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Original Article

Microbiology Section

Incidence of *Staphylococcus Aureus* in Lower Respiratory Tract Infections: An Emerging Trend

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ABSTRACT

Introduction: The pathogen like *staphylococcus* spp. associated with multidrug resistance is one of the major concerns. Prompt diagnosis of staphylococcal Lower Respiratory Tract Infection (LRTI) with its antibiogram plays a vital role in better outcomes of treatment and reducing the cost of hospital stay of patients.

Aim: To find out incidence of staphylococcal LRTIs.

Materials and Methods: This prospective cross-sectional study was conducted in the Department of Microbiology, SMBT Institute of Medical Sciences and Research Centre, Nashik, Maharashtra, India. The duration of the study was 23 months, from June 2017 to May 2019. The specimens were screened for staphylococcal species as per the standard bacteriological procedure. The gram-positive, catalase-positive isolates were further subjected to detection of antimicrobial susceptibility patterns as per Clinical and Laboratory Standards Institute (CLSI) guidelines. The data was analysed by using Statistical Package for Social Sciences (SPPS) version 20.0.

Results: The majority of the patients were of old age groups and the average mean age was 57.95±6.18 years. A total of 22/421 *Staphylococcus aureus (S. aureus)* were isolated from the patients suffering from LRTIs. High incidence was noted in male patients 21/22 (95.5%) than in females 1/22 (4.5%) and 100% of patients were hospitalised with a complaint of LRTIs. The majority of strains were isolated from sputum sample 18/22 (81.8%), followed by 3/22 (13.6%) from pleural aspiration, and 1/22 (4.5%) from endotracheal secretion. Almost 18/22 (81.8%) patients were of pneumonia, followed by 3/22 (13.6%) were of empyema and 1/22 (4.5%) were of sinusitis. All the isolates were sensitive to linezolid, vancomycin, and ceftaroline.

Conclusion: In the present study, LRTI associated with *S. aureus* was found to be (5.22%). It was also observed that, all strains were sensitive to vancomycin, linezolid, and ceftaroline which is unique. Hence, the staphylococcal infection can be treated with low cost and hospital stay if diagnosed in time by microbiological profile, as the clinical presentation and susceptibility to antimicrobial agents vary in different geographical areas.

Keywords: Antimicrobial sensitivity, Bronchitis, Co-morbidities, Gram-positive bacteria, Pneumonia

INTRODUCTION

The LRTI, is a term used for an acute infection of the trachea, airways, and lungs, which make up the lower respiratory system. It includes bronchitis and pneumonia [1]. The consequences of pneumonia lead to empyema, a condition of pus formation in the pleural cavity under the influence of microorganisms [2]. Pleural infection is a common and increasing clinical problem in thoracic medicine, resulting in significant morbidity and mortality [3]. Approximately, 4 million people per year affected by pneumonia and half of them are estimated to develop para-pneumonic effusion. The most common pathogens associated with pleural infections are *Streptococcus pneumoniae*, *Streptococcus pyogenes,* and *Staphylococcus aureus* which often lead to a severe form of infection [3]. *S. aureus* lung infections are often seen in elderly and hospitalised patients with significant co-morbidities that are associated with an abscess, cavitation with empyema containing necrotic debris or fluid caused by microbial infection [4,5]. The emergence of multidrug resistance among *Staphylococcus* species is still challenging, especially methicillin resistant strains [6,7]. In the recent past, several studies carried out around the world show that, methicillin resistant strains of *Staphylococcus* species are not limited to hospital-acquired infection but are significantly associated with community-acquired infection [7,8]. Hence, the clinician faces the challenge while selecting the antimicrobial agents for a better outcome [9].

The continuous screening of *Staphylococcus* species at the institutional level is crucial to plan the treatment protocol from time to time [10]. The Incidence of *Staphylococcus* species and its antibiogram varies with different geographical areas, as the study place belongs to a hilly-tribal area, present study was carried out to see the incidence of *Staphylococcus* species and its antimicrobial susceptibility pattern for judicial use of the drugs and proper institution of the therapy.

MATERIALS AND METHODS

This prospective cross-sectional study was conducted in the Department of Microbiology, SMBT Institute of Medical Sciences and Research Centre Nashik, Maharashtra, India. The duration of the study was 23 months, from June 2017 to May 2019. Ethical approval was granted by the Institutional Ethics Committee as per approval letter no. IEC (Ref: SVIEC/ON/MED/PHD/17007).

Sample size calculation: Where the population is unknown, the sample size can be derived by computing the minimum sample size required for accuracy in estimating proportions by considering the standard normal deviation set at 95% confidence level (1.96), percentage picking a choice or response (50%=0.5) and the confidence interval (0.05=±5) (formula used: n=z 2(p)(1-p)/c 2), (where: z=Standard normal deviation set at 95% confidence level, p=percentage of picking a choice or response, c=confidence interval). Hence, 385 or more samples were necessary to meet desired statistical constraints. In the present study, 421 isolates were taken to meet the criteria.

Inclusion criteria: All clinical samples from all age groups of patients received in the Department of Microbiology were included in the study. These were further screened for *Staphylococcus* species. Present study was intended to isolate *Staphylococcus* spp., Hence, only aerobic culture and sensitivity were done.

Exclusion criteria: Duplicate samples/isolates from the same patient were excluded from the study. Hence, the number of isolates indicates the number of patients.

Study Procedure

Collection of Respiratory specimens: Various clinical specimens were taken from respiratory tract infections, sputum cultures were done primarily to identify the pathogens that cause pneumonia or bronchopneumonia: community-acquired or hospital-acquired.

- **Sputum sample:** Early morning specimen generated after a bout of cough was collected in a wide mouth sterile container. It was further subjected to gram stain and microscopy to rule out the quality of the specimen as per the Q score. Only the presence of a significant number of pus cells in a given sample was processed.
- **Endotracheal Aspirate (ETA):** Endotracheal aspiration was done with a sterile technique using a 22 inch, 12F suction catheter. The catheter was introduced through the endotracheal tube for atleast 30 cm. Gentle aspiration was then performed without instilling saline solution. The first aspirate was discarded. The second aspirate was collected after tracheal instillation of 5 mL saline in a mucus collection tube [11].
- **Bronchoalveolar Lavage (BAL) collection:** In this procedure, 100-300 mL of saline was infused into a lung segment through the bronchoscope to obtain cells and protein of the pulmonary interstitial and alveolar spaces. Its portion was collected in a sterile leak-proof screw-cap container [11].

Lab processing protocol: All the above mentioned clinical specimens, collected in a sterile container by the treating physician/surgeon received in the department of microbiology for culture and sensitivity were included in the study. A medical case report/prescription form was used for the record of age, sex, medical history, clinical presentation, co-morbid condition, associated predisposing factors, and prior antibiotic therapy/antibiotic given. The clinical specimens received in the Department of Microbiology were inoculated on blood agar and MacConkey Agar and incubated at 37^{0}C for 24 hours. Subsequently, a smear was made from the direct specimen and stained with gram stain and examined under an oil immersion lens, and the primary report was sent to the clinician for initial treatment. After 24 hours of incubation of previously inoculated clinical specimens, isolated colonies were taken to make a smear for gram stain to rule out gram-positive cocci arranged in clusters. Confirmed gram-positive cocci were further subjected to the catalase test to differentiate staphylococci from streptococci. The catalase-positive isolated colonies were tested for slide coagulase and incubated for tube coagulase test at 37^{0}C for 4 hours if a clot was not observed at the end of 4 hours; the tube was further incubated at room temperature and read after 18-24 hours [12,13]. Furthermore, a well-isolated colony was taken and suspended in peptone water and incubated at 37^{0}C for 4 hours, the bacterial suspension was compared with 0.5 McFarland turbidity standard, and a comparison was corrected by using the addition of peptone water or further incubation. The 0.5 bacterial suspensions were used for antimicrobial susceptibility testing and biochemical test as per the standard microbiological procedure. The antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method using the different antibiotic disc and E-test strip methods for Minimal Inhibitory Concentration (MIC) detection (vancomycin, ceftaroline) as per CLSI guidelines 2018 [14,15].

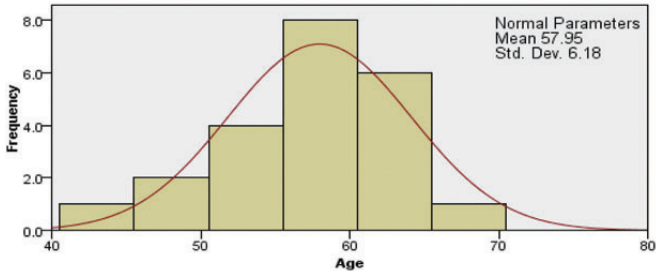
D-test (Disc diffusion test/Disc approximation test): In D-test, erythromycin (15 µg) disc was placed at a distance of 15 mm (edge to edge) from the clindamycin (2 µg) disc on the Mueller-Hinton agar plate previously inoculated with 0.5 McFarland bacterial suspensions and incubated at 37^{0}C, flattening "D shaped" zone of inhibition around clindamycin in the area between two disc, indicated inducible clindamycin resistance. Three different phenotypes were appreciated after testing and then interpreted as Multiple Sclerosis (MS) phenotype, inducible Macrolide-lincosamide-Streptogramin B (iMLSb) phenotype, and constitutive Macrolide-lincosamide-Streptogramin b (cMLSb) phenotype. As MLSb phenotypes are only related to erythromycin-resistant strains, this interpretation was done only for erythromycin-resistant *Staphylococcus* species. All the erythromycin-sensitive strains were excluded [7,8].

STATISTICAL ANALYSIS

The data was analysed by using SPPS version 20.0 software with appropriate statistical tests like a one-sample Chi-square test. The p-value≤0.005 was considered statistically significant.

RESULTS

A total of 22/421 *Staphylococcus aureus* were isolated from the patients suffering from LRTIs. The majority of the patients were of old age groups and the average mean age was 57.95±6.18 years. Age distribution of the study participants is shown in [Table/Fig-1].



Total N		22
Most Extreme Differences	Absolute	.134
	Positive	.084
	Negative	-.134
Test Statistic		.630
Asymptotic Sig. (2-sided test)		.822

[Table/Fig-1]: Overall age distribution.

The majority of the patients suffering from staphylococcal LRTI were males 21/22 (95.5%) and all of them were from in-patient department 22 (100%). The demographic data of the patients having staphylococcal LRTI is shown in [Table/Fig-2].

Demographic data	Frequency (n)	Percentage (%)	Bootstrap for percentage			
			Bias	Std. Error	95% CI	
					Lower	Upper
Male	21	95.5	0	4.5	84.4	100.0
Female	1	4.5	0	4.5	0	15.6
In-patient	22	100.0	0	0	100.0	100.0
Out-patient	0	0	0	0	0	0

[Table/Fig-2]: Demographic data of the patients of staphylococcal LRTI (n=22/421).
p-value: <0.005, CI: Confidence interval

The majority of strains were isolated from sputum sample 18/22 (81.8%), followed by 3/22 (13.6%) from pleural aspiration, and 1/22 (4.5%) from endotracheal secretion. Almost 18/22 (81.8%) patients were of pneumonia, followed by 3/22 (13.6%) were of empyema and 1/22 (4.5%) were of sinusitis. The clinical data of the patients suffering from LRTI is shown in [Table/Fig-3]. All the strains isolated from LRTIs were 100% resistant to penicillin, cefoxitin, tetracycline,

Clinical data		Frequency (n)	Percentage (%)	Bootstrap for percentage			
				Bias	Std. Error	95% CI	
						Lower	Upper
Specimens	Sputum	18	81.8	0.6	8.0	63.6	95.5
	Pleural aspiration	3	13.6	-0.6	6.9	0	27.3
	ET secretion	1	4.5	0	4.5	0	18.2
Department	Medicine	22	100	0	0	100	100
Clinical diagnosis	LRTI	22	100	0	0	100	100
Co-morbidity	Empyema	3	13.6	-0.7	7.0	0	27.3
	Sinusitis	1	4.5	0	4.5	0	15.6
	None	18	81.8	0.7	8.2	63.6	95.5
Prior antibiotics therapy	Azithromycin	14	63.6	-0.2	10.7	40.9	81.8
	Amikacin	3	13.6	0	7.4	0	29.3
	Amoxyclav	1	4.5	0	4.5	0	15.6
	Not given	4	18.2	0.2	8.1	4.5	36.4

[Table/Fig-3]: Clinical data of the patients of staphylococcal LRTI (n=22/421).

erythromycin, chloramphenicol, and ofloxacin. However, linezolid, vancomycin, and ceftaroline were 100% sensitive to *Staphylococcus aureus*, followed by 21/22 (95.5%) sensitive to gentamycin, and 19/22 (86.4%) sensitive to rifampin. The strains isolated from LRTI were 100% resistant to methicillin and were multidrug-resistant strains. The antimicrobial susceptibility of Staphylococcal LRTI is shown in [Table/Fig-4]. All the isolates were erythromycin resistant, No strain was truly susceptible to clindamycin (MSb phenotype) hence, use of clindamycin in LRTI may result in treatment failure, however, inducible clindamycin resistant strains were 18.2% (iMLSb phenotype) and 81.8% of strains belonged to constituents resistant (cMLSb phenotype). The MLSb phenotypes among LRTI is shown in [Table/Fig-5].

Antimicrobial agents	Sensitive		Resistant	
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)
Penicillin	0	0	22	100.0
Cefoxitin (MIC)	0	0	22	100.0
Erythromycin	0	0	22	100.0
Tetracycline	0	0	22	100.0
Chloramphenicol	0	0	22	100.0
Ofloxacin	0	0	22	100.0
Clindamycin	4	18.2	18	81.8
Trimethoprim/ sulfamethoxazole	7	31.8	15	68.2
Rifampin	19	86.4	3	13.6
Gentamycin	1	4.5	21	95.5
Linezolid	22	100	0	0
Vancomycin (MIC)	22	100	0	0
Ceftaroline (MIC)	22	100	0	0

[Table/Fig-4]: Antimicrobial susceptibility of *S. aureus* exhibiting LRTI (n=22/421).

DISCUSSION

The LRTIs are the most common infections in human beings. Worldwide, around 2.74 million deaths occur every year due to LRTIs [16]. Incidence of *S. aureus* LRTI in increasing trend is of the major concern. Emergence of *S. aureus* multidrug-resistant strains is a global concern especially to deal with patients with co-morbid conditions and associated predisposing factors. *Staphylococcus aureus* LRTI with Methicillin-resistant *Staphylococcus aureus* (MRSA) strains left very few therapeutic alternatives to treat such conditions. As the antimicrobial resistance patterns vary from geographical area and even from hospital to hospital. Hence, local

Resistant phenotypes	Frequency (n)	Percentage (%)	Bootstrap for percentage			
			Bias	Std. Error	95% CI	
					Lower	Upper
Erythromycin (S)	0	0	0	0	0	0
$cMLS_b$ (E-R, CD-R)	18	81.8	0	8.6	63.6	97.5
$iMLS_b$ (E-R, CD-S)	4	18.2	0	8.6	2.5	36.4
MS_b (E-R, CD-S)	0	0	0	0	0	0
Total	22	100.0	-2.4	15.2	56.0	100.0

[Table/Fig-5]: Statistics of inducible clindamycin resistance among LRTI (n=22/421).
cMLSb: Constitutive MLSb phenotype; iMLSb: Inducible MLSb phenotype; MSb: Macrolide Streptogramin phenotype; E-R: Erythromycin resistant; CD-R: Clindamycin resistant; CD-S: Clindamycin sensitive

antimicrobial resistance data helps to timely treat such conditions. In the present study, 22/421 (5.22%) *S. aureus* were isolated from LRTIs [Table/Fig-6] [16-23]. High incidence was noted in male patients 21/22 (95.5%) than in females 1/22 (4.5%) and 100% of patients were hospitalised with a complaint of LRTIs. The average mean age group of the patients was 57.95±6.18 years.

Incidence of *S. aureus* LRTI reported by Dopthapa YP et al., 2015, Pravin S et al., 2013, Bajpai T et al., 2013 is in accordance with the present study [17-19]. However, a study conducted by Manikandan C et al., 2013 and Ashina Singla et al., 2021[23] have reported a very high incidence of *Staphylococcus aureus* LRTI [16,23]. A similar study was conducted in Italy for five years to see the yearly trend and observed that, the incidence of *S. aureus* LRTI was raging from 12.7% to 16.2% [16]. It is observed that, the incidence of *Staphylococcus aureus* LRTI increasing year by year [Table/Fig-6] which is alarming, hence, timely isolation of the pathogen and its antimicrobial sensitivity testing is crucial to deal with multidrug-resistant strains of the patients clinically diagnosed as LRTI on clinical background, 3/22 (13.6%) patients were of having empyema (accumulation of frank pus in the pleural cavity). As empyema is a secondary infection to pneumonia or tuberculosis, prior antimicrobial therapy plays a major role to cure the conditions. Despite the widespread availability of antibiotics effective against pneumonia, empyema remains a significant cause of morbidity and mortality even in developed countries due to the emergence of multidrug-resistant strains and inappropriate antimicrobial therapy.

In the present study, prior antimicrobial therapy reveals that, 14/22 (63.6%) patients had taken azithromycin before approaching the tertiary care centre. All the strains were MRSA and resistant to multiple routine antibiotics. The second-line antimicrobial agents like linezolid, vancomycin, and ceftaroline were 100% sensitive. Similarly, Vijay S et al., 43/43 (100%) isolates

Different studies	Place of studies	*Staphylococcus aureus* LRTI Incidence-n(%)
Manikandan C et al., 2013 [17]	Adirampattinam, Tamil Nadu, India	81/337 (24%)
Dopthapa YP et al., 2015 [18]	West Bengal, India	71 (4.83%)
Praveen S et al., 2013 [19]	West Bengal, India	30/750 (04%)
Bajpai T et al., 2013 [20]	Indore, Madhya Pradesh, India	9/253 (3.55%)
Sherchan JB et al., 2020 [21]	Kathmandu, Nepal	14/103 (13.60%)
Singh S et al., 2020 [22]	Jodhpur, Rajasthan, India	44/769 (5.72%)
Ashina Singla et al., 2021 [23]	Jaipur, Rajasthan, India	13/26 (50%)
Santella B et al., 2021 (5 years trend) [16]	Luigi Vanvitelli, Naples, Italy	Year 2015: 105 (12.7%),
		Year 2016: 121 (16.0%),
		Year 2017: 102 (16.2%),
		Year 2018: 113 (15.3%),
		Year 2019: 121 (15.9%)
Present study	Nashik, Maharashtra, India	22/421 (5.22%)

[Table/Fig-6]: Comparison of Incidence of *Staphylococcus* spp among LRTI [16-23].

were sensitive to vancomycin, Gaikwad V et al., reported 30/30 (100%) isolates were sensitive to linezolid and 28/30 (93.33%) isolates were sensitive to ceftaroline [24,25]. All the *S.aureus* strains were also screened for inducible clindamycin resistance by conventional D-test [7,8]. In the present study, all the isolates were erythromycin resistant which was further subjected to rule out inducible clindamycin-resistant strains of *Staphylococcus* species among LRTI. Out of 22 isolates, 18/22 (81.8%) were constitutive resistant, 4/22 (18.2%) were inducible resistant, and none of the isolates was truly susceptible to clindamycin (MSb phenotype). The strains resistant to erythromycin carry *erythromycin ribosome methylase (erm)* genes that enhance the production of methylase enzyme and induce clindamycin resistance. Sharing of the same target site by different antibiotics, resistance to one drug might predict resistance to another related drug and routine antimicrobial susceptibility testing fails to detect true susceptibility of clindamycin among erythromycin-resistant strains. Such inducible-resistant strains should be ruled out to prevent therapeutic failure.

Limitation(s)

The present study was carried out in a hilly-tribal area-based tertiary care hospital hence, the clinical history of the patients and prior medications has limitations.

CONCLUSION(S)

In the present study, LRTI associated with *S. aureus* was found to be significant. It was also observed that all strains were sensitive to vancomycin, linezolid, and ceftaroline, which is unique, yet the studies carried out in India. Hence, the staphylococcal infection can be treated at a low cost and hospital stay, if diagnosed in time by microbiological profile, as the clinical presentation and susceptibility to antimicrobial agents vary from the different geographical areas.

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