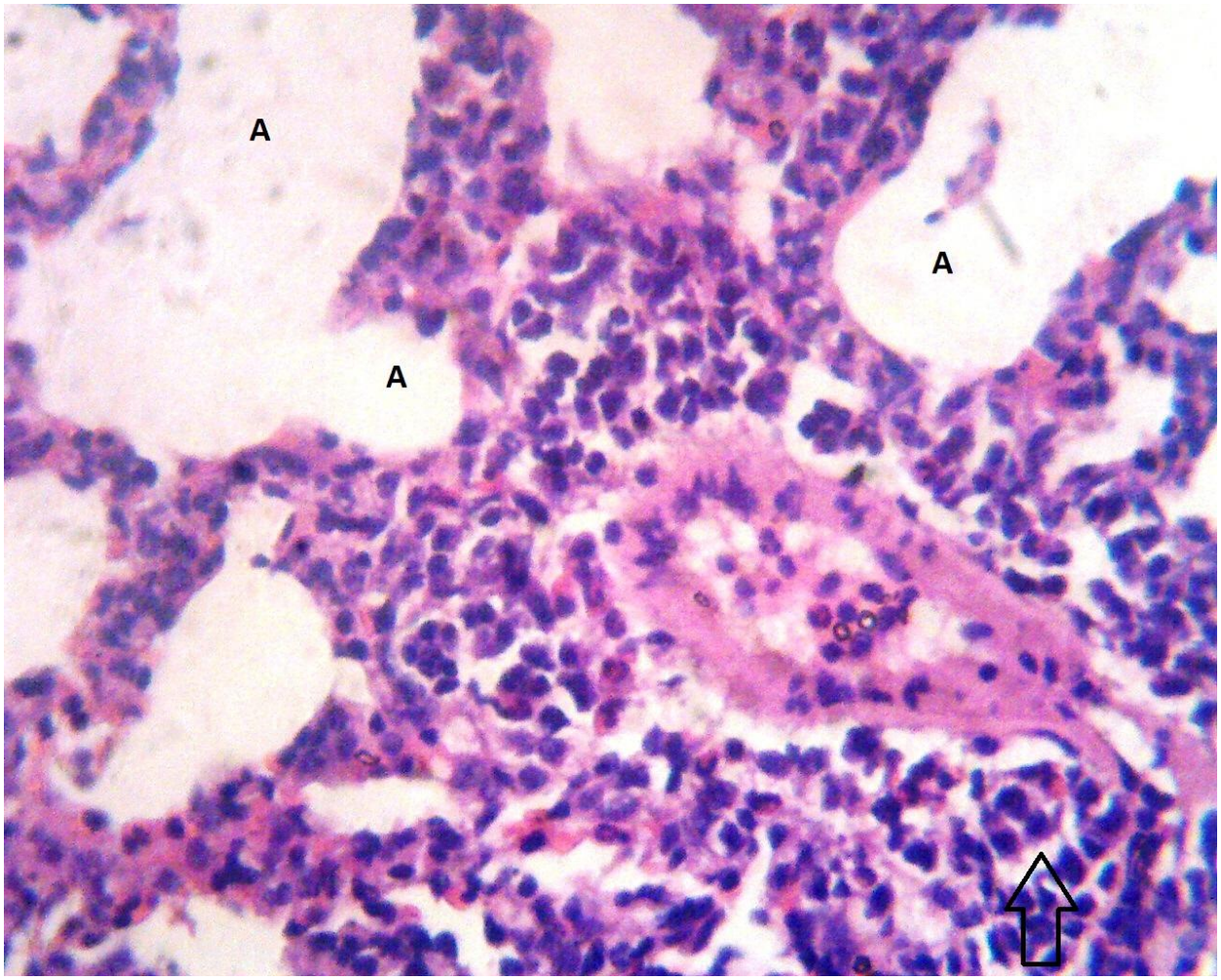
CB 88 **X400** **HE TECHNIQUE**

Photomicrograph shows marked perivascular and interstitial inflammatory cells infiltrates (star) and vascular congestion (CG).



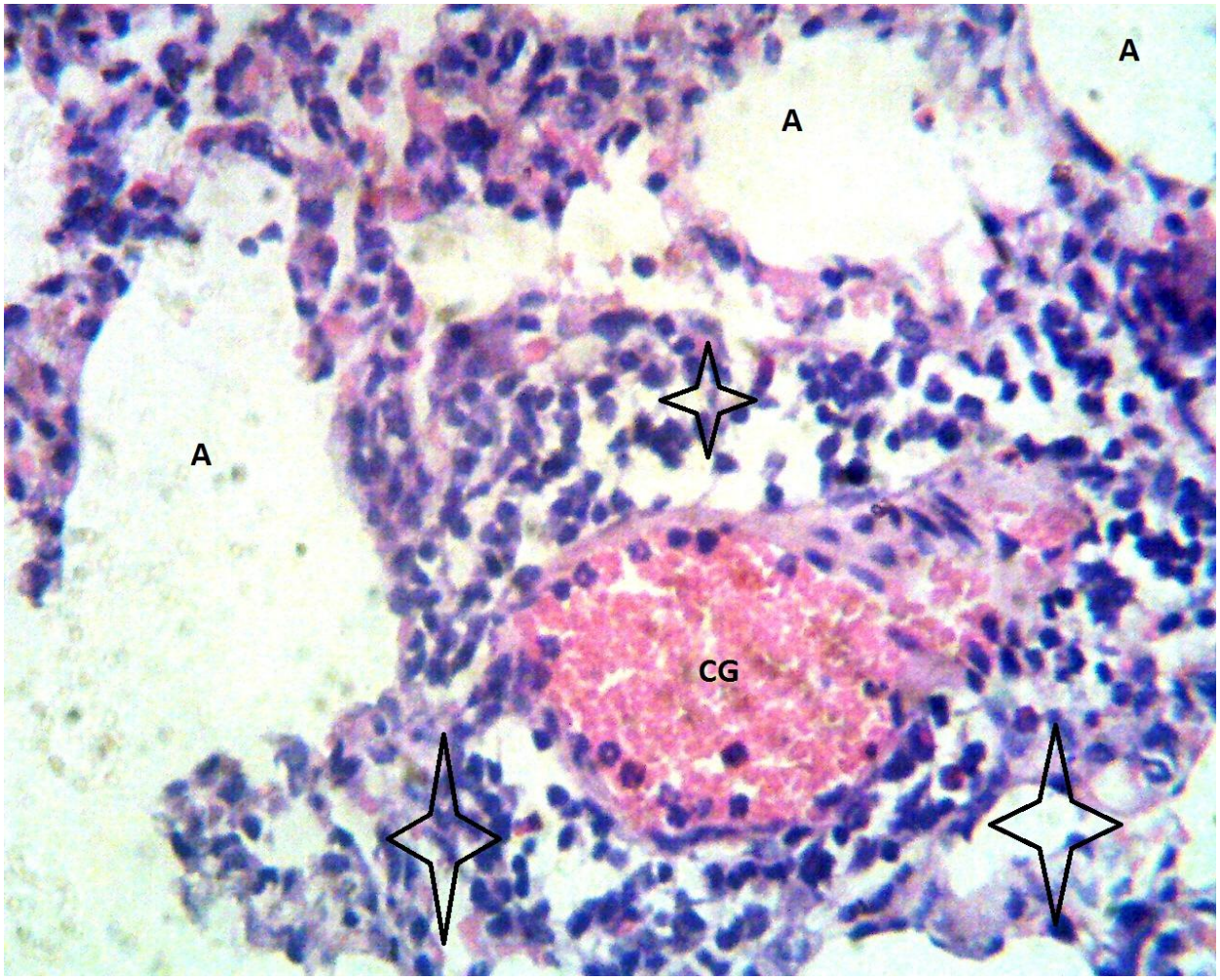
CB 91 X400 HE TECHNIQUE

Photomicrograph shows marked interstitial inflammatory cells infiltrates (circle) and interstitial congestion.



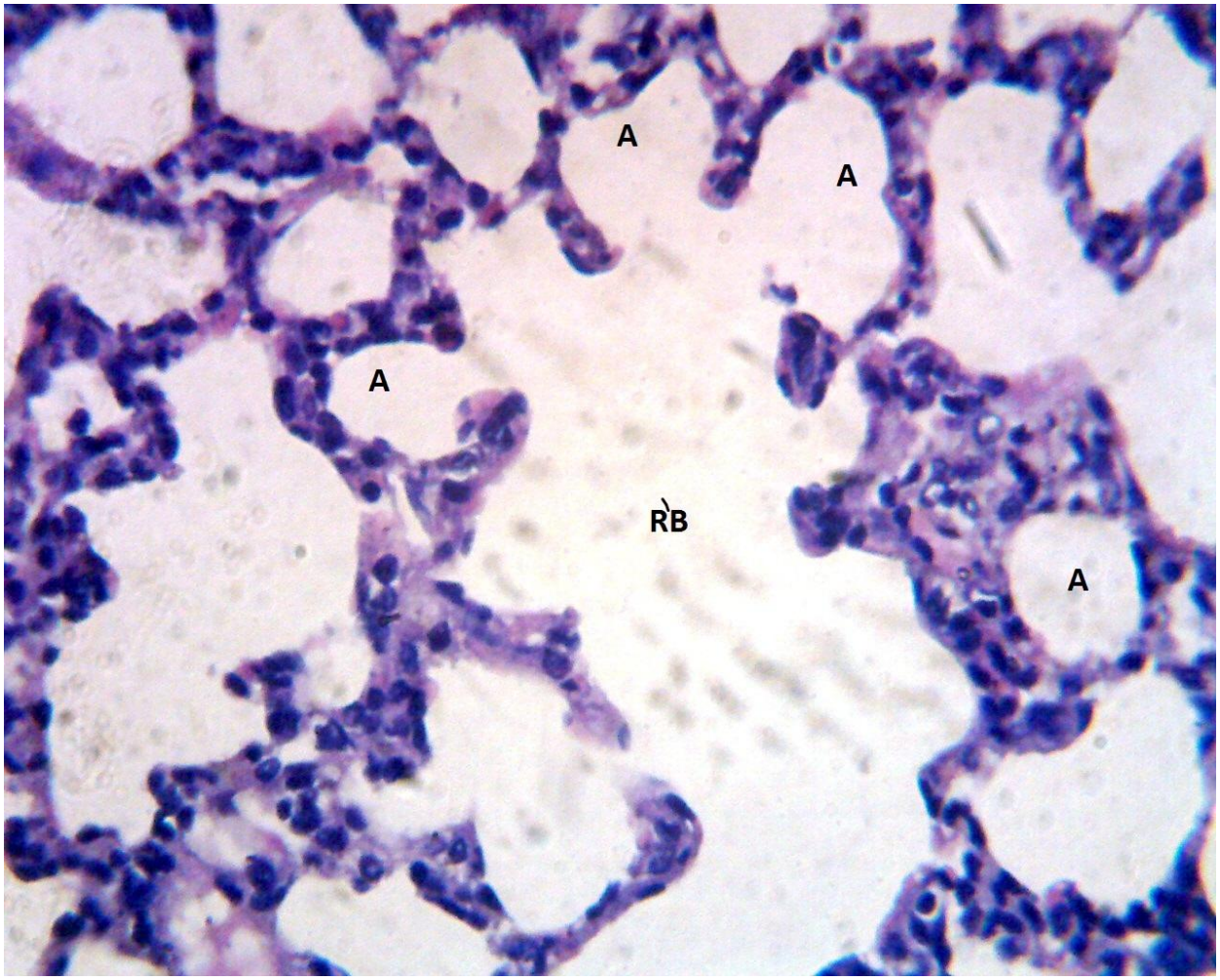
CO 5 X400 HE TECHNIQUE

Photomicrograph shows marked perivascular and interstitial inflammatory cells infiltrates (arrow) and vascular congestion.



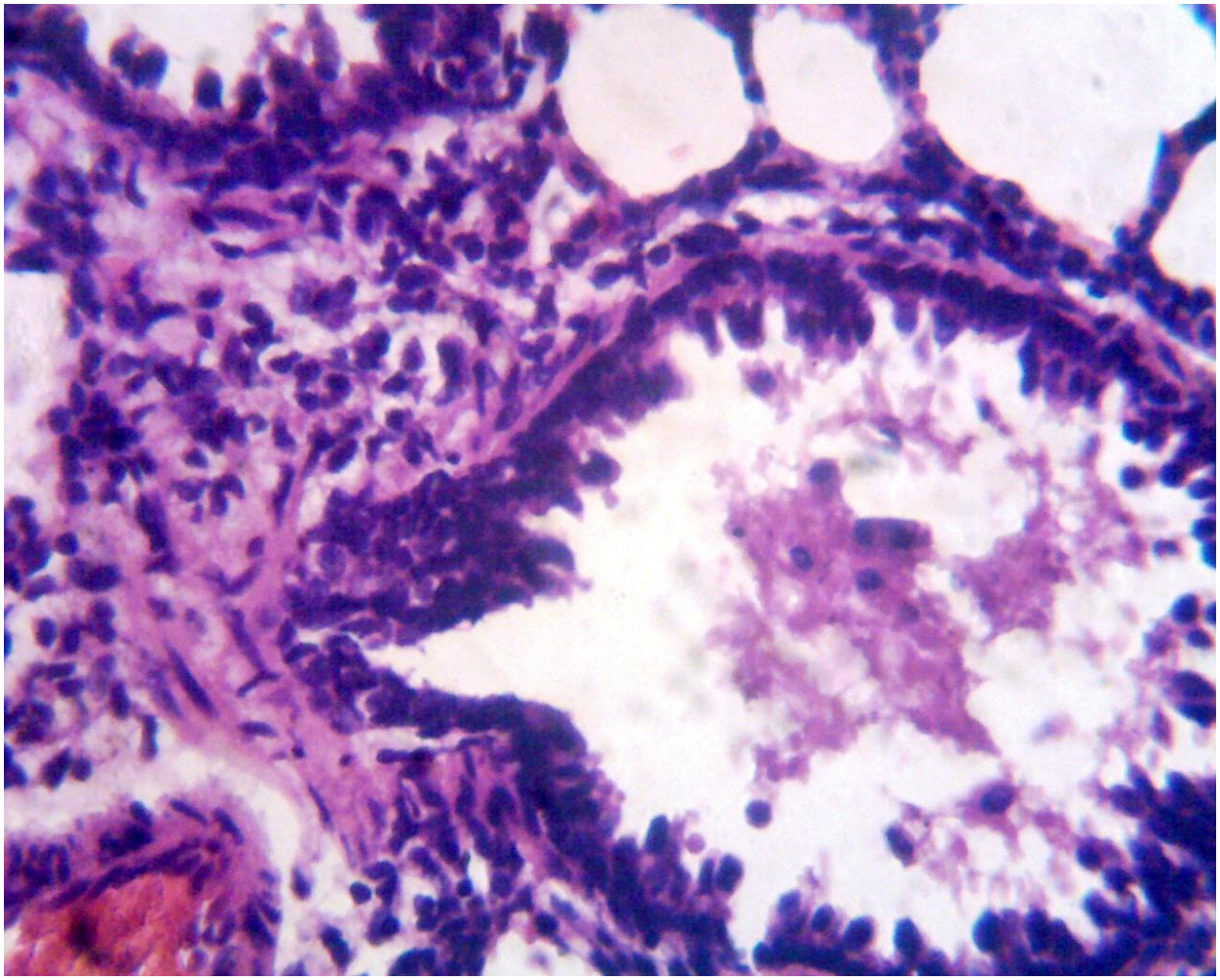
CO 22 X400 HE TECHNIQUE

Photomicrograph shows marked perivascular and interstitial inflammatory cells infiltrates (star) and vascular congestion (CG).



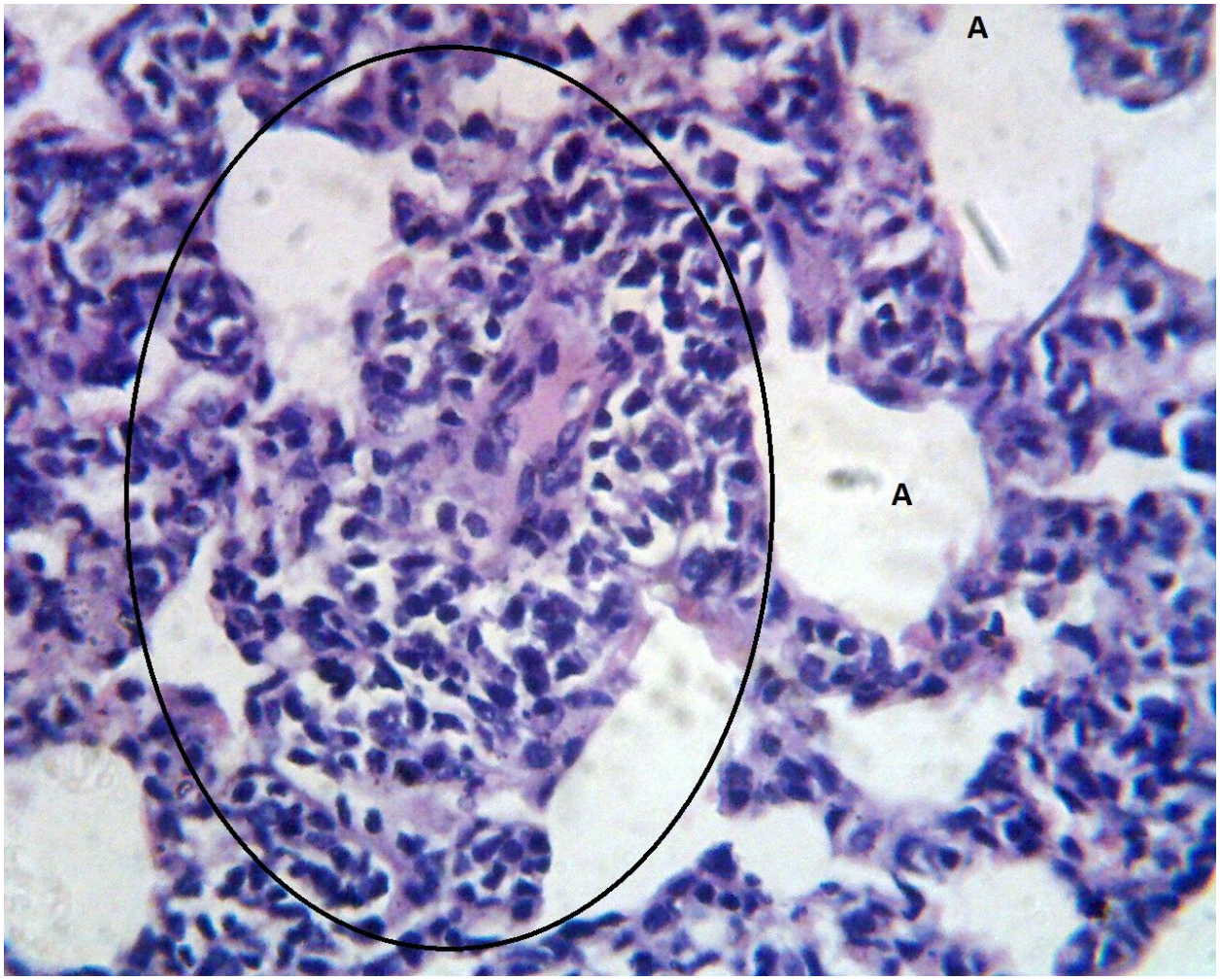
CY 8 **X400** **HE TECHNIQUE**

Sections appear as in control. RB respiratory bronchiole.



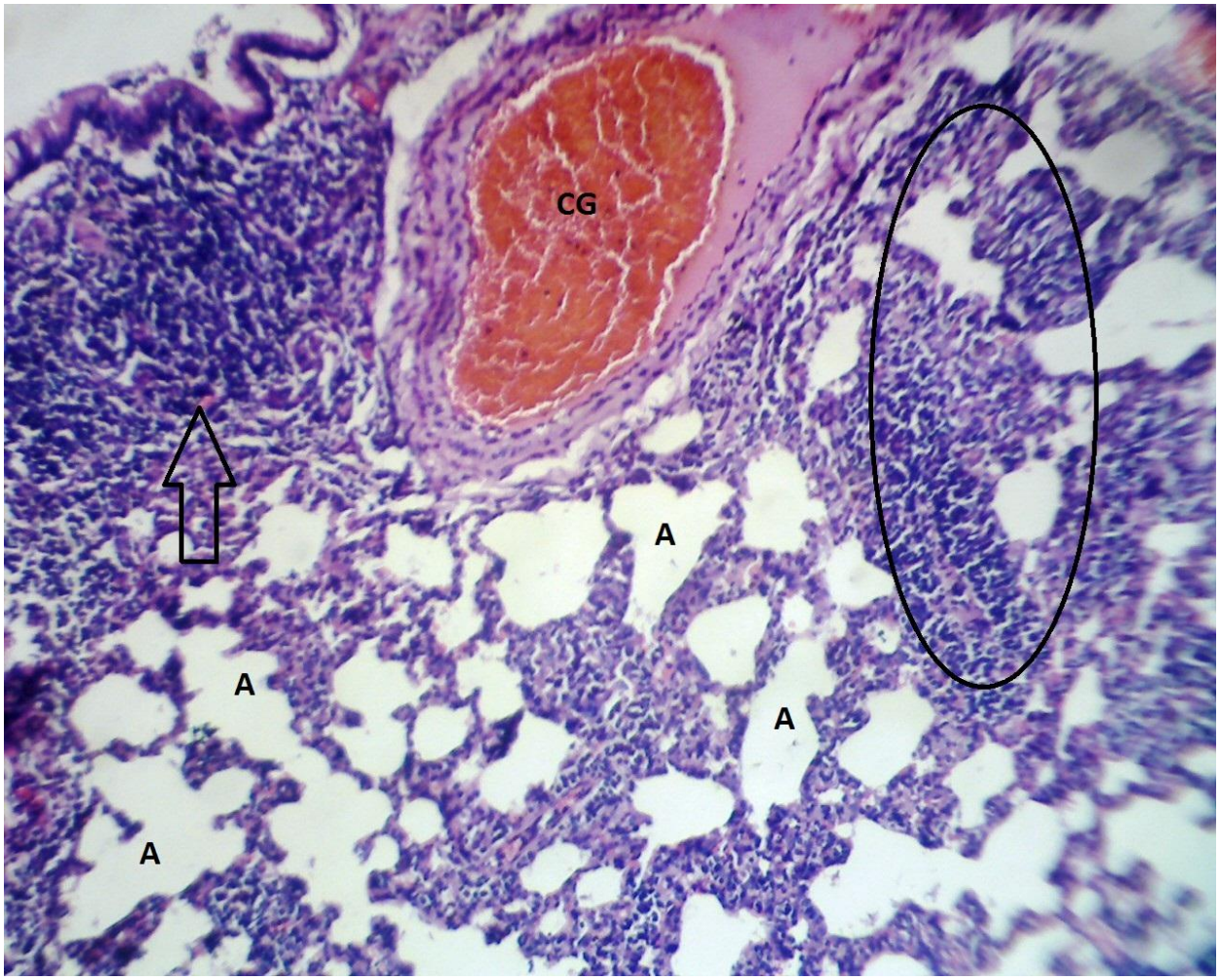
CY 22 X400 HE TECHNIQUE

Photomicrograph shows peri-bronchiole infiltrates,



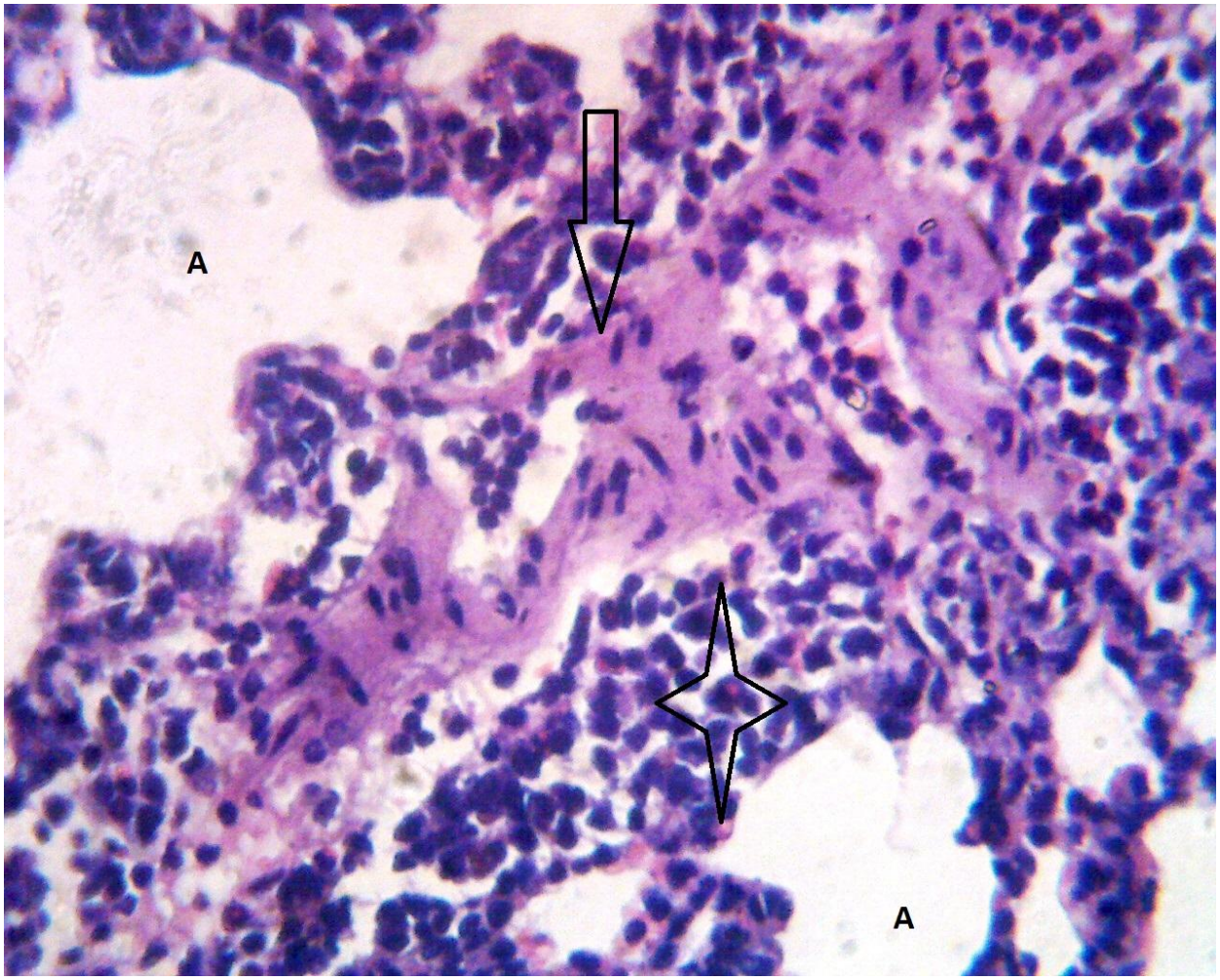
CY 32 X400 HE TECHNIQUE

Photomicrograph shows marked interstitial inflammatory cells infiltrates (circle) and interstitial congestion.



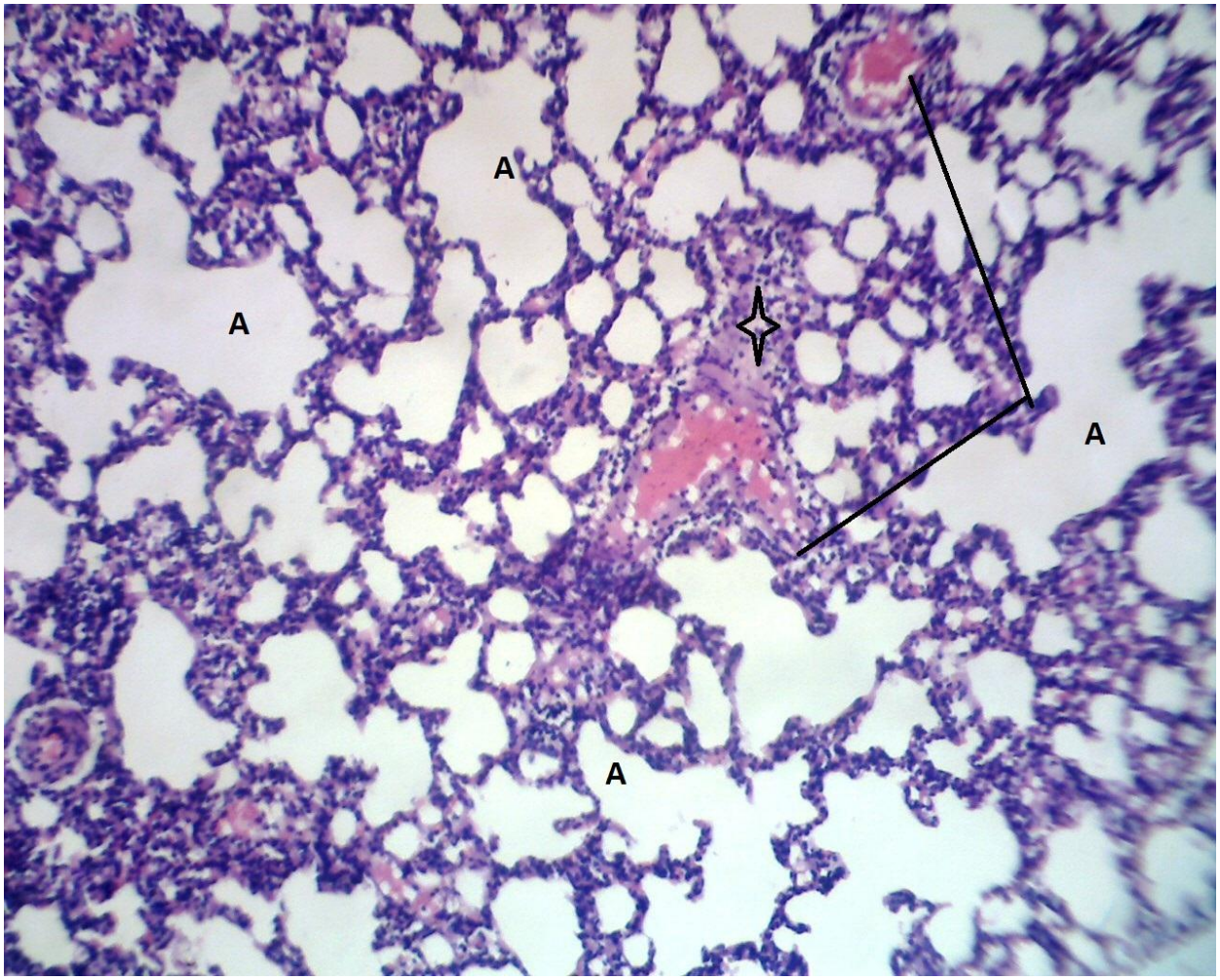
CY 44 **X400** **HE TECHNIQUE**

Photomicrograph shows lymphoid aggregates (arrow), interstitial inflammatory infiltrate (circle) and vascular congestion (CG).



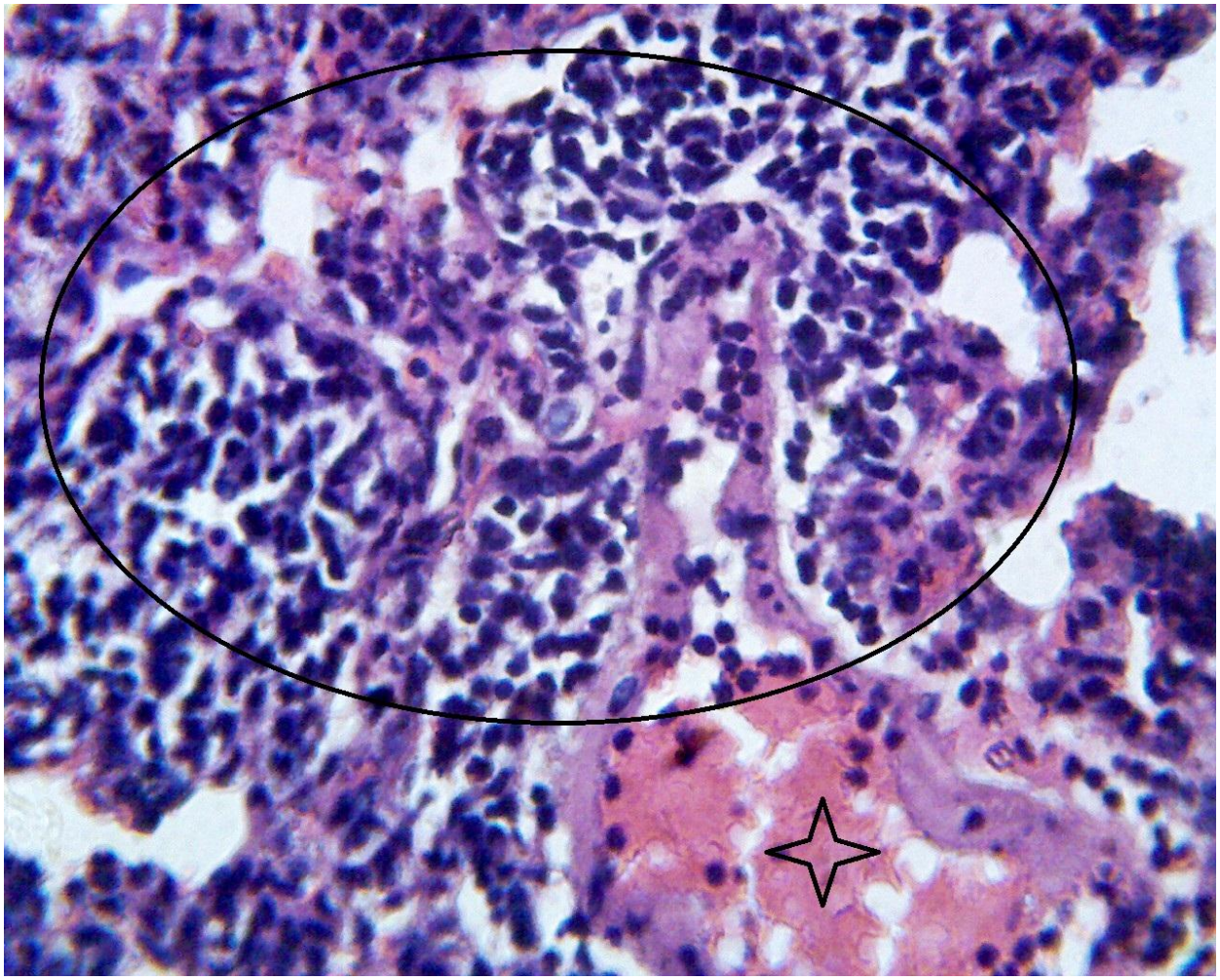
CY 69 X400 HE TECHNIQUE

Photomicrograph shows moderate inflammatory cells infiltrates (Star) and atherosclerosis. (Arrow)



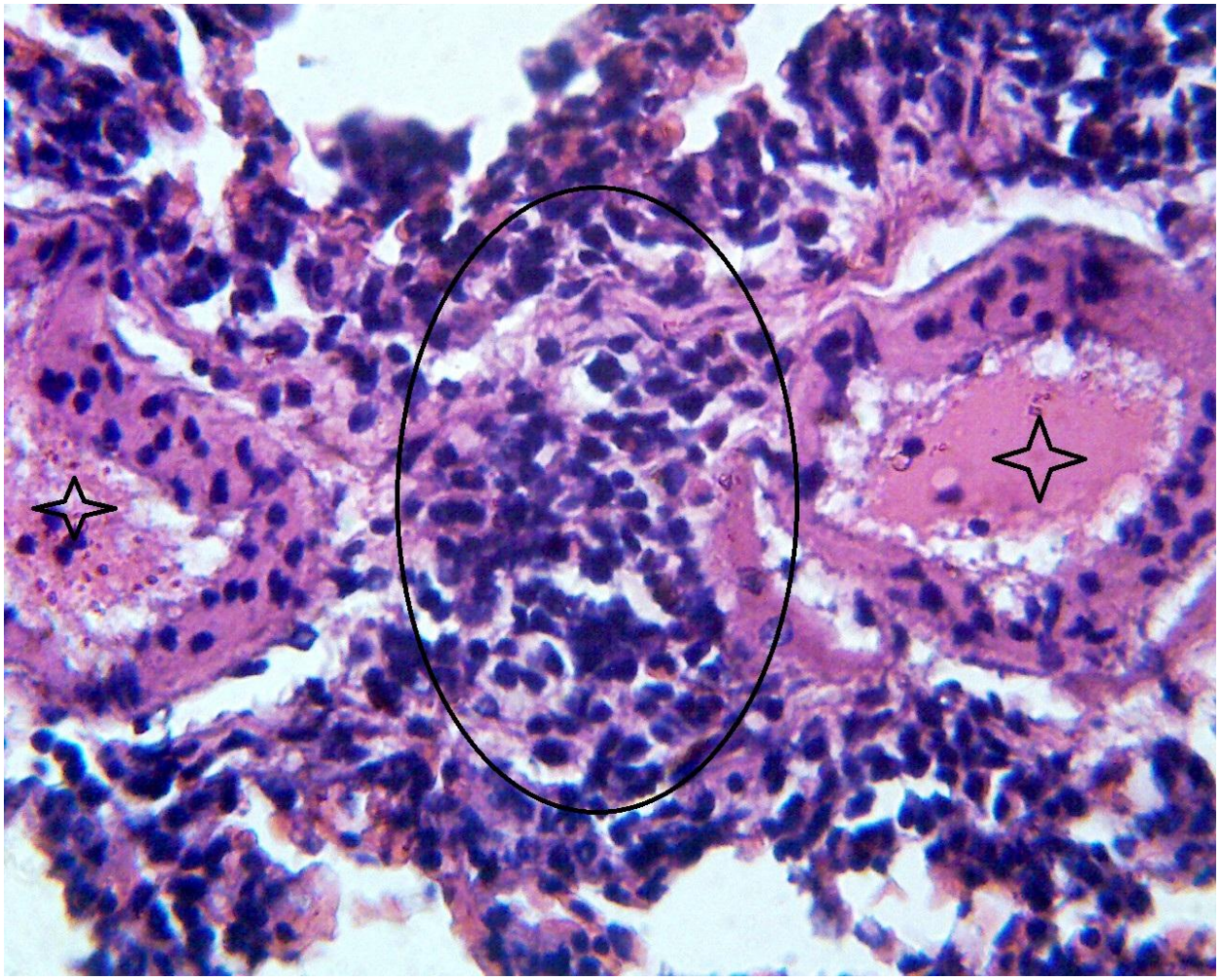
CY 79 **X400** **HE TECHNIQUE**

Photomicrograph shows marked vascular congestion and perivascular inflammatory cells infiltrates (Strar).



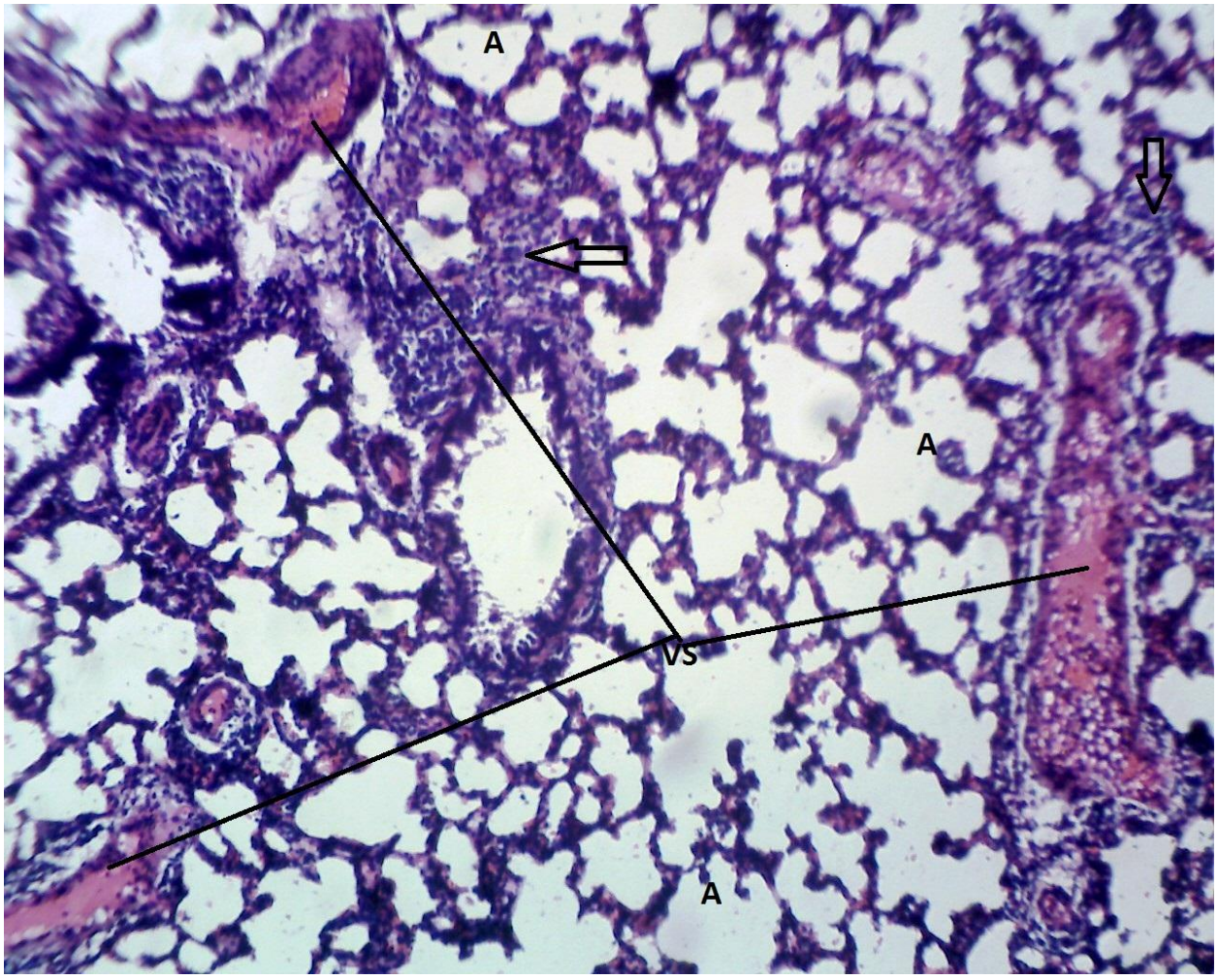
CA 26 **X400** **HE TECHNIQUE**

Photomicrograph shows section of the lungs with marked interstitial inflammatory cells infiltrates (Circle) and vascular congestion (star).



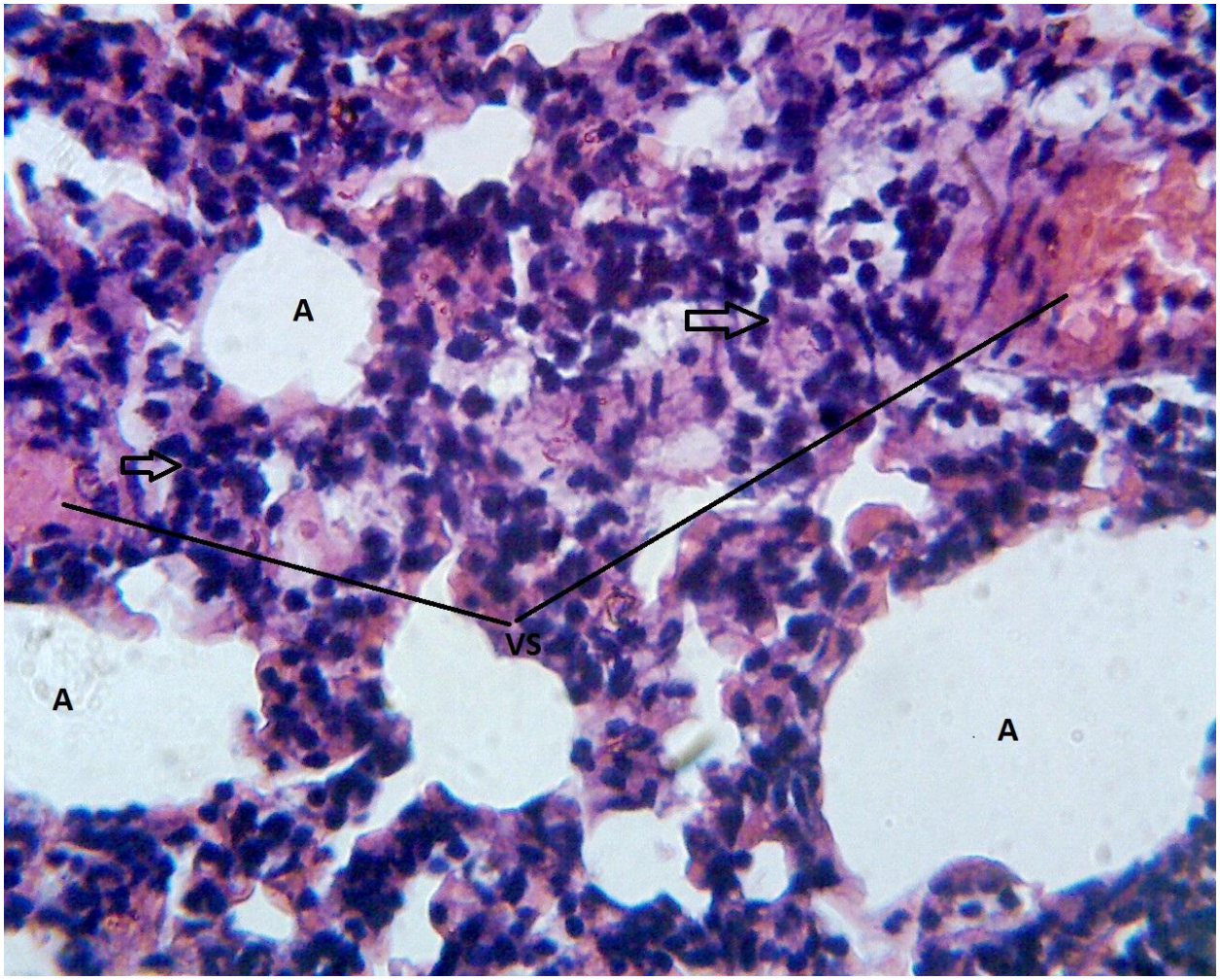
CA 43 **X400** **HE TECHNIQUE**

Photomicrograph shows section of the lungs with marked perivascular inflammatory cells infiltrates (Circle) and vascular congestion (star).



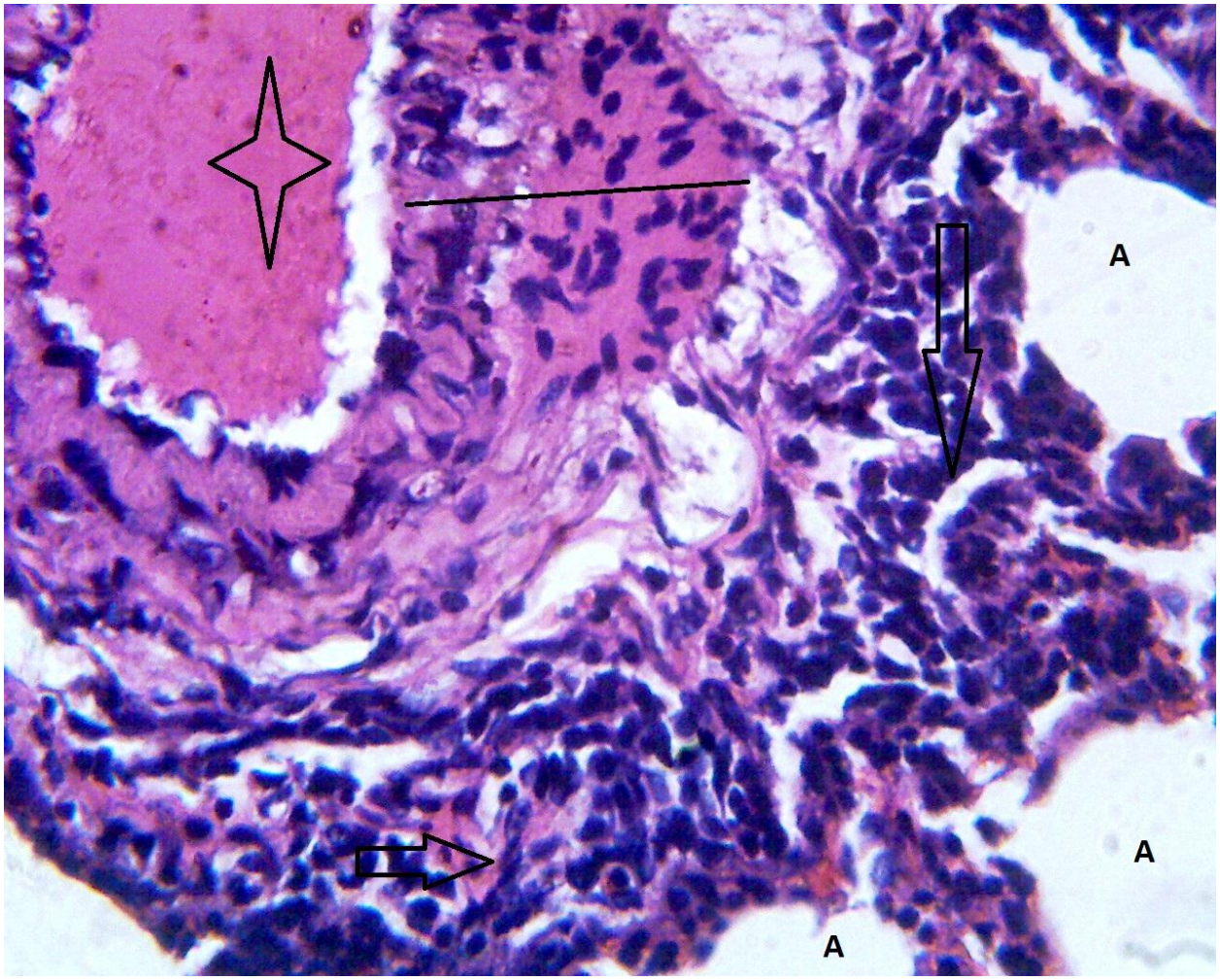
CA 58 **X400** **HE TECHNIQUE**

Photomicrograph shows section of the lungs with vascular congestion (VS), perivascular inflammatory cells infiltrates and peribronchial infiltrates (arrow).



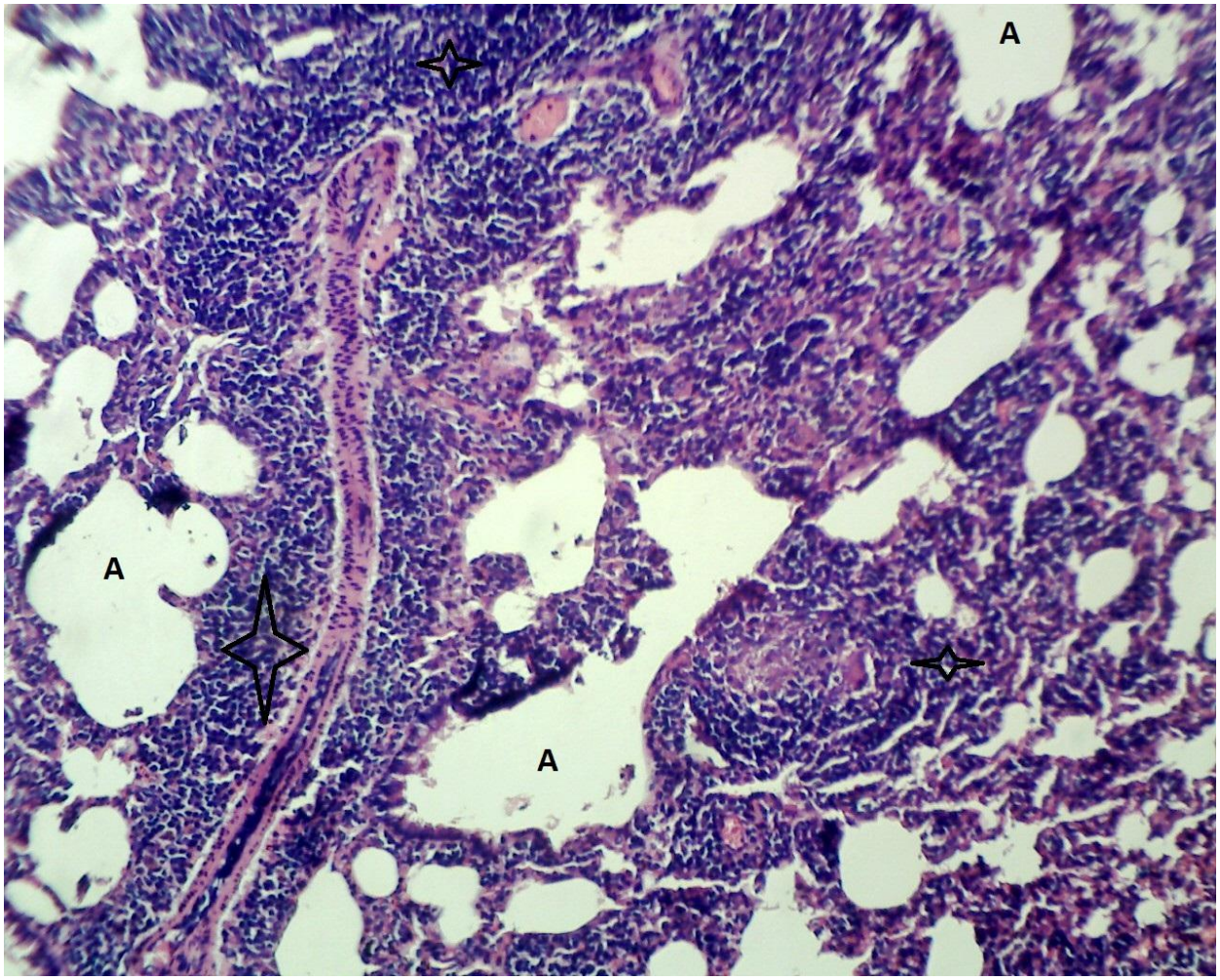
CA 79 **X400** **HE TECHNIQUE**

Photomicrograph shows section of the lungs with vascular congestion (VS) and interstitial inflammatory cells infiltrates.



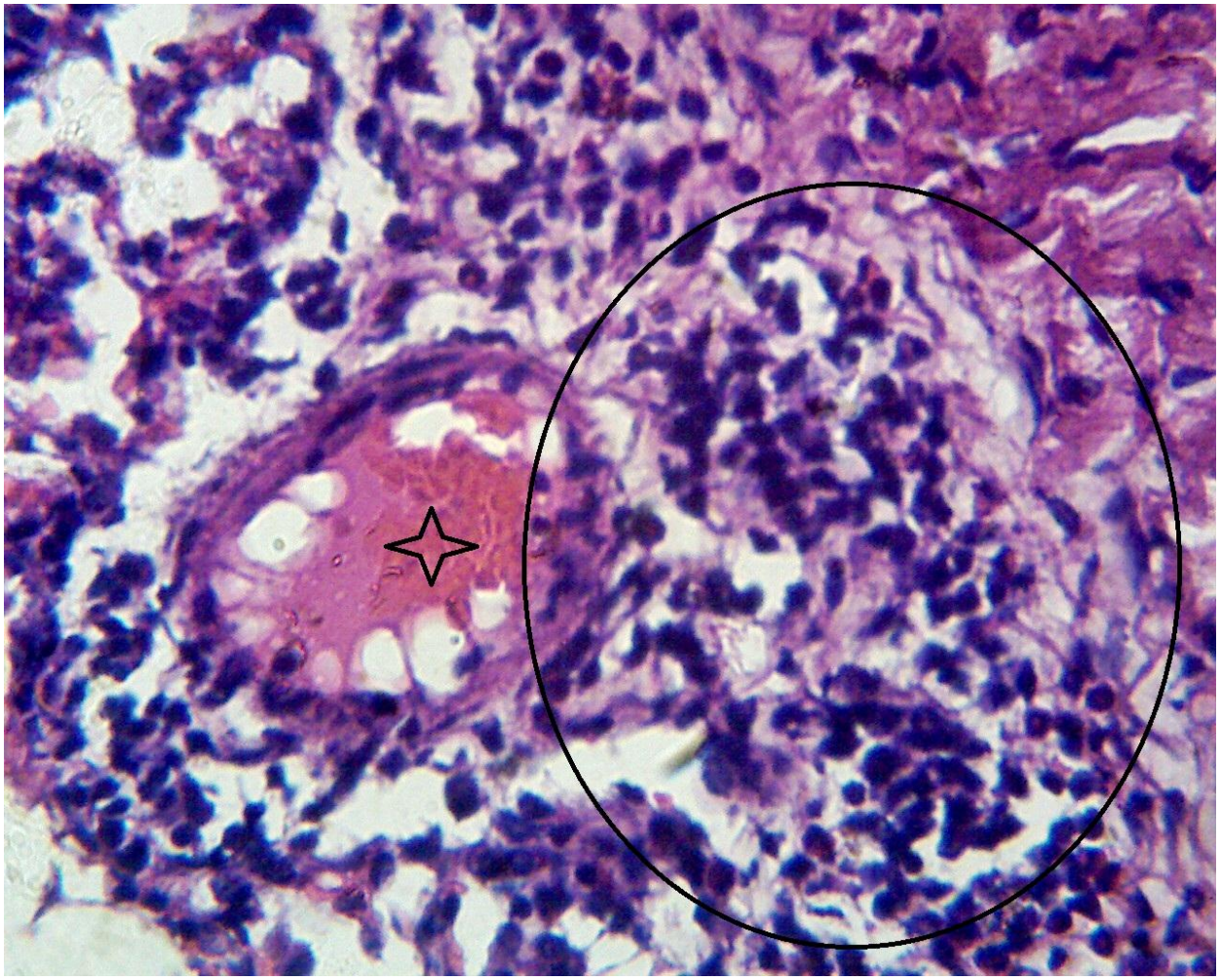
CSA 24 **X400** **HE TECHNIQUE**

Section shows vascular congestion (Star), asymmetrical vascular medial hypertrophy (Line) and perivascular inflammatory cells infiltrations.



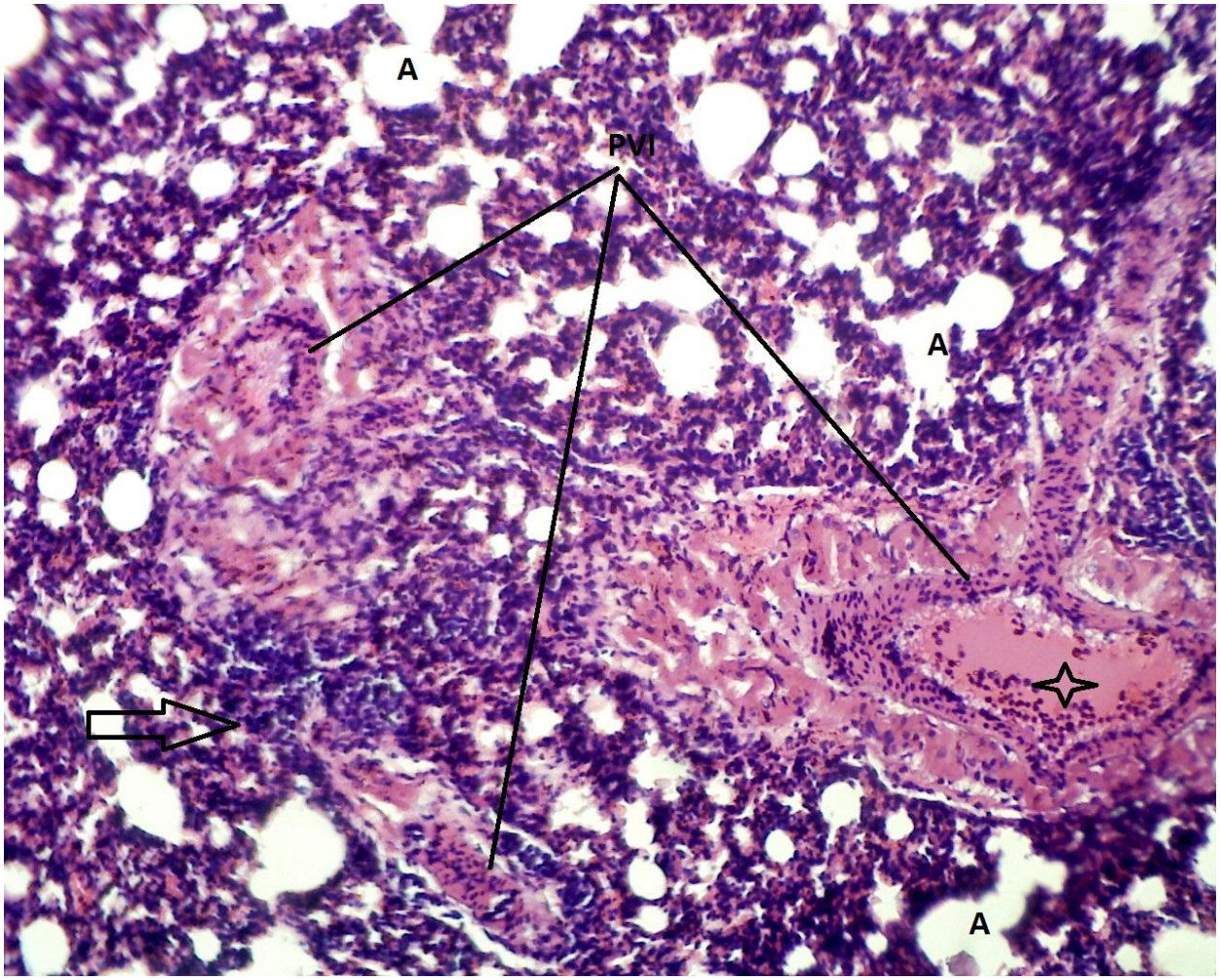
CSA 65 X400 HE TECHNIQUE

Photomicrograph shows marked interstitial lymphoid aggregates (star).



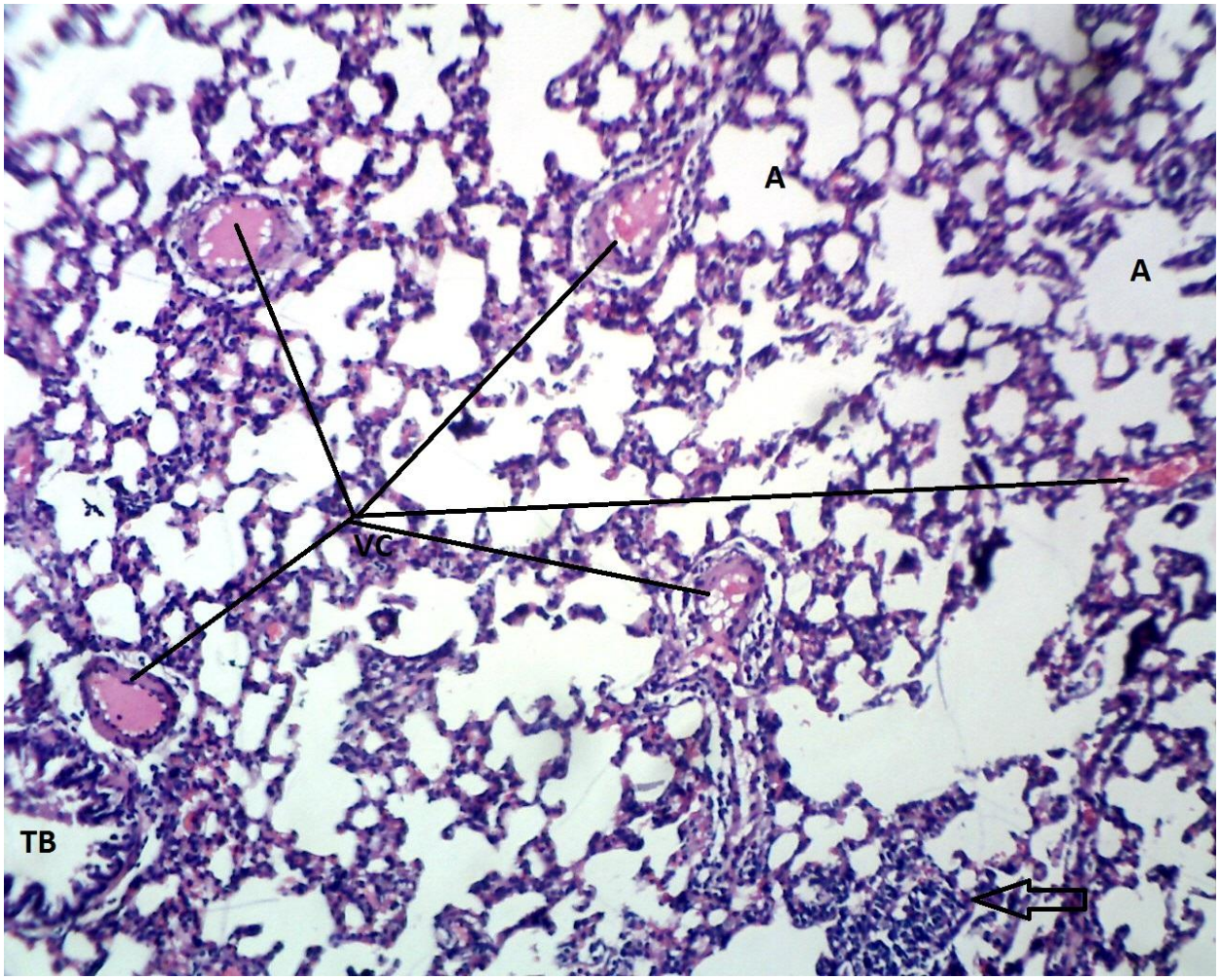
CU 14 **X400** **HE TECHNIQUE**

Photomicrograph shows section of the lungs with marked perivascular inflammatory cells infiltrates (Circle) and vascular congestion (star).



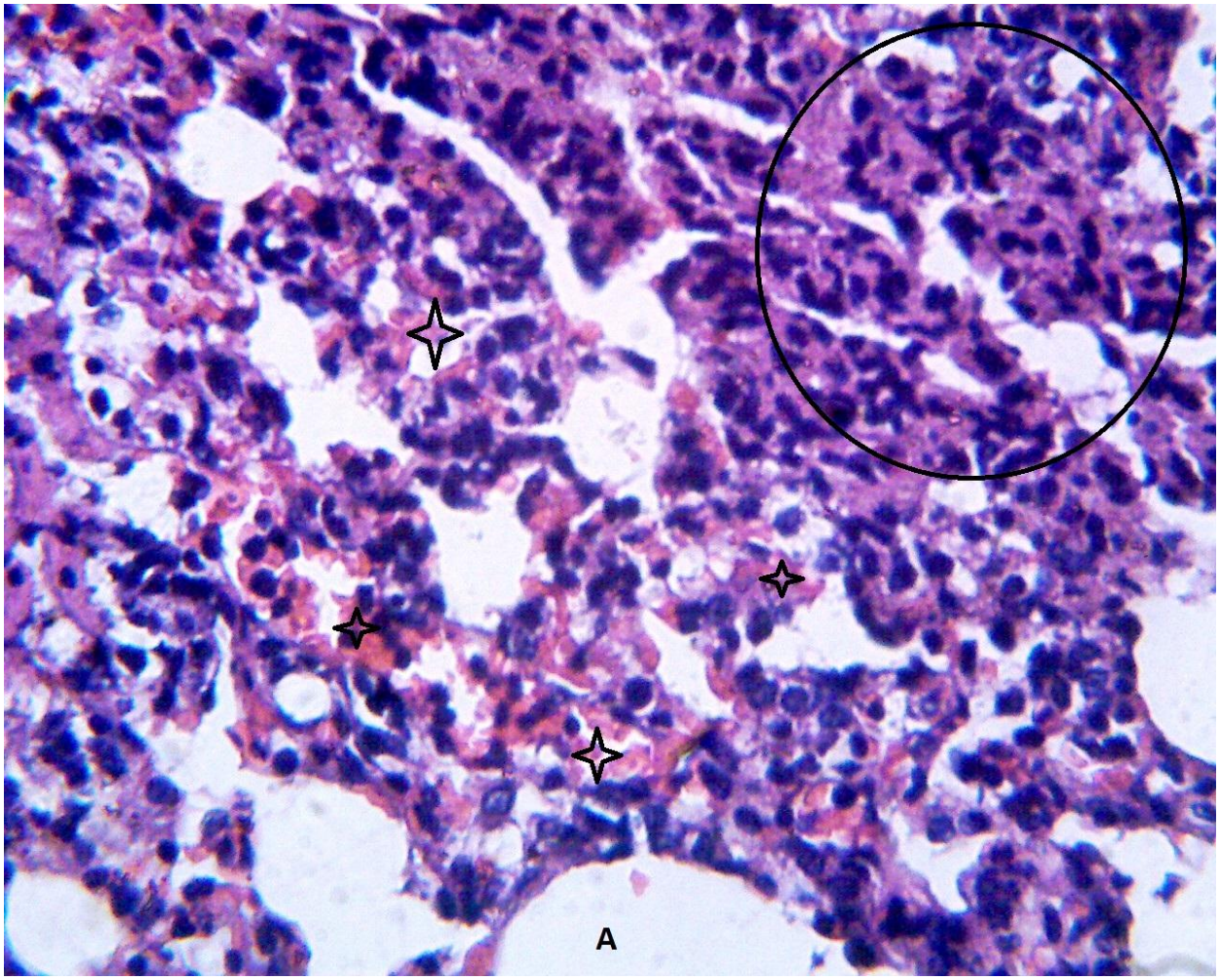
CU 31 **X400** **HE TECHNIQUE**

Photomicrograph shows section of the lungs with marked perivascular inflammatory cells infiltrates (Circle) and vascular congestion (star).



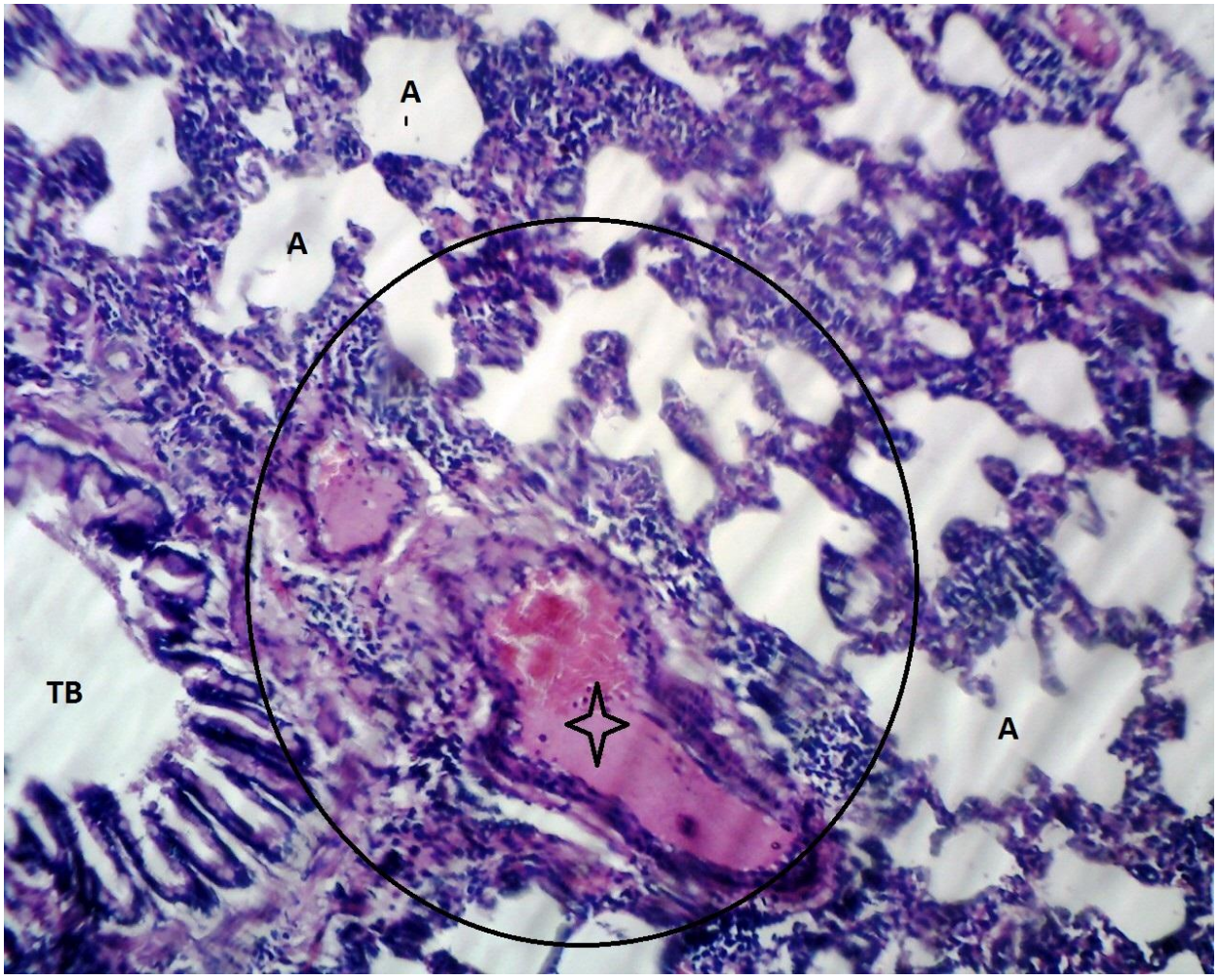
CU 42 **X400** **HE TECHNIQUE**

Photomicrograph shows section of the lungs with marked vascular congestion (vc) and interstitial inflammatory cells aggregations (arrow).



CU 70 X400 HE TECHNIQUE

Photomicrograph shows marked interstitial congestion (star) and interstitial inflammatory cells.



SAP 90 **X400** **HE STAIN**

Section shows the lung tissue with marked congested blood vessel (CG), and chronic infiltrates of inflammatory cells (Neutrophils, circle). The alveoli (A) are lined by regular epithelium.