

Original Research Article

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A Study of Correlation of Sputum Direct Smear and Sputum Culture in detection of Fastidious Organisms in Patients with Bronchitis in a Tertiary Care Hospital at Salem

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ABSTRACT

Sputum examination is a simple and rapid diagnostic tool for the presumptive identification of pathogens. It is one of the oldest and most entrenched technique still in use. Identification of fastidious organisms is important in diagnosis of infections which causes serious diseases whose detection and monitoring is crucial in treating many cases. Direct gram staining places a very important role in identifying these fastidious organisms like *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Legionellae* which may not grow in routine bacterial cultures. Correlation between direct gram staining and sputum cultures helps us to identify these fastidious organism which is crucial for initiation of treatment. This is a descriptive study conducted in the bronchitis patients admitted in the medical ward of GMKMCH, Salem for 3 month period from August, 2022 to November, 2022. 216 sputum samples collected and direct smear and bacterial culture done for the detection of fastidious organisms. 216 sputum samples were collected and direct smear by gram staining done. By bartletts scoring 182 samples were included. Out of 182 samples 76 stained smear showed Gram positive cocci in 23 smears and Gram negative bacilli in 49 smears. Out of 123 plated samples, GPC isolated was 21, GNB isolated was 90 and *Candida* spp was 12. Only 3 *Streptococcus pneumoniae* were isolated. In the present study, good quality sputum samples was obtained in 182 (85%) of 216 patients, which is a higher yield than reported in previous studies. An additional benefit of sputum Gram stain is that it can validate the subsequent sputum culture results. The growth of an organism from a sputum sample does not always indicate the presence of infection. Results of sputum culture can yield false positive findings related to colonization or contamination, and thus should be utilized with the results of the sputum Gram stain when establishing the definitive etiologic diagnosis of pneumonia. Vancomycin is the highest sensitive drug with 100% sensitivity. 33% of the isolates are sensitive to penicillin and 66% are resistant. sensitivity to Tetracycline are 33% and only 33% of the isolates are sensitive to Erythromycin. 66% of the isolates are sensitive for Ofloxacin and least sensitivity is observed for Cotrimoxazole.

Keywords

Pathogens,
infections,
Streptococcus pneumoniae,
Haemophilus influenzae

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Introduction

Sputum examination is a simple and rapid diagnostic tool for the presumptive identification of pathogens. It is one of the oldest and most entrenched techniques still in use (Naoyuki Miyashita *et al.*, 2014). Identification of fastidious organisms is important in diagnosis of infections. A fastidious organism is any organism that requires complex nutritional requirements and conditions. These bacteria cause serious diseases whose detection and monitoring is crucial in treating many cases. Direct gram staining plays a very important role in identifying these fastidious organisms like *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Legionella* which may not grow in routine bacterial cultures.

Correlation between direct gram staining and sputum cultures helps us to identify these fastidious organisms which is crucial for initiation of treatment. Exacerbations of COPD have considerable impact on health care system at both primary and tertiary care levels as they are the major reason for antibiotic use and admissions. WHO has estimated that 600 million people worldwide have COPD. Additionally, exacerbations lead to indirect costs because of days lost from work.

COPD affects 30% of patients seen in chest clinics and constitutes 1-25% of hospital admissions all over India (Thiruvengadam *et al.*, 1974). In the present investigation, we attempted to determine the likelihood that the microbiological study of sputum would identify the causative agent in bacterial pneumonia, specifically by studying the frequency with which microscopic examination of Gram-stained sputum specimens ("Gram staining") and bacterial culture yielded the correct diagnosis in a large number of cases.

Infections are the important cause of acute exacerbation. Bacteria are responsible for causing 60% of exacerbations. Viral infections are the likely cause of approximately 30% of exacerbations. A European survey found that sputum analysis of exacerbated patients is requested only in 10% of

cases (Relationship Between Bacterial Flora in Sputum and Functional Impairment in Patients With Acute Exacerbations of COPD Chest, 1999).

Over 90% of patients with bronchitis are treated with antibiotics, on empirical basis without proper sputum analysis so the effectiveness of treatment is uncertain due to emerging new strains and their resistant pattern thereby leading to recurrent exacerbation (Rennard and Farmer, 2004). A gross underestimate of COPD Prevalence had been estimated as 17 million and it is likely to increase by over 30% in next decade. Highest prevalence (9.4%) was reported from North Indian rural population from a study conducted by Jindal *et al.*, from 1964-1995.

It accounts for 60% of infection. Most common are *Haemophilus influenzae* nontypable, *Moraxella Catarrhalis*, *Streptococcus pneumoniae*. A number of studies have shown that virulent organisms are isolated in severe COPD patients like *Staphylococcus aureus*, *Pseudomonas aeruginosa* and members of Enterobacteriaceae family. (Jindal *et al.*,)

The main aim and objectives of this study to identify the fastidious organisms from sputum in patients with bronchitis, Correlate direct sputum smear and sputum culture results in identifying fastidious organisms

Materials and Methods

Study design- Descriptive study

Study population: Both male and female patients admitted with bronchitis in medical ward in GMKMC Salem.

Place of study: Department of Diagnostic Microbiology, Govt. Mohan Kumaramangalam Medical College, Salem District, Tamil Nadu

Period of study: Aug 2022 to November 2022

Study sample : sputum samples.

Performance of direct Gram stain to assess quality of sputum and presence of bacteria.

All the samples are inoculated in chocolate agar in 5% CO₂ under specified conditions and incubated for 48 hours. Preliminary identification by catalase and oxidase test and other biochemical reactions.

Identification of *Streptococcus pneumoniae* confirmed by bile solubility test

Optochin sensitivity test in 5% CO₂. *Haemophilus influenzae* in direct Gram stain- gram negative pleiomorphic coccobacilli Growth in chocolate agar in 5% CO₂. Show satellitism when streaked with *Staphylococcus aureus*.

Sample collection and Transport: All the samples were collected under strict aseptic precautions in sterile containers, properly labelled and were transported to the laboratory in appropriate conditions and processed within one hour of collection. Samples collected: 1) Sputum (expectorated /induced) 2) Bronchial wash 3) Endotracheal aspirate 4) Blood Procedure of collection & transportation of samples (Murray and Lopez, 1997;. CLSI guidelines M100 January, 2022; Jeremy farrar) Samples were taken to the laboratory for processing, within 1 hour of collection. 2) Bronchial wash: These samples were collected when the pulmonologist, via a fiber optic bronchoscope, was not able to visualize purulent secretions or diseased part of the lung segment. Small amounts of physiological saline were infused and the reaspirated sample is collected into the sterile container for further processing. 3) Endotracheal aspirate: The fraction of inspired oxygen was set at 90% or more. None of the patients received local anesthetics.

A blind endotracheal aspiration sample was obtained first by sterile means using a 22-inch suction catheter and collected in a mucus collector (Specimen trap) for patients with endotracheal tube. (Patrick R. Murray *et al.*) 4) Blood: With strict

aseptic precautions 10ml of blood sample was collected into a sterile screw capped blood culture bottle containing 50 ml of sterile Brain Heart Infusion Broth.

All the sputum samples were prescreened with Gram's stain, using Bartlett scoring system. Only those samples which met the acceptance criteria (a final score of >0) were further processed for culture. Rest of the samples was discarded and repeat sample was obtained in all possible cases. Sputum samples were mechanically homogenized with sterile glass beads using vortex machine. Homogenised sample were plated out onto the surface of a range of different media including blood agar, chocolate agar, MacConkey agar.

MacConkey agar plate, at 37 oC in ambient air for 24 hrs 5% sheep Blood agar plate, with 5-10% Co₂, 37 o C for 24 hrs Chocolate agar plate, with 5-10% Co₂, 37 o C for 24 hrs. Interpretation of bacterial cultures: The isolated colonies were identified by means of Gram's stain, motility, catalase test, oxidase test, coagulase test and by of various other biochemical reactions Optochin Sensitivity (for *Streptococcus pneumoniae*) were performed. Sugar fermentation tests with sugars viz: Glucose, Lactose, Sucrose, Maltose, Mannitol, Xylose, Arabinose and Dulcitol, inositols etc were done to identify the isolate according to standard laboratory procedures.

Fastidious organism: Antimicrobial susceptibility testing was done by disc diffusion method using Kirby bauer technique on Mueller Hinton agar supplemented with 5% sheep blood, using antimicrobial drugs, as directed by CLSI guidelines.

Inoculum

Direct colony suspension, equivalent to a 0.5 McFarland standard

Incubation

35 ± 2 °C; in 5% CO₂ for 20 to 24 hours Quality control tests were done every week for testing the

performance of media & drugs using the standard *Streptococcus pneumoniae* ATCC49619 control strain. (Jane D. seagull *et al.*,) Interpretation of Zone of inhibition diameters were done according to CLSI guidelines for all isolates. Detection methods for drug resistant *Streptococcus pneumoniae*: 1. Screening method: by disc diffusion method using MHA supplemented by 5% sheep blood. Penicillin sensitivity is detected by using oxacillin (1µg) disc. 2. Confirmatory methods: MIC detection methods – Isolates found to be nonsusceptible by oxacillin disk should then be subjected to quantitative MIC testing against penicillin. MIC detection methods – broth/agar dilution method, or antimicrobial gradient E strips using Mueller Hinton broth supplemented with lysed horse blood or defibrinated sheep blood.

For fastidious organisms, if the diameter of the zone of inhibition falls within the resistant range, ie ≤ 19 mm it is confirmed by MIC detection for the concerned drugs before reporting it as resistant, as per CLSI guidelines. b) Penicillin susceptibility were detected by using Oxacillin(1µg) disc. A zone diameter of ≥ 20 mm for oxacillin is taken as penicillin susceptible strains.

Results and Discussion

In this study totally 216 sputum samples obtained during the study period. Out of 216 samples 182 samples were accepted for processing by using Bartlett's scoring after direct gram staining.

Out of the 182 samples showed 76 Gram positive and Gram negative organisms in direct Gram staining.

In direct Gram staining of samples 23 Gram positive cocci identified and 49 Gram Negative bacilli identified in samples.

Out of the 182 samples streaked only 123 showed growth in culture plates.

Gram positive organisms contributed to 21 and 90 were gram negative organisms and 12 were candida

isolates

3 isolates are identified as *Streptococcus* species by and remaining 19 are *Staphylococcus aureus*. Out of the 90 Gram negative isolates the predominant organism was *Klebsiella* followed by *Pseudomonas* spp. The next common isolate was *Acinetobacter baumannii*. Eight *E. coli* isolates were obtained'

Vancomycin is the highest sensitive drug with 100% sensitivity. 33% of the isolates are sensitive to penicillin and 66% are resistant. Sensitivity to Tetracyclines are 66% and only 33% of the isolates are sensitive to Erythromycin. 66% of the isolates are sensitive for Ofloxacin and least sensitivity is observed for Cotrimoxazole. From the 216 samples collected 182 samples are considered good quality samples by Bartlett's scoring system. 85% of the samples were accepted. In this study Out of the 76 samples showed Gram positive and Gram negative organisms in direct Gram staining and 123 were culture positive. The percentage of positivity for gram smear is 41% which is lower than culture positivity which is 67%. Some authors have pointed out that the limited value of sputum Gram stain is due to the difficulty to obtain a good quality sample (Murray and Lopez, 1997; Topley and Wilson's, 1964-1985). In our study, a good quality sputum samples was obtained in 436 (85%) of 512 patients, which is a higher yield than reported in previous studies. An additional benefit of sputum Gram stain is that it can validate the subsequent sputum culture results (Rodriguez-Roisin, 2000). The growth of an organism from a sputum sample does not always indicate the presence of infection. Sputum samples can become contaminated with saliva or upper respiratory tract flora. Results of sputum culture can yield false positive findings related to colonization or contamination, and thus should be utilized with the results of the sputum Gram stain when establishing the definitive etiologic diagnosis of pneumonia. In a study done by Roche *et al.*, 2007 at University Hospital Maastricht in Netherlands most frequently isolated microorganisms were *Haemophilus influenzae* (45%) and *Streptococcus pneumoniae* (27%).

Table.1 Antibiotic susceptibility pattern of *Streptococcus pneumoniae*

Name of the Drug	Sensitive	Resistant
Cotrimoxazole	1	2
Oflaxacin	2	1
Erythromycin	1	2
Tetracyclines	2	1
Vancomycin	3	0
oxacillin	1	2
Optochin	3	-

Fig.1 Panel of antibiotics included for testing antimicrobial sensitivity of gram positive and gram negative cocci

Antibiotic	Disc content µg	Organisms	Diameter of Zone of Inhibition in mm Break points		
			Sensitive	Intermediate	Resistant
Amikacin	30		≥17	15-16	≤14
Penicillin	10units	<i>Staphylococcus aureus</i>	≥29	-	≤28
Amoxicillin/clavulanic acid	20/10	<i>Moraxella catarrhalis</i>	≥20	-	≤19
Ciprofloxacin	5		≥21	16-20	≤15
Cotrimoxazole	1.25 / 23.75	<i>Staphylococcus aureus</i>	≥16	11-15	≤10
		<i>S.pneumoniae</i>	≥19	16-18	≤15
Cefotaxime	30	<i>Streptococcus sp.</i>	≥24	-	-
Chloramphenicol	30	<i>S.pneumoniae</i>	≥21	-	≤20
		<i>Streptococcus sp</i>	≥21	18-20	≤17
Cefoxitin	30	<i>Staphylococcus aureus</i>	≥22	-	≤21
Erythromycin	15	<i>Staphylococcus aureus</i>	≥23	14-22	≤13
		<i>S.pneumoniae</i>	≥21	16-20	≤15
Oflaxacin	5	<i>S.pneumoniae</i>	≥16	13-15	≤12
Oxacillin	1	<i>S.pneumoniae</i>	≥20	-	-
Optochin	5	<i>S.pneumoniae</i>	≥14	-	<14
Tetracycline	30	<i>Staphylococcus aureus</i>	≥19	15-18	≤14
		<i>S.pneumoniae</i>	≥28	25-27	≤24
Vancomycin	30	<i>S.pneumoniae</i>	≥17	-	-

Fig.2

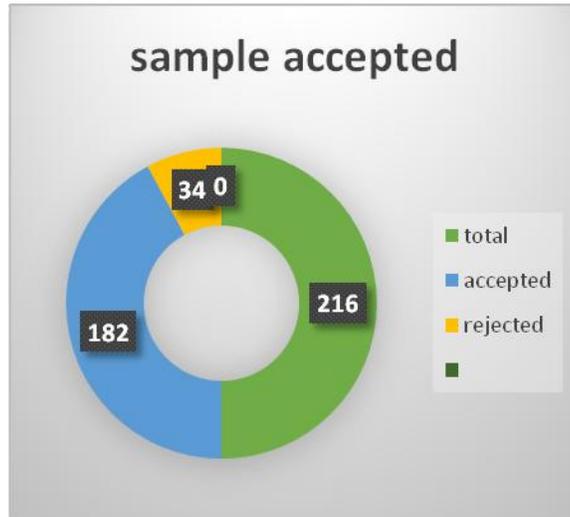


Fig.3

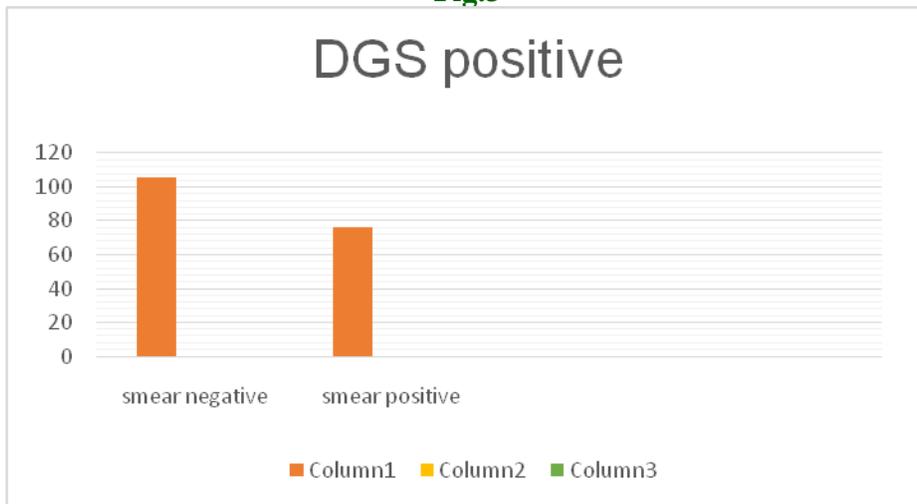


Fig.4

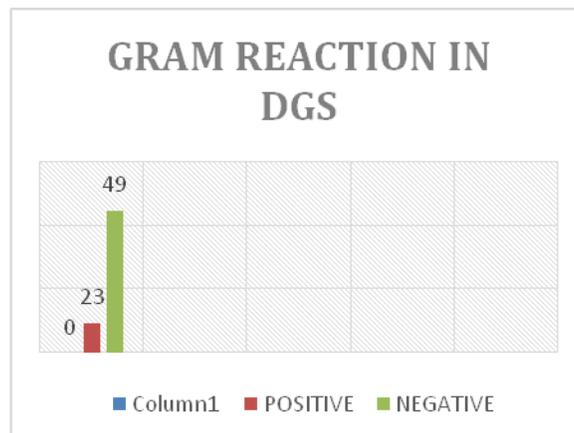


Fig.5

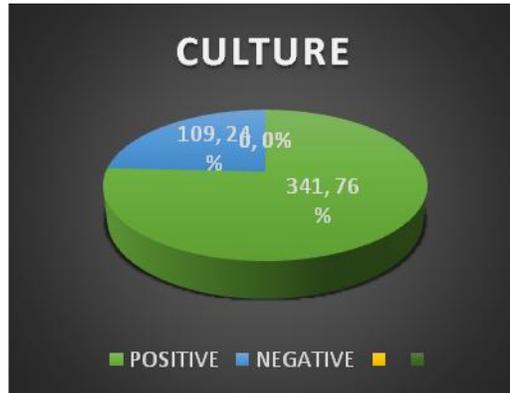


Fig.6

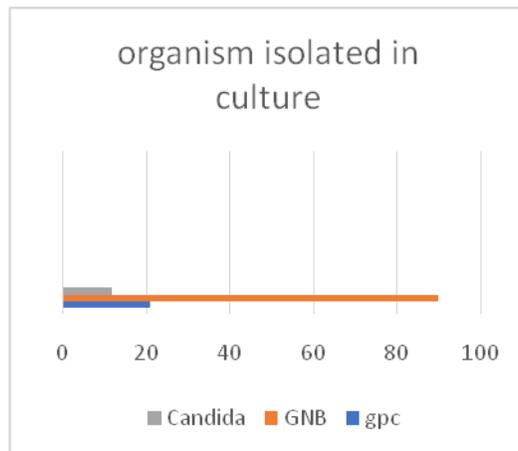


Fig.7

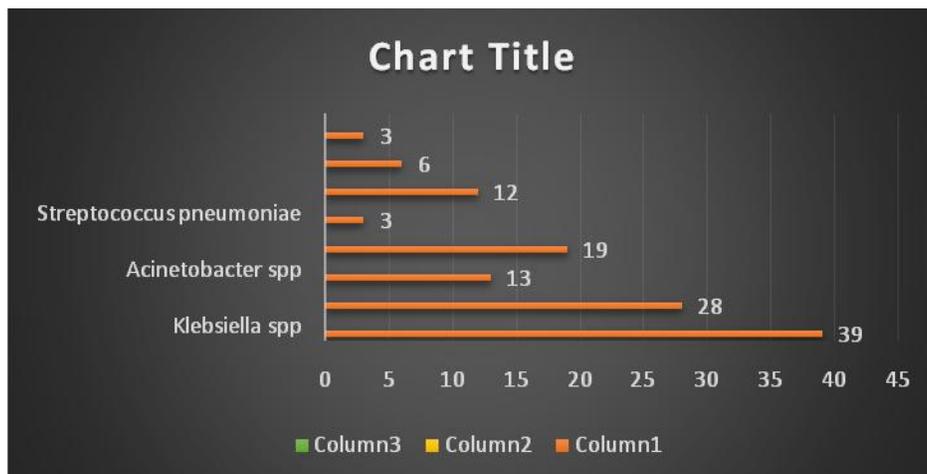
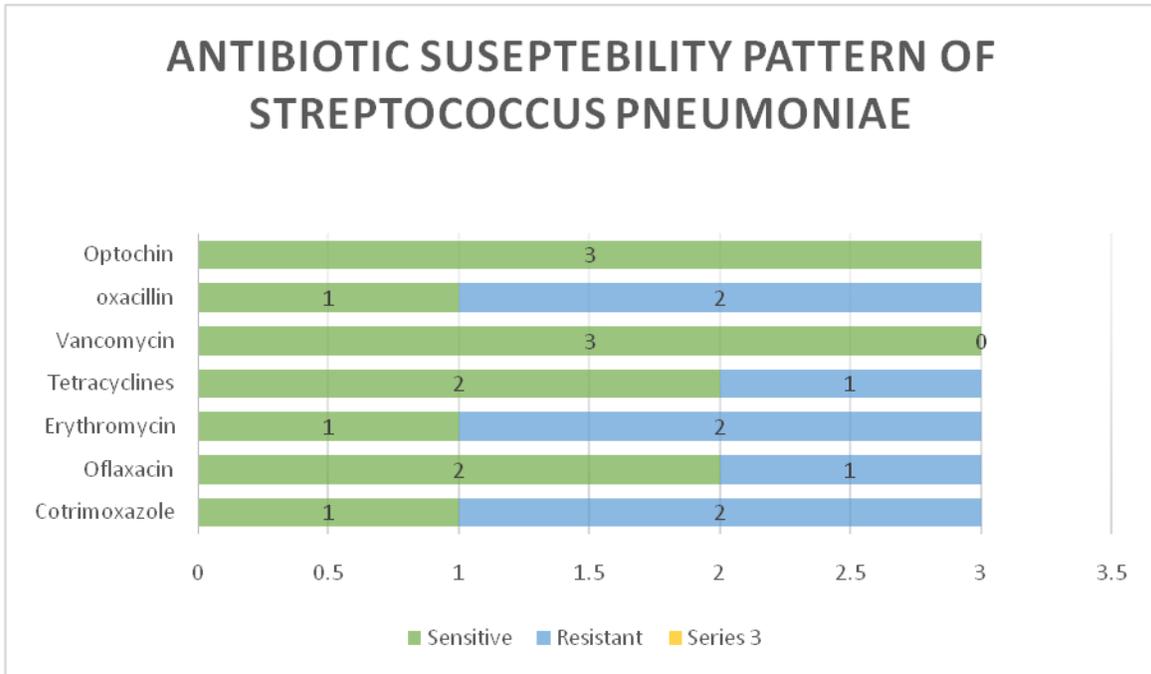


Fig.8



Other pathogens isolated were *Pseudomonas aeruginosa* (15%), *Moraxella catarrhalis* (6%) and *Klebsiella pneumoniae* (5%) (Roche *et al.*, 2007).

A study done by Dalia Saad ElFeky *et al.*, (2016) stated that the predominant organism causing AECOPD was *Streptococcus pneumoniae*, non typable *Haemophilus influenzae* and *Moraxella catarrhalis* (Alamoudi, 2007). All *Streptococcus pneumoniae* isolates from respiratory samples were 100% sensitive to Erythromycin, Cotrimoxazole, Ofloxacin, Tetracycline and Vancomycin. Two of the isolates showed resistance to Oxacillin by disc diffusion. A study done by Goyal *et al.*, (2007) in North India stated that 30 *Streptococcus pneumoniae* isolates were resistant to penicillin by Oxacillin disc diffusion method. Determination of MIC of these strains by broth dilution and E test revealed that 26 of isolates had intermediate resistance and only four isolates had complete resistance to penicillin (INSAR, 2013).

Study conducted by Iain B Catia Cillóniz *et al.*, stated that the prevalence of PRSP (Penicillin Resistant *Streptococcus pneumoniae*) was about 20% (Goyal *et al.*, 2007). Emilio Perez – trallero *et*

al., (2011) in their study stated that *Streptococcus pneumoniae* isolates obtained from AECOPD patients were more resistant to the antimicrobial agents generally used in the treatment of pneumococcal infections than those isolated from patients with pneumonia. This result was expected, as patients with COPD usually receive antimicrobial treatments because of frequent acute bacterial exacerbations and the association between antibiotic consumption and antimicrobial resistance has been demonstrated widely (Goyal *et al.*, 2007). *H. influenzae* was not isolated in the present study. This could be due to prior antibiotic use or seasonal variations in causation. This is in concordance with previous studies conducted by Wilson *et al.*, 1998. In 1915 Dass and Luetscher studied and described the application of bacteriological sputum examination and recognised *Haemophilus influenzae* as a common cause of acute and chronic Bronchitis. In the past, *S. pneumoniae* was almost uniformly susceptible to penicillin, allowing most physicians to treat persons who had severe infections with penicillin alone without testing for resistance. Resistance to penicillin and other antimicrobial agents has spread rapidly and was first reported in Australia in 1967, in New Guinea in 1969, in South

Africa in 1977, and in many other countries throughout Africa, Asia, and Europe. MDR strains resistant to penicillin, tetracycline, erythromycin, cotrimoxazole and chloramphenicol were identified. Investigations of outbreaks by CDC have revealed that Pneumococcal isolates resistance to penicillin in some areas of the United States, as many as 30%. (Prevalence of penicillin-resistant *Streptococcus pneumoniae* - Connecticut, 1992–1993) *Pneumococcal pneumonia*. *S. pneumoniae*, the most common identifiable etiologic agent of pneumonia in virtually all studies, accounts for about two-thirds of bacteremic pneumonia cases, and pneumococci are the most frequent cause of lethal community-acquired pneumonia. Management has been complicated in recent years by the evolution of multidrug resistance. β -lactams (amoxicillin, cefotaxime, and ceftriaxone) are generally regarded as the drugs of choice, although pneumonia caused by resistant strains (MIC, >2 mg/mL) may not respond as readily as pneumonia caused by more susceptible strains. The activity of macrolides and doxycycline or other β -lactams, including cefuroxime, is good against penicillin-susceptible strains but less predictable with strains that show reduced penicillin-susceptibility. Vancomycin, linezolid, and quinupristin/dalfopristin are the only drugs with predictable in vitro activity. Fluoroquinolones are generally active against strains that are susceptible or resistant to penicillin, but recent reports indicate increasing resistance in selective locations that correlate with excessive fluoroquinolone use. The most common etiologic agent identified in virtually all studies of CAP is *S. pneumoniae*, which accounts for about two-thirds of all cases of bacteremic pneumonia cases (Alfred Fishman) this is in contrast to our study where the predominant organism was klebsiella species. Other pathogens implicated less frequently include *H. influenzae* (most strains of which are nontypeable), *Mycoplasma pneumoniae*, *C. pneumoniae*, *S. aureus*, *Streptococcus pyogenes*, *N. meningitidis*, *Moraxella catarrhalis*, *Klebsiella pneumoniae* and other gram negative rods, *Legionella* species, influenza virus (depending on the season), respiratory syncytial virus, adenovirus,

parainfluenza virus, and other microbes. The frequency of other etiologies is dependent on specific epidemiological factors, as with *Chlamydia psittaci* (psittacosis), *Coxiella burnetii* (Q fever), *Francisella tularensis* (tularemia), and endemic fungi (histoplasmosis, blastomycosis, and coccidioidomycosis). The flora may include potential pathogens (leading to false positive cultures), and the normal flora often overgrow the true pathogen (leading to false-negative cultures), especially with fastidious pathogens such as *S. pneumoniae*. In cases of bacteremic *Pneumococcal pneumonia*, *S. pneumoniae* may be isolated in sputum culture in only 40%–50% of cases when standard microbiological techniques are used (Madhavi *et al.*, 2012; Wilson Robert, 1998).

The yield of *S. pneumoniae* is substantially higher from transtracheal aspirates (McHardy *et al.*, 1980; Monso, 1995; Rosell *et al.*, 2005), transthoracic needle aspirates, and quantitative cultures of BAL aspirates. Prior antibiotic therapy may reduce the yield of common respiratory pathogens in cultures of respiratory tract specimens from any source and is often associated with false-positive cultures for upper airway contaminants, such as gram-negative bacilli or *S. aureus*. The most common isolated bacterial pathogen was *Streptococcus pneumoniae* (43.3%) followed by methicillin-resistant *Staphylococcus aureus* (MRSA) (10%), *Haemophilus influenzae* (6.7%) and finally *Acinetobacter* spp and *Moraxella catarrhalis* (3.3% for each).

The etiologic matter which most clearly differentiates Asia from Western countries is the frequency of drug-resistant *Streptococcus pneumoniae* (Goto *et al.*, 2004; Inoue *et al.*, 2004). A recent study found that the frequency of penicillin-resistant and high-level macrolide-resistant *S. pneumoniae* has been increasing gradually in Japan (Goto *et al.*, 2004).

In addition, the incidence of fluoroquinolone resistant *S. pneumoniae* has risen particularly in patients over 60 years of age (Inoue *et al.*, 2004;

Felmingham *et al.*, 2002). The one of the basic policies and main purposes of the Japanese Respiratory Society (JRS) guidelines include prevention of bacterial resistance (Song *et al.*, 2004). Thus, the JRS guidelines recommend pathogen-directed antibiotic treatment as the initial appropriate therapy in cases in which an etiologic diagnosis is established or strongly suspected.

Sputum examination is a simple and rapid diagnostic tool for the presumptive identification of pathogens. In our study, a good quality sputum samples was obtained in 182 (85%) of 216 patients, which is a higher yield than reported in previous studies. An additional benefit of sputum Gram stain is that it can validate the subsequent sputum culture results (Rodriguez-Roisin, 2000).

The growth of an organism from a sputum sample does not always indicate the presence of infection. Sputum samples can become contaminated with saliva or upper respiratory tract flora. Results of sputum culture can yield false positive findings related to colonization or contamination, and thus should be utilized with the results of the sputum Gram stain when establishing the definitive etiologic diagnosis of pneumonia. Vancomycin is the highest sensitive drug with 100% sensitivity. 33% of the isolates are sensitive to penicillin and 66% are resistant. sensitivity to Tetracyclines are 33% and only 33% of the isolates are sensitive to Erythromycin.66% of the isolates are sensitive for Ofloxacin and least sensitivity is observed for Cotrimoxazole. The culture recovery of these fastidious organisms defines a patient having disease with that organism. These fastidious organisms tend to be missed out in routine cultures. Performing a direct gram stain and identifying helps us to plan the appropriate culture conditions and treatment.

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CLSI guidelines M100 January 2022

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