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Analysis of Microbial Profiles and Their Resistance Pattern in Pleural Fluid Samples from a Tertiary Care Centre.

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Abstract:
Background: *Acute respiratory tract infection is the leading cause of morbidity and mortality. Several possible mechanisms of pleural effusion have been proposed accompanying various underlying diseases like Heart failure, Pneumonia, Pleural malignancy & Cirrhosis Liver. One of the major problems world-wide is the increase in antibiotic resistant strains of bacteria, which has proved difficult to control.*
Objectives: *To identify the presence of lactose fermenting bacilli in pleural fluid samples with their resistance pattern along with different enzyme mechanisms responsible for resistance.*
Material and methods: *This study was carried out in Microbiology Division of Central laboratory, SMIH, Patel nagar, Dehradun. A total number of 215 samples, over a period of 12 months from November 2016 to October 2017, were collected for the examination. Out of which, only 31 positive samples were processed to determine the etiology and antimicrobial susceptibility pattern using VITEK 2 compact system.*
All patients suffering from tuberculosis were excluded from the study.
Results*: Out of total samples, only 31 samples showed culture positivity with majority of Gram negative bacilli (23). Among Gram negative bacilli, 12 were lactose fermenters. Enterobacter spp (07/12) was the predominant isolate with high resistance for Piperacillin Tazobactum which is now the only drug left for treatment in most of the cases.*
Conclusion: *The study was carried to find out the microbiological association of pleural effusion in nontubercular cases. Indiscriminate and overuse of broad spectrum antibiotics predisposes the affected patients to develop multi drug resistant virulent micro flora.*
Keywords: *Enzyme resistance, Enterobacter spp., Piperacillin tazobactum*

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I. Introduction

Acute respiratory tract infection is the leading cause of morbidity and mortality in critically ill patients in developing countries. Lower respiratory tract infections (LRTI) are the most common bacterial infections among patients admitted in Intensive Care Units (ICUs) which result in high mortality (1) .

Pleural disorders usually manifest as pleural effusion (PE). Inflammation of pleura leads to increase in the permeability of pleural vessels and fluid accumulation in the pleura. Several possible mechanisms of pleural fluid accumulation have been proposed accompanying various underlying diseases like Heart failure, Pneumonia, Atelectasis, Hypoalbuminemia, Pleural malignancy or Infection, Cirrhosis Liver, & Chylothorax. The most commonly encountered causes in adults are heart failure, malignancy, pneumonia, tuberculosis, and pulmonary embolism, whereas pneumonia is the leading etiology in children (2) (3). Based on the sex, pleural effusion is two times more frequent in males (4).

Information on various lower respiratory tract (LRT) bacterial pathogens and their antibiotic resistance patterns in hospitalized patients is inadequate in our country. It is often witnessed that there is a high rate of culture negativity in pleural fluid or blood cultures (up to 60% of cases) (10). During the last few years, the increase in antibiotic resistance has compromised the selection of empirical treatment (1).

Gram negative bacteria with acquired metallo-β-lactamases production have been increasingly reported in some countries, necessitating their detection. Their high prevalence is of great concern because of their intrinsic and acquired resistance mechanisms, limiting the treatment options. Carbapenems are the drugs of choice for penicillin & cephalosporin resistant bacterial infections. These bacteria are widely distributed in nature and their presence in the hospital environment poses a special risk of opportunistic infections by Multidrug resistant Organisms (MDROs) (5) (6).

Hence this study was undertaken to identify the prevalent lactose fermenting agents of pleural effusion and their antibiotic sensitivity profile in our hospital settings.

II. Material and methods

This study was carried out in Microbiology Division of Central laboratory, SMIH, Patel nagar, Dehradun during November 2016 to October 2017 (one year). The pleural fluid samples were obtained from the patients revealing symptoms of pleural effusion, admitted in the same hospital. The samples were collected aseptically and processed immediately following collection.

Study Design: Cross sectional study.
Study Location: Microbiology Division of Central laboratory, SMIH, Patel nagar, Dehradun ,Uttarakhand.
Study Duration: November 2016 to October 2017 (one year).
Sample size: 215 samples.
Inclusion criteria: All pleural fluid samples (from any cause) collected and transported without delay with universal safety precautions, to the microbiology laboratory.
Exclusion criteria: All patients currently suffering from tuberculosis were excluded.

A total number of 215 samples over a period of 12 months were collected for the examination. Out of which only 31 samples which were positive for any growth were processed to determine the etiology and antimicrobial susceptibility pattern using VITEK 2 compact by BioMereiux, FRANCE.

The present study has been approved by the ethical committee of our institute. Informed consent from all the patients was obtained, before collection of clinical samples.

Methodology in brief: All samples were centrifuged and the deposit was screened for the presence of any microorganism by Gram staining (direct microscopy) & Ziehl-Neelsen stain which helped in making the presumptive diagnosis. All pleural fluid samples were inoculated in aerobic culture vials meant to be used in BacT Alert 3D automated culture system. A subculture from positive vials was then done on 5% sheep blood agar & Mac Conkey agar.

Bacteriological identification was done using Vitek 2 compact automated identification and sensitivity system, after getting pure growth on subculture from the culture vials. The result obtained was statistically analysed using appropriate method.

III. Results

Table 1: Positivity Rate of total Samples (n = 215)

	Number	Percentage (%)
Positive Samples	31	14.41
Negative Samples	184	85.58
Total Samples	215	

Out of the 215 samples, only 31 samples (14.41%) were culture positive, whereas 184 (85.58%) specimens showed no growth as shown in table 1.

Table 2: Age wise distribution of total samples

Age groups (years)	Number (%)
0-20	36 (16.74)
21-40	53 (24.65)
41-60	64 (29.76)
61-80	53 (24.65)
>80	9 (4.20)
Total	215(100)

Figures in parenthesis represent percentage.

Out of the 215 samples studied, maximum number of cases 29.76% (64/215) belonged to 41- 60 years of age group and only 4.20 % (9/215) cases belonged to 81and above years of age group.

Table 3: Gender wise distribution of samples

Gender	Number (%)
Male	151(70.24)
Female	64 (29.76)
Total	**215 (100)**

Figures in parenthesis represent percentage of male and females. Out of total 215 samples studied, 151 samples belonged to Male (70.24%) while the remainder 64 belonged to the female patients (29.76%).

Table 4: Gender wise distribution based on culture positivity

Gender	Culture Positive	Culture Negative	Total
Males	27	124	151
Females	4	60	64
Total	**31**	**184**	**215**

Chi square test =4.52, d.f =1, l.s=0.05.

Table 5 depicts that out of 215 samples studied, 27 /151(17.8%) males and 4/64 (6.25%) females were found to be culture positive, which was found to be statistically significant ($p < 0.05$). This difference in culture positivity result may be due to less number of females in the study.

Table 5: Distribution of Isolates

Isolates	Distribution of isolates (%)
Non fermentating GNB (11)	**35.48**
Fermentating GNB (12)	**38.72**
GPC (6)	**19.35**
Candida albicans (2)	**6.45**
Total (31)	**100%**
Total	**31(100)**

Figures in parenthesis represent percentage.

Figures in parenthesis represent percentage of isolates in pleural fluid samples. Among 23 Gram negative isolates, 12 were lactose fermentating and 11 were non lactose fermenting.

Figure 1: Distribution of isolates (%)

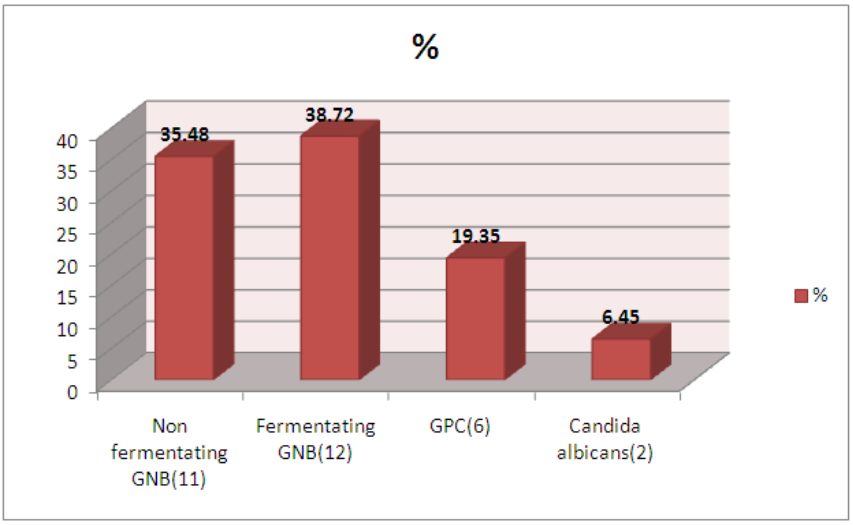
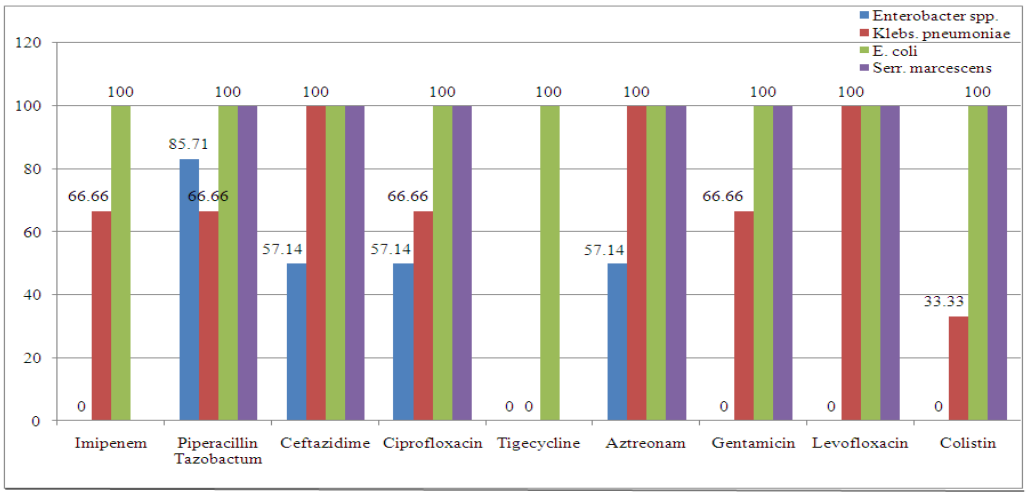


Table 6: Distribution of lactose fermenting Isolates

Lactose Fermenters	%
Enterobacter spp.(n=7)	58.34
Klebsiella pneumoniae (n=3)	25
Escherichia coli (n=1)	8.33
Serratia marcescens (n=1)	8.33

Among lactose fermenters (12), Enterobacter spp (n=7) was found to be maximum followed by Klebsiella pneumoniae (n=3). Single isolate of Escherichia coli and Serratia marcescens was found.

Figure 2: Antimicrobial Resistance pattern of Gram negative Fermenter bacilli (%)



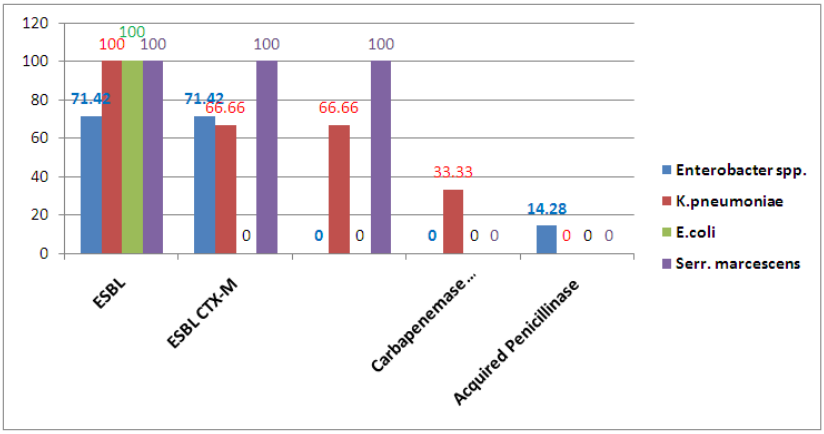
Amongst fermenters, *Enterobacter spp*. showed maximum resistance (85.71%) against Piperacillin Tazobactum while *Klebsiella pneumoniae* expressed 100% resistance against ceftazidime, aztreonam and levofloxacin.

Table 7: Phenotypic Enzyme Resistance Mechanisms of Gram Negative fermenter bacilli (%)

	ESBL	ESBL CTX-M	Carbepenemase METALLO OR OXA	Carbapenemase (impermeability)	Acquired Penicillinase
Enterobacter spp.(n=7)	5 (71.42)	5 (71.42)	0	0	1 (14.28)
K.pneumoniae(n=3)	3 (100)	2 (66.66)	2 (66.66)	1 (33.33)	0
E.coli(n=1)	1 (100)	0	0	0	0
Ser. marcescens(n-2)	1 (100)	1 (100)	1 (100)	0	0

All isolates of *Klebsiella pneumoniae*, *Escherichia coli* and *Serratia marcescens* showed 100% presence of ESBl whereas *Enterobacter spp.* showed 71.42%.

Figure 3: Phenotypic Enzyme Resistance Mechanisms of Gram Negative fermenter bacilli (%)



IV. Discussion

Accumulation of pleural fluid itself is not a disease but it is a reflection of any underlying pathology. Light's criterion has been a constant guideline for most of the clinicians to differentiate between transudates and exudates but still its application with consideration of all the parameters is not proper.

Pleural effusion is the most common pleural disease affecting a significant proportion of population in India. It can be a result of pleural, lung parenchymal, or systemic disease. Despite the impact, antibiotics have on empyema; it still remains a common illness with significant morbidity and mortality.

Culture of pleural fluid showed growth only in 14.41% of patients and no growth in 85.58% of patients. There can be many reasons for such a huge number of samples with no growth, but causes such as high rate of antibiotics pre- treatment or lack of better facilities for culturing fastidious organism are the ones which need special mention. Similar study was done by M.Surya Prasad Rao et al. that showed bacterial growth only in 37.5% of patients and no growth in 62.5% of patients. Sonali Jain et al also observed bacterial growth in 17.7%

only and no growth in 82.3% (table 1).Culture negative cases were not processed further in current study for determining the cause of pleural effusion (7) (8).

Our study revealed that the maximum number of cases of pleural effusion 29.76% (64/215) belonged to 41- 60 years of age group and only 4.20 % (9/215) cases belonged to 80 and above years of age group (table 2). These findings were similar to the study done by Rahul Gupta et al in which majority of patient of pleural effusion was in middle age group between 31 and 40 years of age. It can also be compared to study done by Pujan Parikh et al with 31-40 years of age group being most affected (9) (10).

In the present study pleural effusion was more common among males (table 3). Out of total 215 samples, 151(70.24%) samples belonged to males while 64 (29.76%) belonged to the female patients. Male predominance in our study was similar to studies done by Rahul Gupta et al, Rohit Rungta et al & Maulik P.Saliya et al (9) (11) (12).

Out of 31 culture positive samples, 27/151 (17.8%) were males and only 4/151 (6.25%) were females. This observation when analyzed statistically was found to be significant ($p < 0.05$). γ^2 value was 4.52 with degree of freedom as 1 (table 4). This association was perhaps more significant due to less number of samples from females.

In the present study it was found that majority of the samples were positive for growth of Gram negative isolates, 23 (74.19%) and only 8 (19.35%) were Gram positive isolates including candida spp. (table 5 and fig.1). Similar study was done by Trupti Bajpai et al. and they also found Gram negative bacilli to be the predominant organism (96.04%) with low isolation of Gram positive cocci. Similarity was also seen in the results obtained by Veena Kumari *et al*, Okesola and Ige et al and Goel *et al* who found that percentages of Gram negative bacilli isolated were 92.2%, 93% and 97.4% respectively (13) (14) (15).

Among Gram negative bacilli, fermenters 12/23(52.17%) were grown and rest were nonfermenters 11/23 (47.82%). *Enterobacter spp.* 7/12 (58.33%) was the dominant pathogen among fermenters followed by *Klebsiella pneumoniae* 3/12 (25%), *Escherichia coli* and *Serratia marcescens* 1/12 (8.33%) each. Enterobacter spp. (7/12) was the commonest species isolated among Gram negative fermenter bacilli (table 6) and it showed high degree of resistance (57.14%) towards Ciprofloxacin which used to be the primary mode of treatment for most of the Gram negative isolates. It also showed high degree of resistance (85.71%) towards Piperacillin tazobactum which still belongs to the class of reserve drugs. Where as study done by Joanna Mokracka et al found 60% of strains were resistant to ciprofloxacin and more than 50% were resistant to ceftazidime and piperacillin+ tazobactam (16). However, Colistin, Imipenem and Tigecycline were found to highly sensitive. Classification of Klebsiella pneumoniae as a multidrug resistant pathogen and an important cause of nosocomial infection needs special mention as it still showed some sensitivity towards Tigecycline and Colistin. In present study *Klebsiella pneumoniae* expressed 100% resistance against ceftazidime, aztreonam and levofloxacin whereas study done by Archana et al showed ciprofloxacin was susceptible to *Klebsiella pneumoniae* and cefotaxime showed significant resistance (17). Although we got only single isolate of Escherichia coli and *Serratia marcescens* each but the situation is very alarming because they showed almost 100 % resistance towards all drugs tested against them (fig.2). Almost similar findings were reported in a study done by Shawn R. Lockhart, Murray A. Abramson et al (18).

Presences of ESBL compromise the activity of wide-spectrum antibiotics creating major therapeutic difficulties with a significant impact on the outcome of patients. In the present study all isolates of *Klebsiella pneumoniae*, *Escherichia coli* and *Serratia marcescens* showed 100% presence of ESBL (Table1 7 and fig.3). The results was not completely in accordance with those found in another study conducted by Meeta Sharma et al observed highest ESBL production in *Klebsiella spp.* (67.04%) followed by *Escherichia coli* (56.92%), S Babypadmini et al showed that ESBL production was 41% in *E. coli* and 40% in *K. pneumoniae* and Umadevi S et al observed that 81% of the *E.coli* and 74 % of the *K. pneumoniae* isolates were ESBL producers (19) (20) (21). ESBL production was noted among 44.4% isolates of *Enterobacter spp*. by Shashidhar Vishwanath et al unlike present study which found 83.3%. Due to such extent of ESBL production, our treatment strategies have drastically shifted to the use of reserve drugs in place of conventional treatment (22) (23).

V. Conclusion

The study was carried to find out the microbiological association of pleural effusion of non tubercular origin. We could find out association of aerobic bacteria in 14.41% of cases , leaving behind unknown possible mechanism in 85% of the cases. We therefore recommend enlarged scale of study by including Anaerobic culture methods, Molecular methods, Radiological investigation and detail clinical examination of co morbid conditions to find out the cause of pleural effusion.

It is a well known fact that bacteria are constantly evolving newer mechanisms of resistance which makes the latest group of antibiotics ineffective. This increasing resistance to antibiotics by respiratory pathogens has complicated the use of empirical treatment with traditional agents and a definitive bacteriological diagnosis and susceptibility testing would, therefore, be required for effective management of pleural effusion.

Moreover, no regional or individual hospital data for antimicrobial consumption were available for comparison with the present study.

Based on the current study, a multidimensional approach is suggested to combat and to win this battle against microbes:

- Surveillance of antibiotic use and resistance rates
- Instituting appropriate antimicrobial stewardship programmes (ASP)
- Application and maintenance of health care practices in health institutions
- Application of logic in antibiotic selection for critically ill patients rather than a "tradition-based" approach for selecting treatment
- Lastly, better approach to care of individuals with predisposing factors so that secondary bacterial infections do not set in, thus reducing the use of antimicrobials.

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