AEROBIC BACTERIOLOGICAL PROFILE OF AFB NEGATIVE SPUTUM SAMPLES AT A TERTIARY CARE MEDICAL INSTITUTE IN EASTERN INDIA

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ABSTRACT

BACKGROUND

Lower Respiratory Tract Infection (LRTI) is a leading cause of morbidity and mortality. Many of the patients suspected of suffering from Tuberculosis (TB) and referred to the Revised National Tuberculosis Control Programme (RNTCP) centre usually turn out to be suffering from non-tuberculous LRTIs and end up receiving no specific treatment.

AIMS

1. To isolate aerobic bacterial pathogens other than Mycobacteria spp. from AFB negative sputum samples. 2. To determine the antibiotic sensitivity pattern of the isolates.

SETTINGS AND DESIGN

A cross-sectional study was conducted at the Microbiology Department of a tertiary care medical institute on existing data for a period of four years (2011-2015).

METHODS AND MATERIAL

1015 AFB negative sputum samples were collected. Aerobic bacterial culture identification and antibiotic sensitivity tests were carried out following standard laboratory procedures.

STATISTICAL ANALYSIS

Data analysis was performed by using IBM SPSS version 21 software. Descriptive statistics were derived using frequency, percentage, and proportion. Chi-square test was used to calculate the P-values.

RESULTS

Among the 904 bacterial isolates, 613 (67.8%) were Gram-Negative Bacilli (GNB) and 291 (32.1%) were Gram-Positive Cocci (GPC). The most common organism isolated was Pseudomonas aeruginosa (38.9%) followed by Staphylococcus aureus (30%); out of which 25% were Methicillin-Resistant Staphylococcus Aureus (MRSA) and Klebsiella pneumoniae (15.7%). Pseudomonas aeruginosa was sensitive to carbapenems, aminoglycosides, and β lactam-β lactamase inhibitor combination. The GPCs were highly sensitive to linezolid and vancomycin. The Enterobacteriaceae isolates were highly susceptible to carbapenems and β lactam-β lactamase inhibitor combination.

CONCLUSION

Pseudomonas aeruginosa has been found to be the most commonly isolated aerobic bacteria from the AFB negative sputum samples. Antibiogram helps in specific treatment during the management of non-tuberculous LRTI as most of the isolated bacteria have been observed to be highly resistant to the commonly used antibiotics.

KEYWORDS

Sputum, Bacteriology, Pseudomonas Aeruginosa.

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INTRODUCTION

Lower Respiratory Tract Infection (LRTI) is currently the seventh leading cause of death and disability. It is stated that LRTI will become the third leading cause of death by 2020, next to heart and cerebrovascular diseases. Six hundred million people worldwide have LRTI.[1,2,3]

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Every year, Acute Respiratory Tract Infection (ARTI) in young children is responsible for an estimated 3.9 million deaths throughout the world.[4,5] LRTIs have been attributed to account for almost 20% mortality among the infectious disease deaths in India.[6]

LRTIs are mostly mild, transient, and self-limiting. Due to this, many infected persons tend to disregard them. In developing countries, the situation is more complicated and management is often difficult due to the problem associated with the identification of the aetiological agents and administration of appropriate treatment in cases requiring antibiotic therapy. The aetiology and symptomatology of respiratory disease differ with age, gender, season, the type of population at risk, and other factors. Gram-positive bacteria (GPC) such as Staphylococcus aureus and Streptococcus

pneumoniae and gram-negative bacteria (GNB) like Haemophilus influenzae, Pseudomonas (spp.), Acinetobacter spp., and Klebsiella spp. have been recovered from LRTIs.[7]

With the ever increasing population of immunocompromised patients such as elderly patients, Human Immunodeficiency Virus (HIV) infected, cancer, diabetic patients, etc. the isolation of opportunistic bacteria like Pseudomonas spp., Acinetobacter spp., Haemophilus spp. etc. have been seen to be on the rise.[8] Such opportunistic bacteria are usually resistant to most of the antibiotics used for empirical treatment. The aetiological agents cannot be determined clinically, which vary from area to area and so does their antibiotic susceptibility profile. However, knowledge about the prevalence of microbial agents causing LRTIs in this part of eastern India is sparse. Moreover, many of the patients suspected of suffering from Tuberculosis (TB) attend Revised National Tuberculosis Control Programme (RNTCP) Centre. But, they usually turn out to be suffering from non-tuberculous LRTIs and are left without any specific treatment. In resource, poor settings of an economically developing country like India more so in the eastern states where molecular methods of diagnosis may not be available performing conventional sputum culture and antibiotic sensitivity tests are still effective in providing proper patient care. So, our study aims to detect the aerobic bacterial pathogens of LRTIs other than Mycobacteria spp. and to analyse their antibiogram.

MATERIALS AND METHODS

A cross-sectional study was conducted using laboratory data dating from January 2011 to December 2015 at the Microbiology Department of a tertiary care medical institute in Eastern India. The protocol of the present research work was approved by the institutional ethical committee. A total of 1015 sputum samples were collected from the designated microscopy centre attached to this department under the RNTCP. All patients <18 years of age and those who were sputum Acid-Fast Positive (AFB) were excluded from the study. A detailed clinical history regarding the age, gender, marital status, education, onset, duration and nature of illness, and history of past medication were recorded in a predesigned proforma.

Deeply coughed up sputum samples were collected into a sterile container and were processed immediately. Direct microscopy of Gram stained smear was done and examined for pus cells, epithelial cells, and bacteria. Those specimen with ≥25 pus cells and ≤10 epithelial cells per high-powered field were inoculated onto MacConkey agar (MA), sheep blood agar (SBA), and chocolate agar (CA). MA plates were incubated in ambient air at 37^0C while SBA and CA plates were kept in a candle jar at 37^0C for overnight incubation.

Based upon the Gram stain morphology, colony characteristic, and motility, the biochemical tests and other special tests were done. They were performed according to the standard procedures.[9] The tests performed to identify the GPCs Gram-Positive Cocci (GPCs) were catalase, slide coagulase, mannitol fermentation, novobiocin resistance, optochin sensitivity, bacitracin sensitivity, and bile solubility tests. The first test performed for GNB was oxidase test. Those GNBs, which were oxidase negative (Enterobacteriaceae) were subjected to catalase, indole production, methyl red, Voges-Proskauer, citrate utilisation, urease, triple sugar iron, nitrate reduction, sugar fermentation, and amino acid decarboxylation tests. Those GNBs, which were oxidase positive were further subjected to tests like catalase, nitrate reduction, citrate utilization, and Hugh-Leifson oxidation-fermentation tests.

The antibiotic susceptibility was done on Mueller-Hinton agar except for Streptococcus pneumoniae, which was done on SBA. After overnight incubation, the zone sizes were noted and the results were interpreted as sensitive or resistant comparing with Kirby-Bauer standard chart.[10] Cefoxitin (30 ug) antibiotic disc was used for detection of Methicillin-Resistant Staphylococcus Aureus (MRSA). The antibiotics used are given in Table 1.

All the data was entered into Microsoft Excel 2007 spreadsheet and analysed using IBM SPSS software version 21. Descriptive statistics were derived using frequency, percentage, and proportion. Chi-square test was used to calculate the P-values.

RESULTS

In our present study, a total of 1015 sputum samples negative for AFB were processed and analysed, out of which 689 (67.9%) belonged to IPD (Inpatient Department) and 326 (32%) to OPD (Outpatient Department) Among the IPD patients, maximum sputum samples were received from Medicine and TB and Chest Medicine Departments. (Table 2). The age group of the patients included in the present study ranged from 18 to 80 years. Out of 904 (89%) patients from whom bacteria were isolated, maximum number 278 (27.3%) belonged to the age group of 61-75 years. 595 were males and 309 were females. The male:female ratio is 1.93:1.626 patients were found to be associated with various risk factors and co-morbid conditions (Table 3). 25.3% of the patients were found to be chronic smokers or ex-smokers and 24.1% of the patients were found to be HIV positive. 613 (67.8%) were GNBs and 291 (32.1%) were GPCs. Pseudomonas aeruginosa was the commonest bacteria isolated in 352 (38.9%) cases, followed by S. aureus isolated in 271 (30%) [MSSA=204;MRSA=67], and K. pneumoniae isolated in 142 (15.7%) cases. Other organisms isolated were E. coli in 65 (7.1%), K. oxytoca in 26 (2.8%), St. pneumoniae in 20 (2.2%), C. koseri in 19 (2.1%), and P. vulgaris in 9 (0.9%) of the cases (Table 4).

84% (P=0.033) of Pseudomonas aeruginosa isolates were found to be sensitive to the carbapenems. 74% (P=0.046) of the isolates were sensitive to piperacillin-tazobactam. 90% (P=0.041) of them were resistant to both ceftazidime and cefepime (Table 5). 97% (P=0.009) and 80.5% (P=0.016) of S. aureus were found to be sensitive to linezolid and vancomycin respectively. All of the isolates were found to be resistant to penicillin (Table 6). Antibiotic susceptibility pattern of Klebsiella pneumoniae showed it was most sensitive to the carbapenems, i.e., imipenem and meropenem (97.5%, p=0.031; 95%, P=0.037 respectively) (Table 7).

GPC	Enterobacteriaceae	Pseudomonas
Amoxicillin (10 µg)	Amoxicillin (10 µg)	Gentamicin (10 µg)
AmoxyClav (20+10 µg)	AmoxyClav (20+10 µg)	Amikacin (30 µg)
Co-trimoxazole (25 µg)	Ciprofloxacin (5 µg)	Piperacillin+Tazobactam (100+10 µg)
Tetracycline (30 µg)	Gentamicin (10 µg)	AmoxyClav (20+10 µg)

Erythromycin (15 µg)	Amikacin (30 µg)	Imipenem (10 µg)
Penicillin (10 units)	Cefepime (30 µg)	Meropenem (10 µg)
Vancomycin (30 µg)	Cefotaxime (30 µg)	Ciprofloxacin (30 µg)
Ciprofloxacin (5 µg)	Imipenem (10 µg)	Ceftazidime (30 µg)
Clindamycin (2 µg)	Meropenem (10 µg)	Ticarcillin+Clavulanic acid (75+10 µg)
Linezolid (30 µg)	Piperacillin+ Tazobactam (100+10 µg)	

Table 1: Antibiotics Used

	Medicine and Allied Departments*	**Surgery and Allied Departments****
Wards	451(65.6)	177(25.7)
ICUs	37(5.3)	24(3.4)
Total	488	201

Table 2: Ward Wise Distribution of IPD Patients (N=689)

*Medicine and Allied Departments-Medicine, TB and Chest Medicine, etc.

**Surgery and allied Departments-Surgery, Orthopaedics, Eye, ENT, Obs, and Gynae, etc.

Characteristics	**Number of patients**	**Percentage**
Age		
18-30 yrs.	217	24
31-45 yrs.	199	22
46-60 yrs.	227	25
61-75 yrs.	247	27.5
>76 yrs.	14	1.5
Sex		
Male	595	66
Female	309	34
Male:Female	1.93:1	
Risk Factors		
Smoking	229	25.3
HIV	218	24.1
Past Lung Infections	88	9.7
COPD	55	6
Diabetes	29	3.2
Patients with Neoplasm	7	0.07

Table 3: Patient Characteristics

Organisms	**Number of Isolates**
P. aeruginosa	352 (38.9%)
S. aureus	271 (30%) [MSSA=204; MRSA=67]
K. pneumoniae	142 (15.7%)
E. coli	65 (7.1%)
K. oxytoca	26 (2.8%)
St. pneumoniae	20 (2.2%)
C. koseri	19 (2.1%)
P. vulgaris	9 (0.9%)
Total	**904**

Table 4: Bacteriological Isolates

Drugs	**Sensitivity**
AmoxyClav	138(39.2)
Ciprofloxacin	172(48.8)
Gentamicin	243(69)
Imipenem	296(84)
Meropenem	296(84)
Piperacillin+Tazobactam	261(74)
Ceftazidime	34(9.6)
Amikacin	226(64.2)
Ticarcillin+Clavulanic Acid	157(44.6)
Cefepime	34(9.6)
Colistin	174(48.9)

Table 5: Drug Sensitivity Pattern of Pseudomonas aeruginosa (N=352)

Drugs	**S. aureus**	**St. pneumoniae**
Amoxicillin	90 (33.2)	-
AmoxyClav	97 (35.7)	20 (100)
Co-trimoxazole	150 (55.3)	0 (0)
Ciprofloxacin	218 (80.4)	16 (80)
Clindamycin	90 (33.2)	-
Penicillin	0 (0)	0 (0)
Vancomycin	218 (80.5)	8 (40)
Erythromycin	150 (55.3)	8 (40)
Tetracycline	218 (80.4)	-
Linezolid	262 (97)	-

Table 6: Drug Sensitivity Pattern of GPC Isolates N (%)

Drugs	**K. pneumoniae**	**K. oxytoca**	**E. coli**	**P. vulgaris**	**C. koseri**
AmoxyClav	35 (25)	23 (88.4)	39 (60)	9 (100)	16 (84.2)
Ciprofloxacin	32 (22.5)	23 (88.4)	39 (60)	9 (100)	0(0)
Gentamicin	74 (52.5)	23 (88.4)	47 (72)	8 (88.8)	8(42)
Amikacin	78 (55)	23 (88.4)	23 (35)	8 (88.8)	16 (84.2)
Cefepime	60 (42.5)	15 (57.7)	32 (49)	8 (88.8)	8 (42)
Cefotaxime	53 (37.5)	17(65.3)	36 (55.4)	5 (55.6)	9 (47.3)
Meropenem	138 (97.5)	26(100)	65 (100)	9 (100)	16 (84.2)
Imipenem	138 (97.5)	26 (100)	65 (100)	9 (100)	19 (100)
Piperacillin+ Tazobactam	88 (62.5)	7(26.9)	16 (24.6)	9 (100)	16 (84.2)
Ceftazidime	21 (15)	7 (26.9)	23 (35)	0(0)	0 (0)
Ceftazidime+ Clavulanic Acid	78 (55)	23 (88.4)	23 (35)	8 (88.8)	16 (84.2)
Cefoxitin	53 (37.5)	7 (26.9)	16 (24.6)	0 (0)	8 (42)

Table 7: Drug Sensitivity Pattern of Enterobacteriaceae Isolates N (%)

DISCUSSION

In the present study, the age group of patients ranged from 18-80 years. Majority of the patients belonged to the age group of 61-75 years. Similar findings have been reported in other studies.[4,10] Patients in older age groups are more susceptible to LRTIs because of the effect of ageing on immunity and pulmonary defences, underlying chronic diseases, silent aspiration, increased exposure to institutional care.[11] This was unlike a study where the maximum number of patients belonged to 21-40 years of age.[12] Another study found that patients aged <60 years significantly had more incidence of LRTI than patients aged ≥60 years.[13]

Male:female ratio in our study was found to be 1.93:1, which showed the male preponderance. Our finding is consistent with other studies.[12,14,15] The male preponderance could be due to the fact that they are more ambulatory and more exposed to the associated risk factors like smoking, use of tobacco, Chronic Obstructive Pulmonary Disease (COPD), alcoholism, etc.[14] But, a study conducted in Nigeria found female preponderance than male.[16] The associated risk factors identified in our study were smoking, Human Immunodeficiency Virus (HIV), past lung infections, COPD, diabetes, and neoplasm. Similar findings have already been reported in other studies.[17]

In the present study, bacterial pathogens could be isolated in 89% of the cases, which is similar to other studies.[1,6] The inability to isolate any bacteria in the remaining 11% of the cases could be due to prior antibiotic administration or due to inability to culture other causative agents like anaerobic bacteria, Chlamydia spp., Legionella spp., Mycoplasma spp., etc. Majority of the isolates in our study were GNBs. This is in accordance to other studies.[4,17,18] In our study, the most common organism isolated was Pseudomonas aeruginosa (38.9%) followed by S. aureus (30%; out of which 25% were MRSA) and K. pneumoniae (15.7%). These results obtained were not in accordance to most of the other studies.[12,14,15] The increased incidence of Pseudomonas isolates in our study maybe because of the following reasons. The first reason maybe that a large number of our patients were HIV positive, 218 i.e., 24% of the total 904. Out of these 218 patients, 176 were IPD patients. Manipur is one of the six high prevalence states in India regarding HIV infection.[19] Non-fermentative bacteria like Pseudomonas aeruginosa usually cause disease by colonizing and subsequently infecting the immunocompromised hosts. They have been incriminated as emerging opportunistic pathogen especially from hospital settings.[20] Another reason maybe that majority of the patients (67.9%) belonged to IPD implicating the possibility of hospital-acquired infection. Pseudomonas causes notorious hospital-acquired infections. They are not the common causes of LRTI. We had promptly notified the hospital infection control committee of the institute and stringent actions were taken up to avoid such incidences in future.

In our study, Pseudomonas aeruginosa showed maximum sensitivity to carbapenems, aminoglycosides, and piperacillin-tazobactam combination. They were highly resistant to the third and fourth generation cephalosporins. Similar findings were seen in other studies.[12,14] S. aureus isolates in our study were found to be highly sensitive to linezolid and vancomycin. They were 100% resistant to penicillin, which is also found in other studies.[12,14,18] In the present study, the K. pneumoniae isolates were found to be most sensitive to the carbapenems and piperacillin-tazobactam combination and highly resistant to the third generation cephalosporins and quinolones. This is in accordance to other studies.[12,14,17]

The strength of our study is that a single experienced technician performed the sputum culture identification and antibiotic sensitivity tests relevant in the study, which rendered consistent results. Our study happens to be from a newly established medical institute and is the only one of its kind from this Indian state. Inclusion of anaerobic and fastidious bacterial pathogen culture methods would have been able to further validate our present findings. Inability to include cultivation methods for viral and mycological pathogens is another drawback of our study.

CONCLUSION

Pseudomonas aeruginosa has been found to be the most commonly isolated aerobic bacteria from the AFB negative sputum samples. The result infers the need to strengthen our hospital infection control policies. Antibiogram helps in specific treatment during the management of non-tuberculous LRTI as most of the isolated bacteria have been observed to be highly resistant to the commonly used antibiotics.

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