IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

STUDY OF ANTIBIOTIC RESISTANCE PROFILE OF PATIENTS WITH RESPIRATORY TRACT INFECTIONS IN SMIMER, SURAT, GUJARAT

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Abstract:
Background and Objective: Respiratory tract infections (RTIs) are one of the leading human diseases causing high morbidity and mortality worldwide. Day by day, the prevalent etiological agents which causes Respiratory infection and their antimicrobial resistance patterns varies geographically. It is important to study their recent trends for ideal treatment and effective management.
Materials and Methods: For original research, clinical samples including Sputum, Pleural fluid, Tracheal aspirate, and BAL fluid from respiratory tract were examined for culture and sensitivity reports. The samples were obtained in the microbiology lab, Department of Microbiology, SMIMER during 1st August 2022 - 31st may 2023. The samples were processed by standard methods for isolation and identification. Antibiotic sensitivity test was done by using Kirby Bauer disc diffusion method and results were interpreted according to CLSI 2022 guidelines.
Results: Total 650 samples were collected from which, 439 (67.53%) clinical samples were shown positive for bacterial growth from total 650 clinical samples for. Total 540 isolates were obtained from 439 positive samples in which 450 (83.3%) isolates were gram-negative bacilli (GNB) and 90 (16.6%) isolates were gram-positive cocci. The predominant pathogen isolated was *K. pneumoniae* (31.66%) followed by *P. aeruginosa* (17.40%). The overall susceptibility of GNB was highest towards macrolide followed by penicillin, cephalosporin. Gram positive organisms exhibited highest susceptibility towards Vancomycin and Linezolid. 31% of *Staphylococcus aureus* were Methicillin Resistant (MRSA). Most of the isolates shows high level of resistance against more than two antibiotics. Although multi-drug resistance was also reported in the present study. Gentamicin, Amikacin and Cefuroxime are recommended as the antibiotics of choice for the treatment against bacterial isolates obtained.
Conclusion: Ertapenem and Meropenem is the most sensitive antibiotics followed by Amikacin and Linezolid which can be used for empirical therapy for RTI. The antibiotic therapy should be modified as per the culture and antibiotic sensitivity report. Regular determinations of the type of bacterial pathogens and updating of antibiogram must be followed in every institution to aid in better patient management by helping the clinician in the proper use of antibiotics.

INTRODUCTION

Respiratory tract infections (RTIs) are termed as the infectious diseases of the respiratory tract including nose, sinus, trachea, lungs and bronchia in humans (Miriti et al., 2023). RTIs are classified as upper and lower respiratory tract infections. RTIs are one of the leading causes of morbidity as well as mortality in developing countries and gives heavy burden to counties economic and public health (Safiri et al., 2023) . Respiratory infections cause a serious financial burden to the economy of any country due to loss of productivity and cost of antimicrobial agents are also prescribed by physician, even when bacteria are not the actual cause of respiratory tract infections (CM et al., 2013). The commonly known respiratory bacterial pathogens are *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas spp*., *Klebsiella spp*., *Haemophilus influenzae*, etc. Resistance to antibiotics is a global challenge to the health sectors especially in developing countries leading to the emergence of mutant bacteria strains. Respiratory bacterial pathogens that are associated with reduced susceptibility to multiple classes of antibiotics include *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and *Klebsiella pneumoniae* (Shah et al., 2016). Bacteria may acquire resistance to antibiotics through the different mechanisms like; i) active efflux of the antibiotics, ii) decreased permeability of the cell membrane, iii) modification of drug target or inactivation of the antibiotics (Gebre et al., 2021). Factors that are responsible for the development of resistance bacteria are, a) over usage of antimicrobial agents, b) hospital acquired infections, which are spread by transmission of resistant bacteria among patients and from health care workers to patients and from patients to health care workers and c) poor guidelines in the administration of antimicrobial agents (Ventola, 2015). In developing countries such as India, treatment of respiratory tract infections are done empirically and this may be due to expensive laboratory services and time as some of the tests takes time before the results are out. It results in emergence of resistance bacterial pathogens which is a major and serious problem

in the health sectors. Resistance to antimicrobial agents has led to therapeutically failure of empirical treatment (Khan S et al.; 2015). According to various researches, a better understanding of the resistance mechanism in respiratory pathogens and correct diagnosis of the etiological agents of respiratory infections leads to better patient's health, lowers morbidity, mortality and antimicrobial resistance. Therefore, constant monitoring and surveillance in resistance pattern of respiratory pathogens towards antimicrobial agents will not only guide the physician in the management of these infection but it also helps in the evaluation of these infections (Keith et al., 2010a).

This research study was conducted to determine the presence of bacterial respiratory infections, number of people infected and resistance profile among antibiotic. However, there are minimal reports on the presence of bacterial respiratory infections, number of people infected and the antimicrobial susceptibility pattern among patients presenting with respiratory tract infections.

RESEARCH METHODOLOGY

a) Study design: Hospital based cross sectional study was carried out between the month of August 2022 to June 2023. A total 650 patients were selected who sufferings from respiratory tract infections.

i) Inclusion criteria:

1. Patients with respiratory clinical presentation who consented to participate in the study who had not taken antibiotics for a week prior to sampling on OPD based.
2. Whereas it's not applicable for hospitalized ICU patients.

ii) Exclusion criteria:

1. patients and sputum smears were positive for acid fast bacilli were excluded from the study.

b) Sample size determination:

The sample size was determined using single proportion formula,

$$n = \frac{Z^2 P (1- P)}{\mathcal{E}^2}$$

Where n is the sample size, Z is the statistic corresponding to level of confidence, P is expected prevalence (that can be obtained from same studies or a pilot study conducted by the researchers), and ε^2 is precision (corresponding to effect size).

c) Sample collection and laboratory processing:

Sputum samples were collected into a clean wide mouthed container which was labelled. All patients were guided on how to collect the sputum aseptically and take them to the laboratory immediately for analysis. Throat swabs and Tracheal aspirate were collected using a sterile cotton swab moistened in saline water. Pleural fluid and BAL fluid were collected through sterile syringe by a qualified medical officer and transported immediately to microbiology laboratory for analysis. Sputum samples that were viscous, mucous or purulent were considered suitable for analysis. Gram stain smears were carried out for all samples and were examined microscopically. Ziehl Nielsen staining was done only for sputum samples so that the samples found to be positive for acid fast bacilli were not analysed.

d) Statistical analysis

Collected data were analysed using Statistical Package for Social Sciences version (SPSS) 25. The results obtained were presented in descriptive statistics using tables and percentages with the help of Microsoft excel pilot graph and pilot table tools. One way Analysis of variance (ANOVA) was used for statistical difference between mean of resistant Gram-positive and Gram-negative bacterial isolates to antibiotics. Statistical analysis was done at 95% confidence level and $P<0.05$ was considered significant.

e) Bacterial isolation and identification

All the samples were individually inoculated onto sterile Nutrient agar, Blood agar and MacConkey agar using streak method and incubated at 37 °C for 20 to 24 h. Identification of bacterial isolates was done using colony morphology, colour, haemolysis on blood agar, Gram stain and various biochemical tests (Keith et al., 2010b).

f) Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was carried out using Kirby-Bauer Disc diffusion method on Muller Hinton agar. The antibiotics selected for Antibiotic Susceptibility Test were Azithromycin (15-mcg), Erythromycin (15-mcg), Roxithromycin (30mcg), Clarithromycin (15-mcg), Penicillin (10-unit), Ampicillin (30-mcg), Methicillin (5-mcg), Cefuroxime (30-mcg), Cefotaxime (30-mcg), Amikacin (30-mcg), Chloramphenicol (30-mcg), Clindamycin (2-mcg), Linezolid (30-mcg), Ciprofloxacin (5-mcg), Levofloxacin (5-mcg), Ertapenem (10mcg), Meropenem (10-mcg), Co-trimezole (25-mcg), Vancomycin (30-mcg), Rifampicin (5-mcg). The plates were incubated at 37°C for 24 h and the diameters of the zone of inhibition were measured in millimetres. *Staphylococcus aureus* ATCC 25,923 was used as quality control for Gram positive bacteria while *Escherichia coli* ATCC 25,922 was used for Gram negative bacteria. (Clinical and Laboratory Standard Institute recommendation- C.L.S.I 2022).

IV. RESULTS AND DISCUSSION

a) sample size determination:

sample size was determined with the formula of purposive sampling technique.

Considering that,

z =1.96 for 95% confidence interval corresponding to 5% significance level, while

p = prevalence of the patients (if not known=50%) and margin of error 5% (d=0.05).

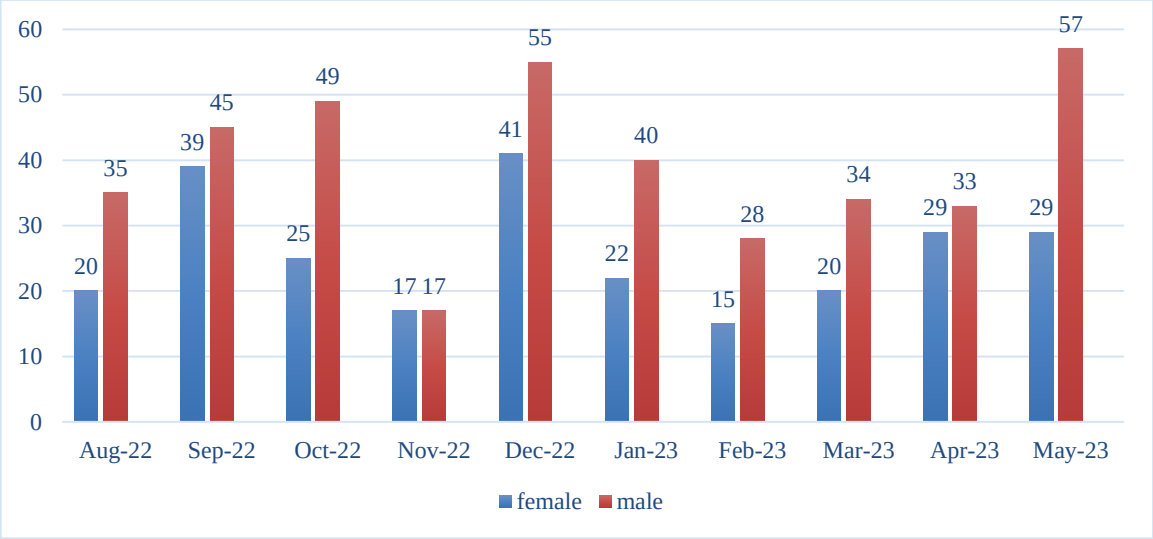
Therefore, $n = \frac{(1.96)^2 \times 0.5 (1- 0.5)}{(0.005)^2} = \mathbf{384}$

b) Specimen collection

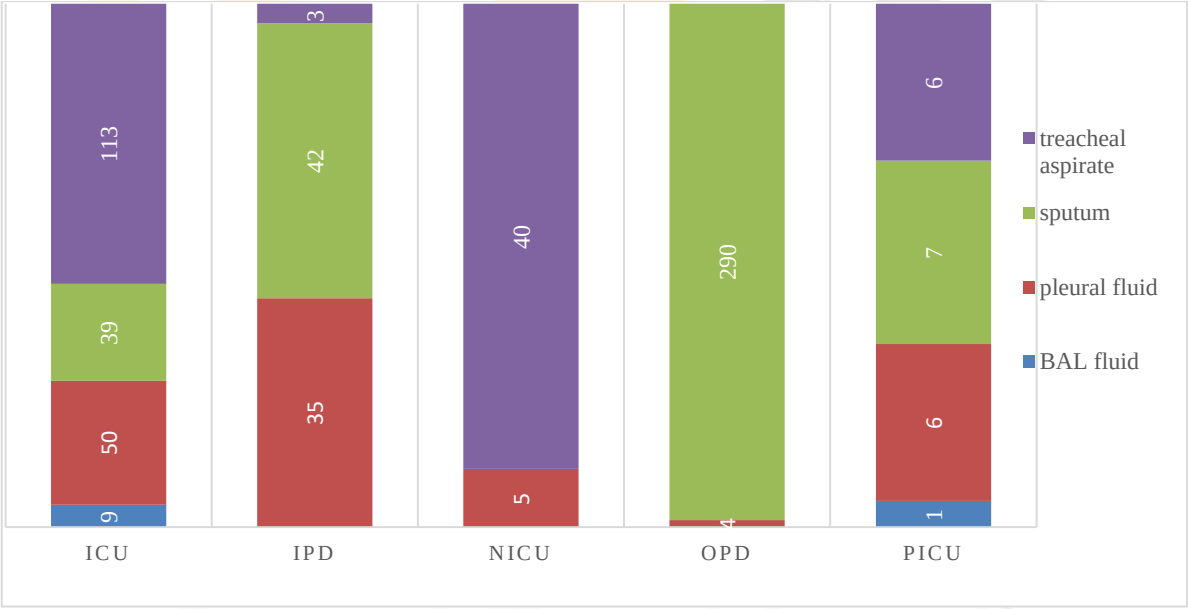
More than 384 sample had to analysed for completion the research work. Total Six hundred and fifty different clinical samples of patients who showing symptoms of respiratory infections, including the sputum, throat swab, tracheal aspirate, pleural fluid and BAL fluid were collected from Microbiology laboratory of SMIMER hospital, Surat.

As shown in below graph Total 650 samples including Sputum, BAL Fluid, Pleural fluid, Tracheal aspirate were collected between August 2022 to May 2023. Prevalence of the samples is shown in graph 1. Highest number of samples (96 samples) were collected in month of December 2022. During collection time different types of samples were collected from OPD (211 sample), IPD (80 samples), ICU (45 samples), NICU (294samples), PICU (20 samples) wards as shown in Graph 2.

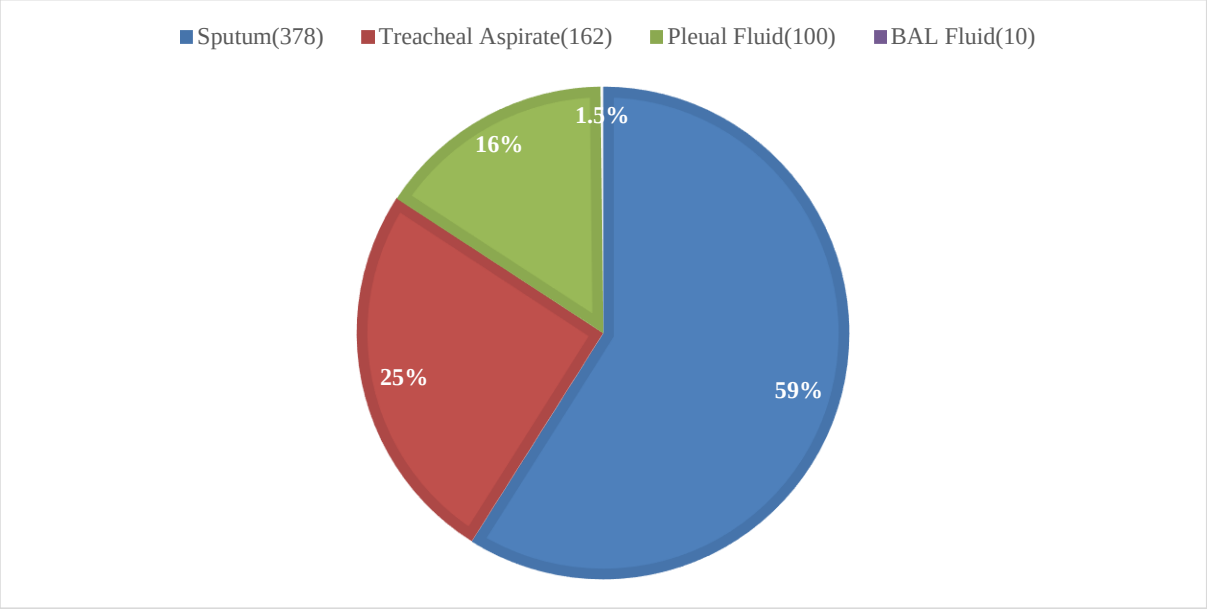
[Graph 1: Monthly distribution of samples]



[Graph 2: Distribution of sample by their collection area]



[Graph 3: Distribution of samples according to the sample type]



Mainly Four different types of samples were obtained i.e, Sputum, Tracheal Aspirate, Pleural Fluid and BAL Fluid; from different wards of hospital area. 59% of samples were sputum form patients complying with RTIs.
Different samples collected from patients in relation to their age group (0-89 years) and gender were determined. The patients age ranged from 0 years to 89 years. The mean age of the patients was 32 years, with a standard deviation of 5.50.

[Table 1: Age & Gender wise distribution of samples]

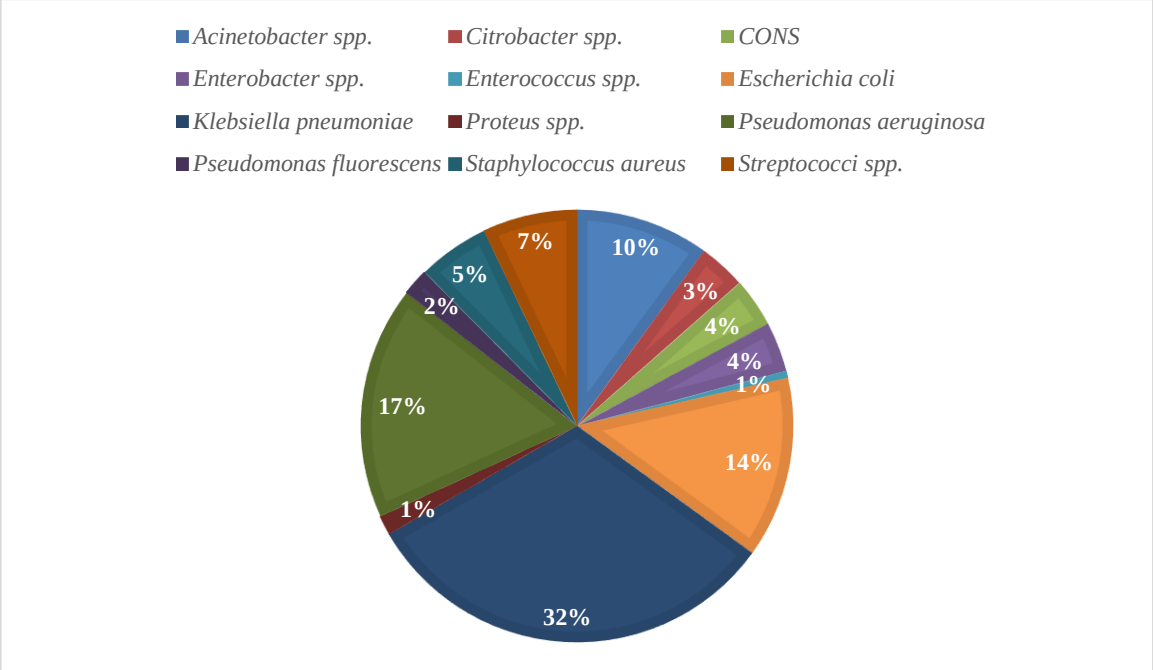
Age group	Female	Male	Grand Total	Relative Frequency
0-9	26	35	61	0.093
10-19	28	21	49	0.075
20-29	56	47	103	0.158
30-39	50	97	147	0.226
40-49	38	85	123	0.189
50-59	27	72	99	0.152
60-69	29	33	62	0.095
70-79	1	2	3	0.004
80-89	2	1	3	0.004
Grand Total	**257**	**393**	**650**	

Positive bacterial growth was recorded in 439/650 (67.53%) of the samples. More samples were obtained from male patients 393/650 (60.46%) while female patients constituted only 253 (38.92%).

c) Isolation and partial Identification of bacterial strain:
All the samples were streaked on various growth supporting media plates and incubated at 37° C for 24h. After the incubation period the growth was observed on various media like, colony morphology on Nutrient agar, haemolysis on Blood agar, lactose fermentation or non-fermentation for gram-negative bacteria. Well isolated colonies were selected for further biochemical test; Various biochemical test was performed for the partial identification of the bacterial strain. *Staphylococcus* spp., *Streptococci* spp., isolated as Gram positive isolates and *Pseudomonas* spp., *Klebsiella* spp., *Escherichia coli*, *Acinetobacter* spp., *Citrobacter* spp., *Enterobacter* spp. Isolated as gram negative species.

d) Prevalence of respiratory bacterial isolates
In this study, the 450 (57.1%) Gram-negative bacteria were obtained whereas 90 (40.6%) were Gram-positive from 650 clinical samples. These isolates include, *Pseudomonas* spp. (65), *Klebsiella* spp. (171), *Escherichia coli* (73), *Acinetobacter spp*. (54), *Citrobacter spp.* (19), *Enterobacter spp.* (20), *Staphylococcus aureus (29), Streptococcus (38)* and other CONS (20) isolates. More than one isolates were also obtained from a single sample.

[Graph 4: Prevalence of Bacterial isolates obtained from the sample]



e) Statistical Analysis

For Biostatistical Analysis Data were analysed using the Statistical Package for Social Sciences software (version 24, SPSS). The association of gender and age groups with the prevalence of bacterial species was assessed using Z-score and Chi-square tests. $P < 0.05$ were considered to be statistically significant. Sample size determination, Distribution of samples (time, collection site, Age, Gender), Prevalence of isolates, Resistance profile was calculated using Microsoft excel tool. Sample size was determined 384 samples by purposive sampling technique. Total 650 samples were collected during August 2022 to May 2023, in which the greater number of samples were collected in December 2022. More number of samples were collected from male patients and age group of 30-39 with relative frequency of 0.22.

d) **Antimicrobial resistance profile of the bacterial isolates**:

This study revealed an association between AMR and MDR bacterial infection and poor outcomes in patients with RTIs. Agar diffusion discs of selected antibiotics were placed aseptically on MHA plate and were incubated at 37 °C for 24 hours. After 24h the diameter of zone of inhibition was measured for each antibiotic disk and recorded in millimetres. The resistance results were compared with the zone diameter interpretive charts provided by the manufacturer. When the spontaneous mutants were present in response to some antibiotics, they were isolated, and tested for the specific antibiotic resistance. High level of resistance was also recorded in Clarithromycin, Erythromycin, Azithromycin, Ciprofloxacin, Ampicillin, Penicillin.

Pseudomonas spp. exhibited high level of resistance to most of the antibiotics used, i.e, Ampicillin (69.0%), Clarithromycin (77.0%), ciprofloxacin (97.5%) and Penicillin (67.0%). On the other hand, it displayed low resistance levels against the Amikacin (35.0%), Ertapenem (35.0%) and Meropenem (35.0%).

Klebsiella spp. were more resistant to Clarithromycin (80.0%%), Penicillin (75.0%), Amphicillin (70.0%) followed by Cefuroxime (49.0%) and Cefotaxime (48.0%). But less resistant to Erythromycin (9.0%), Etrapenem (35.0%) and Merepenem(35.0%).

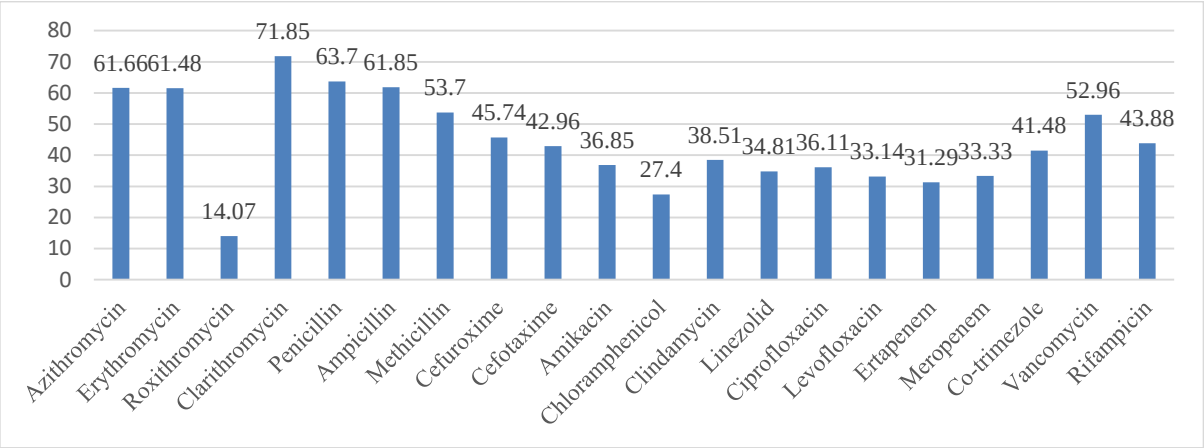
Acinetobacter baumanii isolates were resistant to Penicillin (63.0%), Amphicillin(56.0%), Cefuroxime (52.0%) and Cefotaxime(43).but low resistance to Ciprofloxacin (35.0%), linezolid (38.0%) and Meropenem (38.0%).

Escherichia Coli isolates exhibited high resistance to Clarithromycin (72.0%), Amphicillin(60.0%) and Penicillin (55.5 %) and low resistance to Etrapenem (24.0%), Meropenem (30.0%) and Chloramphenicol (21.0%).

Staphylococcus aureus isolates were resistant to Clarithromycin (92.0%), Penicillin (89.0%) and Ampicillin (67.0%) but lowest resistance to Erythromycin (10.0%), Ertapenem (13.0%) and Meropenem (13.0%).

No singled drug is found more sensitive to any of the isolated pathogen.

[Graph 5: Resistance profile of isolates against selected antibiotics]



A total of 540 bacterial isolates were screened for antimicrobial resistance. Among the screened bacterial isolates, multi-drug resistance was recorded in 300 (55.55%) of the isolates. *Klebsiella pneumoniae* shows highest MDR level 66% followed by *Acinetobacter spp.* 61% >. *Enterobacter spp.* 60% > *Pseudomonas aeruginosa* 57% > *Pseudomonas fluorescens* 55% > *Citrobacter spp.* 53% > *Escherichia coli* 51% > *Staphylococcus aureus* 41% > *Proteus spp.* 38% > *Streptococci* 32% > *CONS* 30%.

[Table No. 2: MDR profile of isolated pathogen]

No.	Name of the isolate	No of isolates	MDR	% OF MDR
1.	*Klebsiella pneumoniae*	171	113	66%
2.	*Pseudomonas aeruginosa*	94	54	57%
3.	*Escherichia coli*	73	37	51%
4.	*Acinetobacter spp.*	54	33	61%
5.	*Streptococci*	38	12	32%
6.	*Staphylococcus aureus*	29	12	41%
7.	*Enterobacter spp.*	20	12	60%
8.	*CONS*	20	6	30%
9.	*Citrobacter spp.*	19	10	53%
10.	*Pseudomonas fluorescens*	11	6	55%
11.	*Proteus spp.*	8	3	38%
12.	*Enterococcus spp.*	3	2	67%
	TOTAL	540	300	56%

Discussion

The etiological agents of RTIs and their susceptibility patterns varies from area to area. The main objective of this study was to find prevalence of various isolates from RTI patients and the antibiotic resistance pattern of bacteria against commonly used antibiotics in Surat, Gujarat. In this study, the bacterial etiology for RTI was noticed in 67.53% of samples. The isolation rates in different study are by Mishra is 44% in 2012, Salman Khan 49.3% in 2015 and Ramana 39.4% in 2013. The National Nosocomial Infections Surveillance (NNIS) of the centre for disease control of USA reports 60% of nosocomial pneumonias to be caused by aerobic Gram-Negative Bacteria (GNB). We found GNB to be the predominant organism (83.33%). These results shows similarity with the result of Veena Kumari et al., Okesola and Ige and Goel et al. who found that GNB isolated was 92.2%, 93% and 97.4% respectively. In this study Gram-Negative bacilli were more frequently isolated than Gram-Positive bacteria. Many other studies also found out considerable predominance of Gram-Negative among respiratory pathogens. (Thomas AM; 2016, Ratna S; 2017). The Gram-Negative predominance might may be due to the unequal distribution of patients with community acquired and hospital acquired infections and also due to the spread of antibiotics resistance in hospital settings. There was an overall preponderance of GNB among the RTIs isolates with *K. pneumoniae*, *P. aeruginosa* and non-fermenting GNB as the common isolates as also confirmed from the studies made by Veena Kumari et al. (2007). Pneumonia is a frequent complication in patients admitted in the ICU. It is frequently polymicrobial with multi-drug resistant such as *P. aeruginosa, K. pneumoniae, Acinetobacter spp.* and *Escherichia coli*. In this study the predominant pathogen isolated was *K. pneumoniae* (31.1%). This is in concordance with Ratna S; 2017, Verma D et al; 2006, Madhavi et al; 2012 and Mokkapati A et al., 2013. But in some other studies the predominant pathogen was *P. aeruginosa* followed by *K. pneumoniae* (Thomas AM; 2016, Salman Khan; 2015). *P. aeruginosa* was the second most common isolate in the present study as shown by Viswanath S et al; 2013. Among gram positive bacteria, *S.aureus* (4%) and *Streptococci spp.* (14%) were most frequently isolated. Incidence of mixed bacterial infection in this study was 15.84% and this is consistent with the fact that the incidence of mixed infections does not usually exceed 30% as has observed by de Roux et al. in 2006. The identification of polymicrobial infections is important for treatment and to avoid a false impression of critically resistant strains.

Antibiotic resistance is a major problem in admitted patients with RTIs. Carbapenems are frequently used as a last choice in treating serious infections caused by Gram Negative Bacteria. Our study showed 31.29% resistance towards Ertapenem and 33.33% Meropenem in accordance to observations made by Akhtar that showed 26.1% resistance and Fatima et al. that showed 24%

resistance. The resistance of some GNB to aminoglycosides to a longer extent to linezolid than to amikacin has been well-recognized in many hospitals. In this study, the resistance of Gram-negative isolates toward amikacin (36.85%) and linezolid (34.84%), which is again alarming. Due to high rate of progression of resistance to amikacin and linezolid a strategy of limited and prudent use of antibiotics is urgently needed. Against GNB, the most active antibiotics were Amikacin, Ertapenem and Meropenem. *K. pneumoniae* and *P. aeruginosa* showed comparatively good susceptibility rate towards these antibiotics. According to our findings, carbapenem group drugs are the most suitable drugs for empirical therapy for RTI in our settings. 36% of GNB isolates were resistant to Ciprofloxacin. Recent studies from various parts of India demonstrated prevalence was shown by some other authors. 56% of multi drug resistant (MDR) bacteria were isolated in this study which is high as compere with others. We conclude that multidrug resistant Pseudomonas and Klebsiella are the most common etiological agents of RTIs. There is high rate of resistance to cephalosporins, beta lactam-beta lactamase inhibitors and carbapenem against predominant organism. The 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 RTI. Now it is well known that critically ill and elderly patients are at greater risk of contracting GNB-RTI. Antimicrobial resistance monitoring helps in optimization of antimicrobial therapy and is more important in the ICUs as infection and antimicrobial consumption are significantly higher.

Conclusion

The study revealed Gram-Negative bacteria as major pathogens causing respiratory tract infections. *K. pneumoniae* was the predominant respiratory pathogen followed by *P. aeruginosa*. Isolated pathogens showed high level of resistance towards the Macrolide and penicillin group drugs whereas chloramphenicol was the most sensitive drug, next being ertapenem and meropenem and should be used for empirical therapy for RTI. The treatment should be modified as per the culture and sensitivity report from the microbiology lab. Antibiotic resistance among respiratory bacterial pathogens is alarming for concern. Strict implementation of the concept of 'antibiotic stewardship' has become necessary for conservation of the already available antibiotics. Hospitals should have a proper '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 updating of their antibiograms should be done in every institution, so that changing trends can be identified and therapy adjusted accordingly.

Ethics approval

This study was approved by the Research Ethics Committee in Surat Municipal Institute of Medical Education and Research (SMIMER), Surat, Gujarat. approval No: (Reference no-120/2024).

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