Original Research Paper

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Microbiology

WHICH BACTERIA ARE CAUSING LRTI NOW? – A POST-COVID ERA STUDY AT A TERTIARY CARE HOSPITAL IN SOUTH GUJARAT.

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ABSTRACT We are presenting an original research article, depicting the specimen profile and prevalent bacterial profile of suspected Lower respiratory tract infection presenting in tertiary care hospital in south Gujarat. A retrospective study was conducted for sputum samples collected at Surat Municipal Institute of Medical Education and Research, Surat, Gujarat during a span of 12 months, with a total of 729 samples collected. The specimen which showed maximum culture positivity was endotracheal tip. followed by sputum and pleural fluid. Among the organisms isolated, gram negative organisms were prominently dominant, among which most common organism was Klebsiella pneumoniae followed by Pseudomonas aeruginosa, Acinetobacter spp. and Escherichia coli. Among the gram-positive organisms, Staphylococcus aureus was most common followed by Streptococcus pneumoniae

Aims

- To isolate most common bacterial pathogen causing Lower respiratory tract infections in our region
- To study the specimens and culture positivity with each specimen collected for suspected LRTI

KEYWORDS : Lower respiratory tract infections, Klebsiella pneumoniae, sputum, Endotracheal tip, Pleural fluid

INTRODUCTION:

Lower respiratory tract infection (LRTI) is one of the common major health problems in developing countries. [8]. LRTIs are any infection in the lungs, trachea, bronchi, bronchioles, alveoli, or below the larynx. LRTI's is not only one disease, but a cluster of specific infections each with different epidemiology, pathogenesis, clinical presentation, and outcome Acute manifestations of LRTIs that may or may not involve lungs include acute bronchitis, bronchiolitis, influenza, pneumonia either with or without radiological evidence, acute exacerbation of Chronic Obstructive Pulmonary Disease (COPD), and acute exacerbation of bronchiectasis. A 4.4% of all hospital admissions and 6% of all general practitioner consultations are due to infections of the lower respiratory tract[5] They are some of the leading causes of morbidity and mortality in young children and the elderly. [8]

Hospital-acquired and community-acquired LRTI's have been on the rise as seen with other immunocompromised conditions which include asthma, COPD, diabetes, chronic kidney disease. [5] It has been seen that 13.3% of Disability–Adjusted Life Years (DALYs) are due to respiratory diseases [10]. As reported by the World Health Organisation (WHO), among the infectious disease deaths in India LRTI's have been attributed to account for almost 20% mortality. [11]

The problem is much greater in developing countries where pneumonia is the most common cause of hospital attendance in adults [15]. 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 [12]. Since the aetiological agents of LRTI cannot be determined clinically, microbiological investigation is required for both treatment and management of the individual case and epidemiological purposes [13,14]

Overtreatment of acute uncomplicated bronchitis, which is largely due to viruses, has led to unparalleled levels of multidrug resistance among invasive pathogens. [9] With emerging drug resistance of organisms to commonly used antibiotics, it is imperative to study their recent trends, for effective management of these cases[4] Data related to the offending organisms and their antibiotic sensitivity pattern is limited. Therefore, this study was designed to identify the bacterial pathogens in patients with lower respiratory tract infections.

MATERIALS AND METHODS:

This was a laboratory based observational study which was carried out between January 2022 to December 2022 at Surat Municipal Institute of Medical Education and Research, Surat, Gujarat India, after informed consent. A total of 729 respiratory specimens were received during the study period.

Inclusion Criteria: Lower respiratory tract sample (Sputum, pleural fluid, Broncheoalveolar lavage, Endotracheal aspirate and tip, Tracheostomy aspirate and tip) of patients admitted to SMIMER hospital with complaints of LRTI infections were included in the study.

Exclusion Criteria:

(i) Sample other than lower respiratory tract sample were excluded from the study.
(ii) Sample which were positive for Pulmonary Tuberculosis were excluded in the study (AFB positive)
(iii) Samples which were positive for COVID-19 or other established viral infections were excluded from the study.
(iv) Samples with oral contamination by Bartlett's grading score on gram stain.

On the basis of exclusion criteria, 24 specimens were excluded, total study sample size – 705 .

Study Procedure:

All the lower respiratory tract samples (sputum, ET aspirate, BAL etc.,) of patients coming to the outpatient and in-patient department of SMIMER with symptoms of LRTI were collected for bacteriological culture into a wide-mouthed sterile containers, transported to the laboratory and processed within two hours.

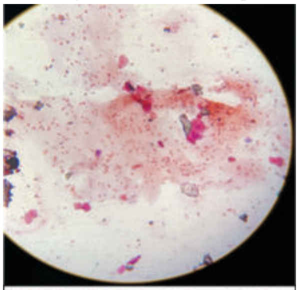
Gram's staining was done for all the received samples and sputum quality was assessed by Bartlett Grading Score.

Then inoculated onto Blood agar, Chocolate agar and MacConkey agar. Streaked culture plates were incubated at 37°C overnight. On the next day, the bacterial growth was observed, and was further processed for identification.

Identification of Isolated Organisms: The identification of significant isolates were carried out using the manual standard microbiological techniques, which involved morphological study of colonies, Gram staining reactions, and a battery of biochemical tests as required [16]. A colony count of $\geq 10^4$ CFU/ml was considered to be significant for bronchial washing [17] while for other specimens, $\geq 10^5$ CFU/ml was suggestive for infection [18]. Bacterial isolates were identified on the basis of colony characteristics, gram-staining, and a battery of biochemical tests.

Statistical Analysis

The data was entered in Microsoft office excel worksheet. The data was analysed as number and percentage (descriptive analysis)



IMG-1 : Epithelial cells <10/LPF, Pus cells >25/LPF
BARTLETT'S GRADING SCORE : +2
SPUTUM QUALITY : GOOD
IDEAL SPUTUM .

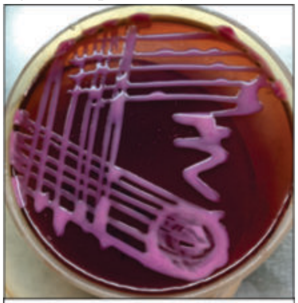


IMAGE 2 - Growth of Klebsiella pneumoniae on MacConkey agar

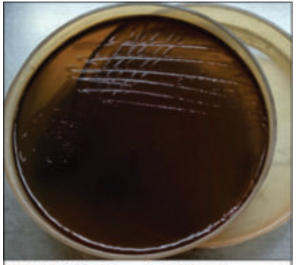


IMAGE 3 - Growth of *Pseudomonas aeruginosa* on MacConkey agar

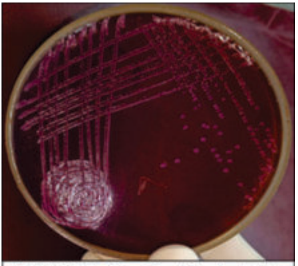


IMAGE 4 - Growth of *Escherichia coli* on MacConkey agar

RESULTS:

Number of specimens and result pattern

A total of 705 specimens were included in the study. Out of which 447 (63.40%) were culture positive[fig-1]. Patients enrolled in the study included 409 males and 296 females , out of which 281 (62.86%) males and 166 (37.14%) females were culture positive.

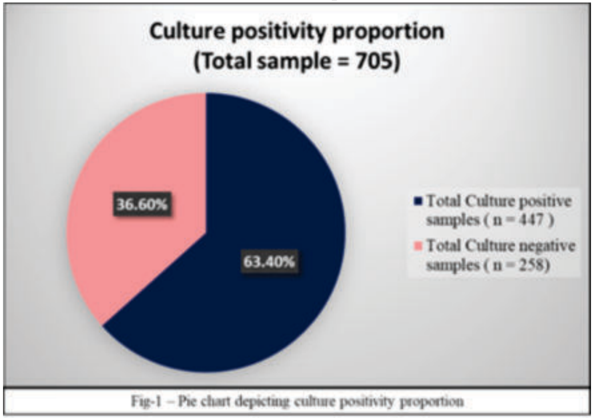


Fig-1 – Pie chart depicting culture positivity proportion

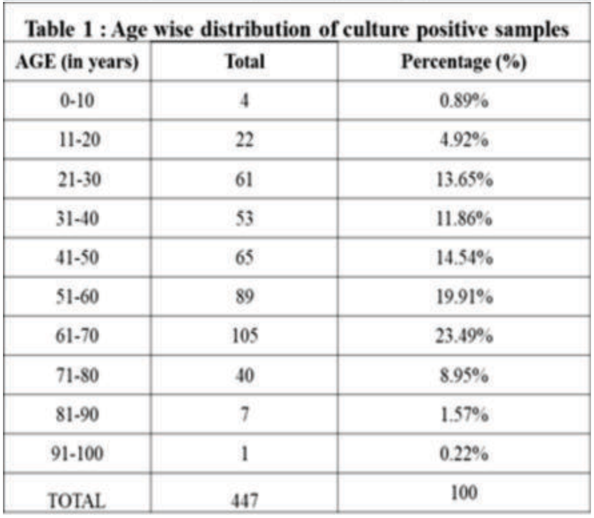


Table 1 : Age wise distribution of culture positive samples

AGE (in years)	Total	Percentage (%)
0-10	4	0.89%
11-20	22	4.92%
21-30	61	13.65%
31-40	53	11.86%
41-50	65	14.54%
51-60	89	19.91%
61-70	105	23.49%
71-80	40	8.95%
81-90	7	1.57%
91-100	1	0.22%
TOTAL	447	100

Most of the culture positive isolates were from patients in the age group of 61-70 years (n=105, 23.49%) [Table-1]

Out of 447 culture positive specimens, Endotracheal tip 96.23% (n=255/265) showed maximum culture positivity. 61.54% of Bronchioalveolar lavage samples (n=16/26), 60.96% of sputum samples(n=89/146) and 26.97% of pleural fluid samples (n=65/241) were culture positive. [Table-2, Fig-2, 3]

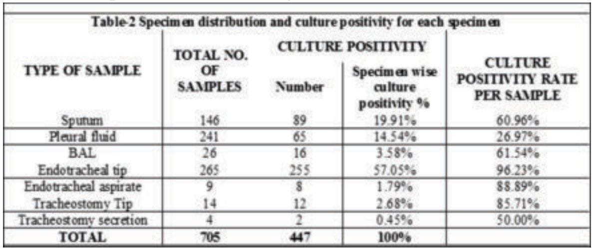


Table-2 Specimen distribution and culture positivity for each specimen

TYPE OF SAMPLE	TOTAL NO. OF SAMPLES	CULTURE POSITIVITY		CULTURE POSITIVITY RATE PER SAMPLE
		Number	Specimen wise culture positivity %	
Sputum	146	89	19.91%	60.96%
Pleural fluid	241	65	14.54%	26.97%
BAL	26	16	3.58%	61.54%
Endotracheal tip	265	255	57.05%	96.23%
Endotracheal aspirate	9	8	1.79%	88.89%
Tracheostomy Tip	14	12	2.68%	85.71%
Tracheostomy secretion	4	2	0.45%	50.00%
TOTAL	705	447	100%	

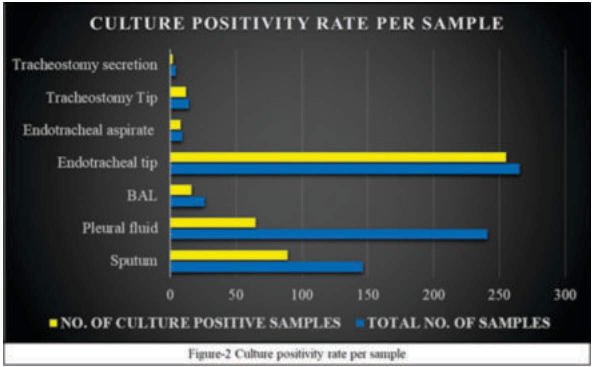


Figure-2 Culture positivity rate per sample

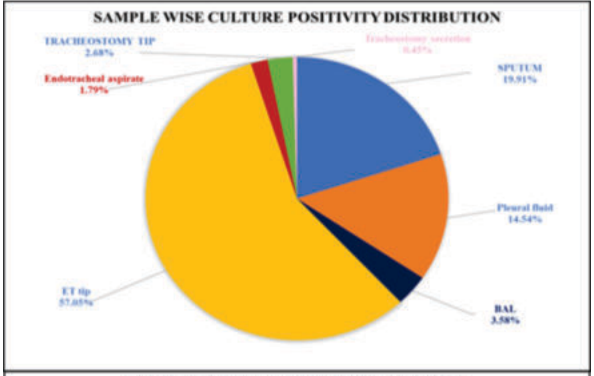


Fig-3 Sample wise culture positivity distribution
(Culture positive samples *n*=447)

Trend of Microbial Isolates

Out of 447 culture positive samples, 420 were gram negative organism (93.96%) and 27 were gram positive organisms (6.04%). [Table-3]

Table 3 - Gram distribution of culture positive samples

	No. of organisms	% of organisms
Gram negative organisms	420	93.96%
Gram positive organisms	27	6.04%
Total Culture positive samples	447	100%

Distribution of Bacterial Isolates

Among gram negative organisms (*n*=420), *Klebsiella pneumoniae* (*n*=127, 28.41%) was the most common organism, followed by *Pseudomonas aeruginosa*(*n*=88, 19.69%) , *Acinetobacter spp.* (*n*=78, 17.45%) and *Escherichia coli* (*n*=66, 14.77%). Among gram positive organisms (*n*=27), *Staphylococcus aureus*(*n*=8, 1.79%) and *Streptococcus pneumoniae*(*n*=7, 1.57%) were most common. [Table-4, Fig-4]

Table-4 Distribution of organisms (culture positive n = 447)

ORGANISM	Number Of Isolates	% Of Isolates
Klebsiella pneumoniae	127	28.41%
Pseudomonas aerugniosa	88	19.69%
Acinetobacter	78	17.45%
Escherichia coli	66	14.77%
Citrobacter	30	6.71%
Enterobacter	27	6.04%
Staphlococcus aureus	8	1.79%
Streptococcus pneumoniae	7	1.57%
CONS	7	1.57%

Enterococci	5	1.12%
Proteus mirabilis	2	0.45%
Proteus vulgaris	1	0.22%
Burkholderia	1	0.22%

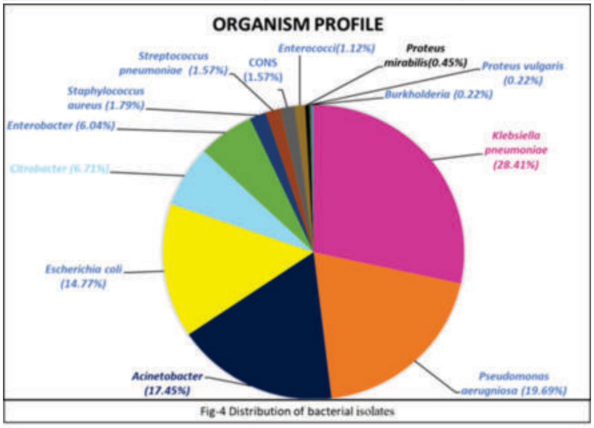


Fig-4 Distribution of bacterial isolates

DISCUSSION :

The primary goal of this study was to ascertain the current prevalence/trend of bacteria causing LRTI among patients in the region of Surat, western Gujarat.

In this study, LRTI were more common in males(62.86%) than in females(37.14%), which is similar to studies done by Ashina Singla et al.[5] , Panda S et al.[19], and Akingbade OA et al.[20]. .Higher rates of isolation among males indicates increased susceptibility of male sex to lower respiratory tract infections than female sex. The reason might be owing to associated risk factors like cigarette smoking, chronic alcoholism, COPD and cultural differences in communities. [6]

Maximum cases of LRTI in this study has been encountered in the age group of 61-70 years (n=105, 23.49%) and minimum cases were found in 91- 100 years (n=1, 0.22%). The findings are supported by studies done by Ahmed SM et al., [21]. Elumalai et al., found maximum culture positivity in 40-50 years age group [4]

In this study, overall culture confirmed cases were found to be 63.40% (*n*=447) of which ET tip 57.05% (*n*=255) showed higher positivity rate than sputum 19.91% (*n*=89) and pleural fluid 14.54% (*n*=65). Study done by Mishra S et al. [22] and Ashina Singla et al.[5], also showed greater positivity in ET aspirates than sputum samples . The main limitation with sputum samples is the difficulty to obtain good-quality, purulent sputum. Many pneumonia patients do not produce sputum, particularly older patients. [23] Furthermore, the results of sputum bacterial cultures are strongly influenced by specimen collection, transport, rapid processing, satisfactory use of cytologic criteria, and the skill in interpretation. [4]

Out of 447 culture positive samples, 93.96% were gram negative organism and 6.04% were gram positive organisms . This is in concordance with study by Ashina Singla et al. [5] which showed 92.19% gram negative and 4.23% gram positive organisms in Jaipur. The predominant pathogen that was isolated in this study from Gujarat was *Klebsiella pneumoniae* (28.41%, *n*=127) . This is in accordance with the findings in the study by Nurahmed et al. [6] , Ashina Singla et al.[5] and Nithya Chinnnusamy et al. [9]. The second most common organism in this study was *Pseudomonas aeruginosa* (19.69%, *n*=88) followed by *Acinetobacter spp.* (17.45%, *n*=78) and *Escherichia coli* (14.77%, *n*=66). Arthi Elumalai et al [4] in their study from Puducherry (South India) found 52% *K. pneumoniae* , 20% *P.aeruginosa* , 18% *E.coli* , 5% *Acinetobacter spp.* Whereas Ashina Singla et al. [5] in their study from Jaipur, Rajasthan found that among the gram negative isolates, *Klebsiella pneumoniae* 30.15% (n=171) was the most common organism isolated, followed by *Acinetobacter baumanii* 28.21% (n=160), *Pseudomonas aeruginosa* 19.40% (n=110), *Escherichia coli* 15.16% (n=86). Patients in the older age group are more susceptible to gram negative pneumonia because of waning immunity and pulmonary defence mechanisms, underlying chronic diseases and silent aspiration. Institutional care also makes the patients more susceptible to gram negative pneumonia. [24]

This study showed 8 isolates of *Staphylococcus aureus* (1.79%) and 7 isolates of *Streptococcus pneumoniae* (1.57%). *Streptococcus pneumoniae* can cause pneumonia by the spread of infection from the pharyngeal mucosa. [24]

Limitation(s) :

Atypical pathogens like Mycoplasma pneumoniae, Chlamydia trachomatis etc., and viruses have not been reported in this study. For the detection of these atypical pathogens, advanced techniques like molecular assays are required which were not done in this study

CONCLUSION:

63.40% culture positivity was seen in clinically suspected patients of LRTI . Culture testing becomes important to confirm the presence of bacterial infection in clinically suspected LRTI. The specimen which showed maximum culture positivity was endotracheal tip , owing to the invasiveness of the sample (less contamination) followed by sputum and pleural fluid. Among the organisms isolated, gram negative organisms were prominently dominant , among which most common organism was *Klebsiella pneumoniae* followed by *Pseudomonas aeruginosa, Acinetobacter spp.* and *Escherichia coli*. Among the gram positive organisms, *Staphylococcus aureus* was most common followed by *Streptococcus pneumoniae.* Since bacterial aetiology may vary in different geographical regions and even over time in the same location and population, routine surveillance of microbial aetiology of LRTI is important.

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