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Antibiogram Assessment of Gram-negative Bacterial Isolates of LRTI Suspected Patients Visiting Tertiary Care Hospital

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Abstract

Lower respiratory tract infection is a common cause of morbidity and mortality worldwide. A cross-sectional study was carried out with an objective to assess the antibiogram pattern of the Gram-negative bacterial isolates from LRTI suspected patients visiting tertiary care hospitals. The study was carried out in a tertiary care hospital laboratory from January 2022 to March 2022. A total of 108 sputum samples from LRTI suspected patients were processed for Gram-negative bacteria isolation. Out of 108 sputum samples, 17 (15.74%) showed significant growth for Gram- negative bacteria and 91 (84.26%) showed insignificant growth. Male suspected patients were more predominant over female suspected patients, showing (11)10% and (6)6% significant growth respectively. The most common bacterial pathogens among Lower respiratory tract infection (LRTI) suspected patients are *Mycobacterium tuberculosis* (47.05%) followed by *Pseudomonas aeruginosa* (17.64%), *Klebsiella pneumoniae* (17.64%), *Escherichia coli* (11.76%), and *Klebsiella oxytoca* (5.88%). Antibiotic susceptibility tests (AST) were done by the Kirby-Bauer disc diffusion method. The antibiotic susceptibility patterns of isolates revealed high resistivity against most of the used antibiotics whereas the isolates showed good in vitro activity against amikacin, ofloxacin and ceftriaxone. Therefore, Culture and Susceptibility tests are important for management of LRTI in patients.

Keywords: Lower respiratory tract infection, Sputum, Bacterial isolates, Antibiogram patterns

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Introduction

Respiratory tract infections (RTIs) are infections ofbody parts which are involved in breathing such as sinuses, throat, airways or lungs. It includes both upper and lower respiratory tract infections [1]. Upper respiratory tract infections include common cold, tonsillitis, laryngitis, acute rhinosinusitis & others. While lower respiratory tract infections include acute bronchitis, bronchiolitis, pneumonia, tracheitis, & others [2]. Respiratory tract infections are the most commonly occurring health conditions where 5% to 10% death reported in CDC, [3]. The bacteria which are most commonly found in respiratory infections includes; *Streptococcus pneumoniae, Klebsiella pneumoniae, Streptococcus pyogenes,Staphylococcus aureus, Haemophilus influenzae,Escherichia coli* & others. Besides that, Acid fast bacilli may also be present in some individuals which have respiratory tract infections. *Mycobacterium tuberculosis & Mycobacterium leprae* are two examples of acid-fast bacteria, where *Mycobacterium tuberculosis* cause the respiratory disease called Tuberculosis and *Mycobacterium leprae* causes the disfiguring disease called Leprosy. Some viruses also cause respiratory infections which include human coronavirus, adenovirus, parainfluenza virus 1&3, influenza virus A&B, respiratory syncytial virus & others [4]. Also, fungi species like *Aspergillus* spp., *Cladosporium* spp., *Alternaria* spp., *Penicillium* spp., *Fusarium* spp., *Blastomyces dermatitidis, Histoplasma capsulatum, Candida albicans, Nocardia* spp. and Parasites such as *Paragonimus* spp., *Ascaris lumbricoides, Entamoeba histolytica* [5].

Lower respiratory tract infections are those infections in the lungs or below the voice box which include pneumonia, bronchitis & tuberculosis.Usually, the lower respiratory tract is free from microbes but they can enter at any time if they can defeat the host immune system. It differs from upper respiratory tract infections by the area of the respiratory tract where they affect. Lower respiratory tract infections involve the airways below the larynx while upper respiratory tract infections involve the larynx or above. The symptoms of lower respiratory tract infection varies & depends on the severity of the infection. Sputum is the most common sample which is collected from patients suffering from lower respiratory tract infections. Sputum is the thick fluid made in the lungs & in the airways leading to the lungs. It is made up from secretions from cell lining of the respiratory tract, dead cells, and foreign matter i.e. breathed into the lungs such as tar from cigarettes & air pollutants, white blood cells (WBC) & othersimmune cells. In case of infections, bacteria may also be present in the sputum. Sputum which is secreted into the airways of the respiratory tract

Table 1. Bacterial isolates recovered from patients

Isolated Organisms	No. of isolates	Percentage
Mycobacterium tuberculosis	8	47.05%
Pseudomonas aeruginosa	3	17.64%
Klebsiella pneumonia	3	17.64%
Escherichia coli	2	11.76%
Klebsiella oxytoca	1	5.88%
Total	17	100%

differs from the saliva. Saliva is produced in higher amount in the mouth. Sputum can be in any color including clear, white, yellow, green, pink or red & blood tinged withvarious medical conditions [6]. The aim of sputum collection is to identify the bacterial, viral, or fungal cause of suspected infection and its sensitivities to antibiotics. Those infections are spread through respiratory droplet which includes the mucus & saliva from an infected individual, which made airborne due to coughing or sneezing. It can remain airborne for hours in a closed room. These droplets which are infective, are then inhaled by a person who is uninfected. Then, it enters the respiratory tract, attaches to the respiratory lining (epithelium) & then causes an infection. Less commonly infectious agents may enter through the mouth while eating contaminated food, drinking water, sharing eating utensils, mouth to mouth contact and hand to mouth contact. Even in these cases the infectious agent is secreted from an infected person in saliva and respiratory mucus.

Materials and methods

This study was conducted in the Microbiology Laboratory of Tertiary Care Hospital from Januaryto March 2022. During this period, 108 sputum samples from LRTI suspected patients of all age- groups and both sexes were collected. The sputum samples were collected in a sterile, clean, wide-necked, leakproof containers and processed for Acid Fast staining for Mycobacterium and onto MacConkey agar (MA)and Blood agar (BA), a semi-quantitative culture technique for Gram- negative bacteria using standard calibrated loop and incubated aerobically at 37ºC for 24 hours [7]. The isolates were identified using routine conventional methods based upon microscopic findings, colony morphology and biochemical properties according to the standard microbiological protocol [8]. Antibiotic susceptibility test of Gram-negative bacterial isolates against various antibiotics were performed by the Kirby Bauer disk diffusion method using Muller's Hinton agar as recommended by CLSI, [9]. The statistical analysis was done by calculating chi square test.

Results

Total isolates

In this study, out of 108 sputum samples 15.74% showed significant bacterial growth whereas 84.26% possessed insignificant growth of bacteria (**Figure 1**).

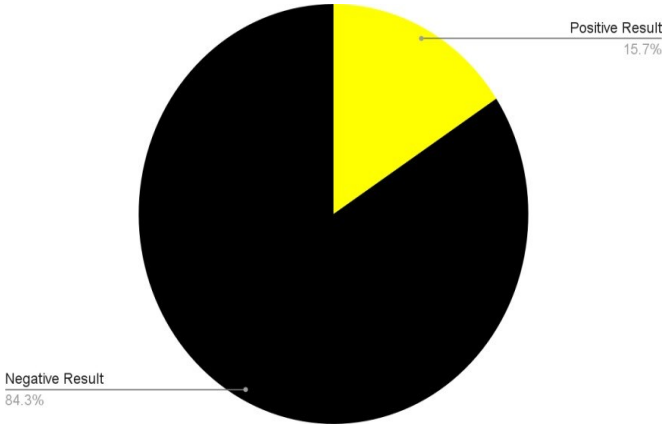

Figure 1. Pie chart showing percentage of significant and insignificant growth.

Bacterial pathogens in patients

Mycobacterium tuberculosis (47.05%) was found to be most prevalent among the patients followed by *Pseudomonas aeruginosa* (17.64%), *Klebsiella pneumoniae* (17.64%), *Escherichia coli* (11.76%), and *Klebsiella oxytoca* (5.88%) respectively. Five microbial isolates were identified from the patients.

Gender wise distribution of sputum culture report

Among 17 positive sputum samples, 11(65%) were male suspect and 6 (35%) were female.

The association between male and female bacterial growth rate is statistically non-significant atP>0.05 which is shown in **Table** 2.

Table 2. Gender wise distribution of sputum culture report

Gender	Growth		p-value
	No	%	
Male	11	65	>0.05
Female	6	35	
Total	17	17	

Incidence of LRTIs on the basis of age-group

Out of 17 bacterial isolates, the highest bacterial load was found in the age group greater than 60 (35.3%) and age-group between 21-40 (35.29%) Followed by age group 41-60 (25.41%). There is no bacterial growth obtained from the age group less or equal to 20 which was shown in **Figure 2**.

Antibiotic Susceptibility Testing

Among the bacterial isolates recovered from the sputum samples of patients, most of the isolates showed

Table 3. Antibiotic Susceptibility test of isolated organisms

Antibiotics Used	Organisms							
	P. aeruginosa		*K. pneumoniae*		*E. coli*		*K. oxytoca*	
	R	S	R	S	R	S	R	S
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Amikacin	-	3 (100)	1(33.33)	2(66.66)	1(50)	1(50)	-	1(100)
Ofloxacin	-	3 (100)	3 (100)	-	2(100)	-	1(100)	-
Amoxicillin	3 (100)	-	1(33.33)	2(66.66)	2(100)	-	1(100)	-
Amoxicillin /Clavulanic acid	3 (100)	-	2(66.66)	1(33.33)	2(100)	-	1(100)	-
Co-trimoxazole	3 (100)		2(66.66)	1(33.33)	1(50)	1(50)	1(100)	-
Ceftriaxone	-	3 (100)	1(33.33)	2(66.66)	1(50)	1(50)	1(100)	-
Ceftazidime	2(66.66)	1(33.33)	1(33.33)	2(66.66)	2(100)	-	1(100)	-
Ciprofloxacin	3 (100)	-	1(33.33)	2(66.66)	2(100)	-	1(100)	-

resistance against the antibiotic used. While the few isolated pathogens show sensitivity towards the used antibiotics. The Antibiotic susceptibility patterns of isolated organisms were shown in **Table 3**.

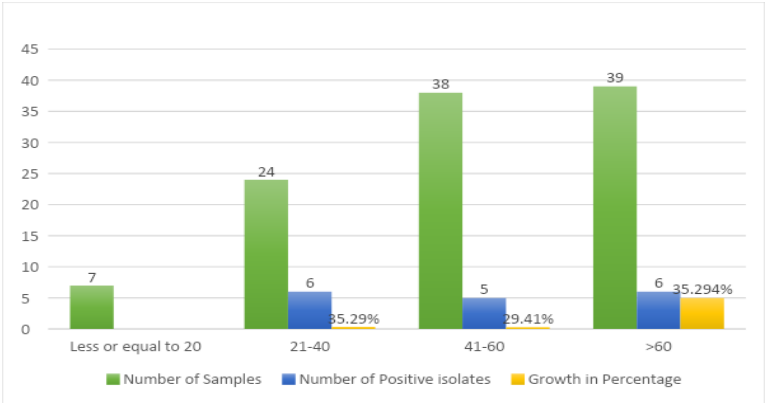

Figure 2. Bar- graph showing number of positive isolates and growth in percentage on the basis of Age- group (Inyears).

Discussion

This study aimed to find out the Gram-negative bacteriological profile of lower respiratory tract infection suspected patients and the sensitivity pattern of the isolates. This study showed a culture positivity rate of 17/108(15.74%) which is quite similar with result of Egbe et al. [6] i.e. (18.91%), whereas other similar studies done by Khan et al. [5] had shown higher growth rate i.e. (24.66%). The low growth rate and culture negativity of the LRTI suspected patients may depend upon various factor like prior use of antibiotics [10]. Viruses like adenovirus, respiratory syncytial virus, parainfluenza virus and rhino virus, which are significant contributors of LRTI, were not looked for in our study due to limitation of resources. Likewise, common pulmonary pathogens such as *Mycobacterium tuberculosis*, *Mycoplasma*, *Chlamydia*, *Pneumocystis*, Fungi, *Legionella*, and anaerobes could not be cultured by routine methods. In our study the Mycobacterial positive samples were referred to TB referral center for further processing.

Sputum samples received from patients of OPD, IPD & ICU were included in study & further processed in the laboratory. Out of 108 sputum samples, *Mycobacterium tuberculosis* was reported in high rates in 47% and Gram-negative bacterial isolates; *Pseudomonas aeruginosa* (17.64%), *Klebsiella pneumoniae* (17.64%), *E. coli* (11.76%), & *Klebsiella oxytoca* (5.88%) respectively. A study done by Khan et al. [4] has also reported the highest prevalence of *Pseudomonas aeruginosa* among the Gram-negative isolates. *M. tuberculosis* was identified by gold standard staining method i.e., Acid-fast staining.

In relation with gender, male population were more prone to this infection showing bacterial growth of 65% in male patient's sputum followed by 35% female patients' sputum which may be due to exposure to external environment but statistically there is no relation between growth and gender. The bacterial growth was found highest i.e., 6 (35.29%) in the age-group category of (21-40) and above 60 years which was followed by 5 (29.41%) among age-groups between (41- 60). No infection was seen in patients of age-groupbelow 20 years i.e., among 7 samples, no growth of isolates was seen. In this study, it was found that the age- group between (21-40) followed by the age-group above 60 years and age-group between (41-60)were found to be more prone to LRTIs respectively. Whereas age-groups below 20 years were not detected with LRTIs which shows that patients below 20 years were healthy as compared to other age-groups. This shows the correlation between age and LRTI which is also supported by a study done by Egbe et al. [6].

Antibiotic susceptibility tests (AST) were done by the Kirby Bauer disc diffusion method on Muller Hinton Agar (MHA). Inoculum was prepared & adjusted to 0.5 McFarland's turbidity standard. We have used eight antibiotics (amikacin, ofloxacin, amoxicillin, and amoxicillin/clavulanic acid, co-trimoxazole, ceftrixone, ceftazidime and ciprofloxacin) against isolates among

which we got variable results. Among used antibiotics, amikacin, ofloxacin and ceftriaxone were 100% affective towards P. *aeruginosa* whereas rest of the antibiotics are fully as well as partially ineffective [11]. In case of *K. pneumoniae,* amikacin, amoxicillin, ceftriaxone, ceftazidime and ciprofloxacin, bacteria were found only 66.66% susceptible. Whereas *E. coli* showed 50% susceptibility towards amikacin, co-trimoxazole and ceftriaxone. All the antibiotics were ineffective except amikacin for *K. oxytoca.* Among various antibiotics used in antibiotic susceptibility tests, amikacin, ofloxacin and ceftriaxone were highly effective against the isolates of Gram- negative pathogens. In some studies, resistant strains have occurred more frequently in isolates of sputum, suggesting emergence of resistance at sites more likely tohave poor penetration and sub-inhibitory level of aminoglycosides [12]. Gram- negative organisms were the most common cause of LRTIs in our study. Antibiograms suggest that the most common antibiotics that were used as the first choice of drugs to treat the LRTIs were found to be less effective [13]. Thus, this study highlights the need for the development of novel drugs.

Conclusion

The most common causative agent of LRTIs was found to be *M. tuberculosis* acid fast staining but due to our limitation we could not proceed it. Among the Gram-negative pathogens, *P. aeruginosa* and *K. pneumoniae* were the most common cause of LRTIs in the study. The antibiogram pattern in our study suggests that the Gram-negative bacterial pathogens were highly sensitive towards amikacin and ofloxacin. The emergence of drug resistance is a major hurdle in antibiotic therapy and it can be prevented by avoiding the practice of administration of prophylactic antibiotics before culture reports are available.

Author's contributions

All the authors have equal contribution for this research work and manuscript preparation.

Competing interests

No competing interests were disclosed.

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