

# Bacterial pathogens from lower respiratory tract infections: A study from Western Rajasthan

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## ABSTRACT

Respiratory tract infections are the most common diseases that are associated with social burden for the patient. Western Rajasthan has cases of Cystic fibrosis due to migrant population. The dry and dusty environment has led to prevalence of silicosis and COPD. As per IDSA (2018) guidelines, patients attending Out-Patient Department do not need microbiological investigations for lower respiratory tract infections (LRTI) except for influenza and tuberculosis. **Aims:** This study was conducted to identify the bacterial aetiology of LRTI among patients who attended AIIMS, Jodhpur, and to ascertain the current scenario of bacterial susceptibility in respiratory tract infections in order to optimize empiric therapy in Hospitals and community. **Methods and Material:** In total, 1,775 lower respiratory tract samples were received in Bacteriology Section of Microbiology Department (January 2017 to December 2018). Bartlett's criteria were stringently used to assess quality of specimen. Semiquantitative cultures were done for tracheal aspirate and bronchoalveolar lavage samples. Following culture, the isolated organisms were identified and antimicrobial sensitivity was performed according to CLSI. **Results:** Total 769 bacterial pathogens were isolated from 1,775 samples collected from cases of VAP, HAP, CAP, COPD, and cystic fibrosis. *Pseudomonas* species was the commonest isolate (31%), followed by *Klebsiella pneumoniae* (21.3%), *Acinetobacter* species (17.5%), *Escherichia coli* (15.4%), and *Staphylococcus aureus* (5%). Others include Group A  $\beta$ -hemolytic *Streptococcus*, *Burkholderia cepacia* complex, *Stenotrophomonas maltophilia*, and *Nocardia*. Gram-negative organisms showed increased resistance to routinely used antibiotics. Gram-positive organisms showed 100% susceptibility to vancomycin, linezolid, and clindamycin. **Conclusions:** Cotrimoxazole,  $\beta$ L- $\beta$ LI, aminoglycosides, and all second-line antibiotics tested were effective for treatment of RTIs.

**Keywords:** Chronic obstructive pulmonary disease, cystic fibrosis, lower respiratory tract infections, and nonfermenters

## Introduction

Lower respiratory tract infections (LRTI) are among most-common infectious diseases affecting humans' worldwide causing significant morbidity and mortality for all age groups. It is responsible for 4.4% of all hospital admissions and 6% of physicians' consultation. It accounts for 3%–5% of deaths in adults.<sup>[1]</sup> LRTI are often misdiagnosed, mistreated, and underestimated due-to its nonspecific presentation in

community or hospital-setting. Etiological agents of LRTI vary geographically and timely.<sup>[2,3]</sup> The problem is much greater in developing countries.<sup>[1]</sup> Recognition of the possible existence of lung microbiome has been a major recent revelation in medicine.<sup>[4]</sup> The increase in antibiotic-resistance has compromised selection of empirical treatment and choice of effective-antibiotic.<sup>[5]</sup>

## Objectives

The objective of the present study was to identify the bacterial aetiology of LRTI among patients who attended AIIMS, Jodhpur from January 2017 to December 2018 and to ascertain the current scenario of bacterial susceptibility in respiratory tract infections in order to optimize empiric therapy in patients presenting with

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cases of community acquired pneumonia (CAP), hospital acquired pneumonia (HAP), ventilator-associated pneumonia (VAP), chronic obstructive pulmonary disease (COPD), and cystic fibrosis in various healthcare centers.

## Materials and Methods

### Setting

This is a retrospective study conducted at the Department of Microbiology, All India Institute of Medical Sciences, Jodhpur, for the duration January 2017 to December 2018. The study was approved by Institutional Ethics Committee wide letter number AIIMS/IEC/2019/1767 (06.04.2019).

### Patient's enrolment

All the patients enrolled in the study were from Out-Patient Department (OPD), In-Patient Department (IPD), and Intensive Care Unit (ICU). The respiratory tract samples (sputum, bronchoalveolar lavage [BAL], endotracheal aspirate, gastric lavage, etc.) were obtained from the patients of all the age and sex groups, with clinical presentation of COPD, cystic fibrosis, CAP, VAP, HAP, post-influenza, old tuberculosis, cavitary lesions, lung abscess, neoplasm, prolonged hospital stay, etc.; suggestive of LRTI. History of antibiotic consumption was also noted.

### Sample collection and processing

In total, 1,775 samples of sputum, BAL, endotracheal aspirate, gastric lavage, etc., were obtained.

Sample selection was done: sputum-quality of sample was assessed based on Bartlett's scoring. Satisfactory sputum samples were further processed. BAL-microscopically percentage of neutrophils with engulfed bacteria was determined, and semiquantitative analysis  $\geq 10^4$  colony forming unit (CFU)/mL was done. Endotracheal aspirate: semi quantitative analysis  $\geq 10^5$  CFU/ml was done.

Samples were further processed for routine bacterial culture and sensitivity. Following culture, the isolated organisms were identified and antimicrobial sensitivity was performed as per laboratory standards and antibiotic interpretation was done as per Clinical and Laboratory Standard Institute (CLSI) guidelines.<sup>[6]</sup>

## Results

In total, 1,775 respiratory specimens were received during the study period out of which 769 cultures yielded a significant pathogen and 1,006 cultures had growth of normal oropharyngeal flora.

It was realized that almost 50% of these isolates were in poorly collected samples. Swabs from endotracheal tubes were refused as it represents only colonization. Many a times, tracheal aspirates were mislabelled as BAL sample.

Among 769 positive cultures, 112 samples showed polymicrobial infection. *Pseudomonas* species 31.2% (275) was the most common isolate followed by *Klebsiella pneumoniae* 21.3% (188), *Acinetobacter baumannii* 17.5% (154), *Escherichia coli* 15.4% (136), and *Staphylococcus aureus* 5% (44). Others were as follows: Group A  $\beta$ -hemolytic *Streptococcus* 3.2% (28), *Burkholderiacepacia* complex 1.1% (10), *Stenotrophomonas maltophilia* 0.4% (4), and *Nocardia* 0.2% (2) [Table 1].

Oxacillin (1  $\mu$ g) disc was used as surrogate marker to identify penicillin resistance in *Streptococcus pneumoniae*. Group A  $\beta$ -hemolytic *Streptococci* was identified presumptively with Bacitracin (0.04 U) disc. Demographic and clinical details (age, sex, location of patients, sample distribution) of the patients are provided in Table 2. Antibiotic resistance pattern of Gram-negative bacteria and Gram-positive bacteria are shown in Tables 3 and 4, respectively.

## Discussion

In our study, LRTI were more common in males 73% than in females 27%. Male prevalence of LRTI may be due to their exposure to different group of population and due to some associated risk factors of respiratory tract infection, such as smoking, alcohol consumption, and COPD. Similar to other studies, our findings corroborated with the results accomplished by Shah *et al.*, Panda *et al.*, Saha *et al.*, and Akingbade *et al.*<sup>[3,7-9]</sup> It was observed that adults and the elderly males were most at risk of a severe respiratory condition. Almost one-third of cases were of pulmonary Kochs.

In this study, single and multiple organisms were isolated in 86.67% and 13.33%, respectively, of study population. These findings are similar with the study conducted by Saxena *et al.* and Narayanagowda *et al.*<sup>[10,11]</sup>

**Table 1: Distribution of organisms presenting to OPD, IPD, and ICU**

Organism name	OPD	IPD	ICU	Number, n=769 (%)
<i>Pseudomonas aeruginosa</i>	79	103	44	226 (25.6)
<i>Pseudomonas</i> species	22	18	9	49 (5.6)
<i>Acinetobacter</i> species	21	60	73	154 (17.5)
<i>Burkholderia cepacia</i> Complex	0	7	3	10 (1.1)
<i>Stenotrophomonas maltophilia</i>	0	2	2	4 (0.5)
<i>Klebsiella</i> species	41	88	59	188 (21.3)
<i>Escherichia coli</i>	31	81	24	136 (15.4)
<i>Citrobacter freundii</i>	0	3	2	5 (0.6)
<i>Enterobacter aerogenes</i>	2	8	4	14 (1.6)
<i>Proteus</i> species	0	3	1	4 (0.5)
<i>Serratia marscescens</i>	0	2	0	2 (0.2)
<i>Staphylococcus aureus</i>	13	23	8	44 (5)
<i>Streptococcus pneumoniae</i>	14	1	0	15 (1.7)
Group A $\beta$ -Hemolytic - <i>Streptococci</i>	22	6	0	28 (3.2)
<i>Nocardia</i>	0	2	0	2 (0.2)

**Table 2: Demographic and clinical details of the patient (n=769)**

	Number (%)
Age	
<20	136 (17.7)
21-40	133 (17.3)
41-60	231 (30)
>61	269 (35)
Gender	
Male	561 (73)
Female	208 (27)
Location of patients	
OPD	154 (20)
Wards	397 (51.7)
ICU	218 (28.3)
Sample	
Sputum	456 (59.3)
Bronchoalveolar lavage	205 (26.6)
Endotracheal aspirate	105 (13.7)
Gastric aspirate	1 (0.1)
ET tube	2 (0.3)

In this study, Gram-positive 10.1% (89) and Gram-negative 89.9% (792) organisms were isolated. Similar observations have been shown in other studies as shown in Table 5. Among which nonfermenting Gram-negative bacilli (NFGNB) were isolated in 50.3% (443/881) of respiratory samples. The importance of isolation of nonfermenters has increased in last decade, after more and more reports are correlating them with the either infection outbreaks in hospitals or healthcare-associated infections. Most of the patients were having prolonged hospital stay for more than a week. It supports the fact that these patients may have acquired some of these multidrug resistant pathogens in hospital settings as HAP and VAP. Identification of nonfermenters used to be considered as commensal flora, but due to increased awareness of their pathogenicity in certain patient population and improvement in diagnostic criteria, they are increasingly being reported.

Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa*, *Acinetobacter* species, *Klebsiella* species and *Staphylococcus aureus* are depicted in Figures 1-4 respectively.

In this study, 28.3% bacterial strains were isolated from ICU, whereas 51.7% were isolated from wards from which most isolates were *Acinetobacter baumannii* 73 (47.4%), followed by *Klebsiella pneumoniae* 59 (31.4%) and *Pseudomonas aeruginosa* 44 (19.4%) similar to that seen in Ullah et al.<sup>[15]</sup> In other Study by Nishat et al.,<sup>[15]</sup> nonfermenters (61.11%) were the predominant isolates from Surgical ICU, whereas in the medical ICU, along with nonfermenters (47.91%), enterobacteriaceae (41.66%) was the most common organisms isolated.

*Pseudomonas aeruginosa* is more commonly found in patients with chronic lung cavities or as a complication of treatment with immunosuppressive drugs. *Pseudomonas aeruginosa* was isolated in 25.6% (226) cases, similar to Saha et al., Sethi

**Table 3: Antibiotic resistant (%) Gram-negative organism**

No.	Pn	AMC	PIT	CTX	CTR	CAZ	CAT	CPM	AT	MRP	IPM	ETP	CIP	LE	GEN	AK	COT	TGC	NET
<i>Pseudomonas aeruginosa</i>	226	IR	29 (12.8)	IR	IR	22 (9.7)	24 (10.6)	14 (6.2)	26 (11.5)	18 (8)	71 (31.4)	IR	20 (8.8)	23 (10.1)	36 (15.9)	34 (15.0)	IR	IR	16 (7.1)
<i>Pseudomonas</i> species	49	IR	2 (4.1)	4 (8.1)	-	4 (8.1)	1 (2)	4 (8.1)	5 (10.2)	2 (4.1)	6 (12.2)	-	5 (10.2)	4 (8.1)	7 (14.3)	4 (8.1)	3 (6.1)	-	3 (6.1)
<i>Acinetobacter</i> Species	154	IR	103 (66.9)	4 (2.6)	73 (47.4)	59 (38.3)	52 (33.7)	100 (64.9)	IR	51 (33.1)	103 (66.9)	IR	38 (24.6)	63 (40.9)	105 (68.2)	88 (57.1)	14 (9.1)	6 (3.9)	-
<i>Burkholderia cepacia</i> complex	10	IR	IR	IR	IR	7 (70)	-	IR	IR	1 (10)	IR	IR	1 (10)	8 (80)	IR	IR	4 (40)	-	6 (60)
<i>Stenotrophomonas maltophilia</i>	4	IR	IR	IR	IR	1 (25)	2 (50)	-	IR	IR	IR	IR	-	1 (25)	IR	IR	2 (50)	-	-
<i>Klebsiella</i> species	188	IR	15 (8)	9 (4.8)	120 (63.9)	29 (15.4)	56 (29.8)	122 (64.9)	69 (36.7)	40 (21.3)	94 (50)	39 (20.7)	38 (20.2)	76 (40.4)	96 (51)	83 (44.1)	81 (43)	9 (4.8)	1 (0.5)
<i>Escherichia coli</i>	136	5 (3.7)	21 (15.4)	35 (18.6)	99 (72.8)	21 (15.4)	25 (18.4)	102 (75)	37 (19.7)	14 (10.3)	42 (30.9)	18 (13.2)	46 (33.8)	54 (39.7)	50 (36.8)	19 (13.8)	43 (31.6)	1 (0.5)	-
<i>Citrobacter</i> species	5	IR	IR	-	2 (40)	-	-	2 (40)	-	-	-	-	1 (20)	-	1 (20)	-	1 (20)	-	-
<i>Enterobacter aerogenes</i>	14	IR	IR	-	7 (50)	5 (35.7)	2 (14.3)	6 (42.8)	4 (28.6)	4 (28.6)	5 (35.7)	3 (21.4)	2 (14.3)	4 (28.6)	4 (28.6)	3 (21.4)	2 (14.3)	1 (7.1)	-

Pn=Penicillin, PIT= Piperacillin-tazobactam, CTX=Cefotaxime, CTR=Ceftazidime, CAZ=Ceftazidime, CAT=Ceftazidime, CPM=Ceftazidime, AT=Aztreonam, MRP=Meropenem, IPM=Imipenem, ETP=Ertapenem, CIP=Ciprofloxacin, LE=Levofloxacin, GEN=Gentamicin, AK=Amikacin, COT=Corrimoxazole, TGC= Tigecycline, NET=Netilmicin, IR=Intrinsically resistant as per CLSI guidelines.

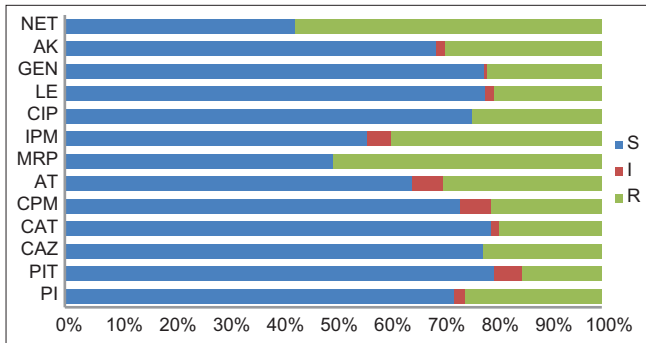
**Table 4: Antibiotic resistant (%) gram positive organism**

Organism	No.	Pn	AMP	OX	CX	COT	TOB	GEN	AK	CIP	LE	E	CD
<i>Staphylococcus aureus</i>	44	40 (91)	-	-	25 (56.9)	10 (22.7)	1 (2.2)	5 (11.4)	2 (4.5)	18 (41)	9 (20.5)	31 (70.4)	24 (54.5)
Group A $\beta$ -Hemolytic- <i>Streptococci</i>	28	1 (3.6)	1 (3.6)	-	-	1 (3.6)	-	-	-	-	-	1 (3.6)	-
<i>Streptococcus pneumoniae</i>	15	-	2 (13.3)	6 (40)	-	5 (33.3)	-	-	-	-	2 (13.3)	2 (13.3)	-

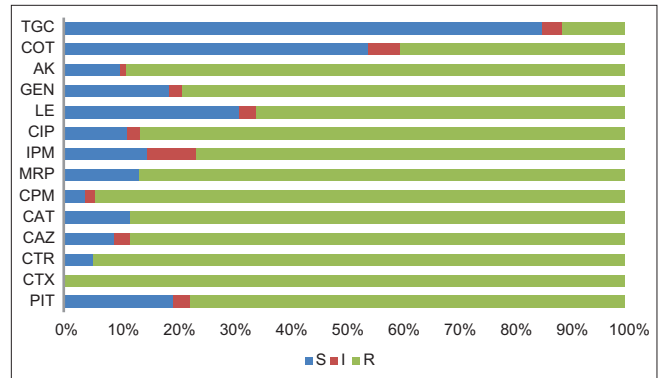
Pn=Penicillin, AMP=Ampicillin, OX=Oxacillin, CX=Cefoxitin, COT=Cotrimoxazole, TOB=Tobramycin, GEN=Gentamicin, AK=Amikacin, CIP=Ciprofloxacin, LE=Levofloxacin, E=Erythromycin, CD=Clindamycin

**Table 5: Comparison with other similar studies**

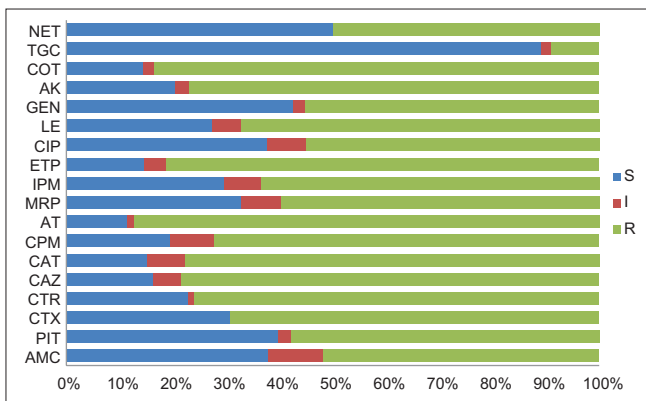
	Year	Gram-positive cocci	Gram-negative bacilli	Nonfermenter Gram-negative bacilli (NFGNB)	Enterobacteriaceae	n
This study (Jodhpur)	2018	89 (10.1%)	792 (89.9%)	443 (50.3%)	349 (39.6%)	881
Regha et al. <sup>[1]</sup> (Kerala)	2018	44 (15.3%)	244 (84.7%)	136 (55.7%)	108 (37.5%)	288
Anup saha et al. <sup>[8]</sup> (Tripura)	2018	5.10%	92.86%	28%	67%	100
Ravichitra et al. <sup>[2]</sup> (Andhra Pradesh)	2016	-	65.5%	35 (10.1%)	191 (55.4%)	345 (58.9%)
Sarmah et al. <sup>[12]</sup> (Guwahati)	2016	13	407	80	327	597 (49.4%)
Vishwanath et al. <sup>[13]</sup> (Karnataka)	2013	-	-	830 (16.4%)	-	5056 (54%)
Ullah et al. <sup>[5]</sup> (Bangladesh)	2015	57 (89.06%)	7 (10.92%)	2 (3.12%)	5 (7.80%)	64
Kulkarni et al. <sup>[14]</sup> (Nashik)	2014	29%	71%	26.7%	20%	45



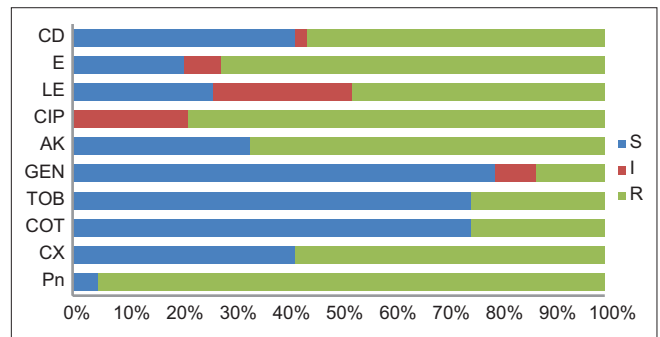
**Figure 1: Pseudomonas aeruginosa antimicrobial susceptibility pattern**



**Figure 2: Acinetobacter species antimicrobial susceptibility pattern**



**Figure 3: Klebsiella species antimicrobial susceptibility testing**



**Figure 4: Staphylococcus aureus antimicrobial susceptibility testing**

et al., Sethi et al., and ElKorashy et al.<sup>[8,16,17]</sup> *Pseudomonas* was found to be sensitive to amikacin, piperacillin–tazobactam, ceftazidime–tazobactam, cefepime, gentamicin, levofloxacin, and ciprofloxacin. Similar findings were observed by Narayanagowda et al. and Vishwanath et al.<sup>[11,13]</sup>

In this study, *K. pneumoniae* was the second most common Gram-negative isolate and was tested to be sensitive to

piperacillin–tazobactam, amikacin, ceftriaxone, ceftazidime, ceftazidime-tazobactam, cefepime, levofloxacin, gentamicin, ciprofloxacin, and amoxiclav. These findings are same as observed in the study carried out by Regha et al., Saha et al., Saxena et al., Vishwanath et al., and Kulkarni et al.<sup>[1,8-10,13,14]</sup>

Malini et al.<sup>[18]</sup> from Kolar in India, have documented the isolation of 6.8% (25 of 365) of NFGNB in respiratory samples. *Stenotrophomonas maltophilia* is considered as a common



nonfermenter to cause infection in hospital settings. Correct identification of this NFGNB assumes importance as it shows inherent resistance to commonly used broad spectrum  $\beta$ -lactam group antibiotics and even to imipenem.<sup>[6]</sup> Our study has shown the isolation of this bacterium in four cases. *Burkholderia cepacia* complex is another NFGNB colonizing and infecting patients with chronic respiratory illness. It is known to cause disease in cystic fibrosis patients, and once infected, it is very difficult to treat due to multiple intrinsic resistance to many  $\beta$ -lactam drugs, aminoglycosides, colistin and polymixin B, the first-line therapeutics of choice against serious pseudomonal infections.<sup>[6]</sup> In our study, *Burkholderia cepacia* complex was isolated in 10 cases from IPD (7) and ICU (2). Rahbar *et al.*<sup>[19]</sup> have shown the isolation of *Burkholderia cepacia* complex as 4.66% of all the nonfermenters isolated from different types of specimens (respiratory, blood, urine, wound, etc.).

NFGNB have shown resistance to amikacin, gentamicin, imipenem, cefepime, ceftriaxone, and piperacillin–tazobactam [Table 3]. The aminoglycosides that are considered as good option for life-threatening lower respiratory infections have shown high resistance in the present study for these nonfermenters wherever tested. There is poor penetration of aminoglycosides from blood into infected respiratory tissues so as to reach the local drug concentration above the minimum inhibitory concentration necessary for the infecting organisms. This observation has also been discussed by earlier studies. All these nonfermenters are known for their inherent resistance to multiple groups of antibiotics. Hence, correct identification of these nonfermenters is very important for choosing correct antibiotic so as to reduce the morbidity and mortality. Any NFGNB culture isolate from respiratory tract infection should not be ignored as just contaminant but correlated clinically for its pathogenic potential and identified using standard methods, so as to institute appropriate and timely antibiotic coverage. It is equally important not to treat commensal NFGNBs.

In this study, among Gram-positive organisms, *S. aureus* 5% (44) was the most common pathogen isolated followed by Group A  $\beta$ -hemolytic *Streptococci* from OPD patients and *Streptococcus pneumoniae*. *Staphylococcus aureus* was sensitive to tobramycin, cotrimoxazole, and gentamicin, 56.9% were Methicillin Resistant *Staphylococcus aureus* (MRSA). All isolates were vancomycin and linezolid sensitive. Similarly in the study conducted by Narayanagowda *et al.*,<sup>[11]</sup>  $\beta$ -Hemolytic *Streptococci* was second frequently identified gram positive organism and was sensitive to penicillin group of antibiotics, erythromycin, clindamycin, and levofloxacin. *Staphylococcus aureus* and Aspergilli are known to cause secondary infections post-influenza. Increasingly fungal pathogens are being reported as cause of LRTIs from ICU.<sup>[20]</sup>

It is necessary to have policies regarding restrictive use of antibiotics such as carbapenems and colistin. Regular monitoring of such resistant isolates would be important for infection control in critical units.<sup>[21]</sup>

Strict implementation of the concept of ‘antibiotic stewardship’ has become necessary to conserve the already available antibiotics. Hospitals should have an “antibiotic policy” and facilities for proper monitoring of antibiotic usage along with effective infection control practices to check the issue of antibiotic resistance worldwide. Periodic analysis of types of respiratory pathogens and regular updation of their antibiograms should be done in every healthcare setting, so that changing trends can be identified and therapy adjusted accordingly.<sup>[1]</sup>

The trend towards increased use of molecular diagnostic tools will probably continue with increased availability of point of care testing.<sup>[4]</sup>

## Conclusion

This study reveals that a variety of pathogens are responsible for LRTI and antibiotics resistance has become a great public health issue. Gram-negative organisms showed increased resistance to routinely used antibiotics. Gram-positive organisms showed 100% susceptibility to vancomycin, linezolid, and clindamycin.

Proper identification of the probable pathogens and their antibiotic susceptibility pattern can help our health professionals to choose the right antibiotic therapy and improve the outcome. Do not report everything that grows, knowledge of colonizers and contaminants in different clinical conditions is important. This year CDC (Center for Disease Control) has proposed theme during Fungal Disease Awareness week (September 23–27, 2019) think fungus if a case of pneumonia does not improve with appropriate antibiotics.

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## Conflicts of interest

There are no conflicts of interest.

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