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Original Article

Microbiology Section

Prevalence of LRTI in Patients Presenting with Productive Cough and Their Antibiotic Resistance Pattern

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ABSTRACT

Aim: To find out the prevalence of Lower Respiratory Tract Infection (LRTI) such as bacterial, fungal, mycobacterial infections etc. in patients with productive cough of duration less than 15 days and to rule out the patients having previous history of tuberculosis or having treatment of tuberculosis.

Materials and Methods: Outdoor and Indoor patients of Department of Medicine and Chest & TB, SRG hospital and Jhalawar Medical College, Jhalawar were included. After sample collection the specimens were sent to the Microbiology department, for processing of Gram staining, Acid fast staining, KOH mount and bacteriological culture and sensitivity.

Results: A total of 200 samples were obtained from the outpatient and inpatient Department of Medicine and Chest & TB of which 66% were male and 34% were female. Seventy seven percent of samples were culture positive for both single pathogen and mixed infection of which 56.5% were male and 20.5% were female as males are more at risk for LRTI. *Klebsiella pneumoniae* was the most prevalent pathogen (71/193), followed by coagulase positive *Staphylococci* i.e. COPS (43/193). More resistant pattern was found in coagulase negative *Staohylococci* (CONS) showed 61.11% Methicillin Resistant *Staohylococci* (MRS) incidence compared to 41.86% in COPS, also regarding Extended Spectrum Beta Lactamase (ESBL) production *Escherichia coli* showed incidence of 36.36% as compared to other gram negative bacilli. *Pseudomonas aeruginosa* was the most resistant organism found based on the antibiotic susceptibility pattern while *Proteus mirabilis* was the most sensitive organism.

Conclusion: Lower respiratory tract infections can spread easily among community and indiscriminate use of antibiotics contributes to their therapeutic failure. Area-wise studies on antimicrobial susceptibility profiles are essential to guide policy on the appropriate use of antibiotics to reduce the morbidity and mortality and also to control the emergence of antimicrobial resistance in local area.

Keywords: ESBL, Klebsiella pneumoniae, Mixed Infection, MRSA, Sputum culture

INTRODUCTION

Lower respiratory tract infection (LRTI) is an important cause of morbidity and mortality among humans affecting all age groups worldwide and describes a range of symptoms and signs, which vary in severity from non-pneumonic LRTI in young healthy adult to pneumonia or life threatening exacerbation in a patient with severe disabling chronic obstructive pulmonary disease (COPD). LRTI along with pneumonia, a disease of developing countries, have an incidence of about 20–30% in developing countries as compared to 3–4% in developed countries [1].

The risk factors implicating to affect the prevalence of LRTI are age, gender, nature of work, environment and season. Respiratory tract infections, ranging from reduced input in workplaces, frequent prescription of antibiotics, a serious economic burden on society, even when the causative agents is not bacteria. The causative agents of LRTI's are not well recognized, most are caused by viruses and atypical pathogens secondarily infected by bacterial pathogen [2].

A better understanding of the pathogens causing infection allows a logical approach to treatment [3]. In developing countries like India, there is a need to formulate an strategy for timely diagnosis and rapid use of empirical therapy and change of suggested therapy immediately based on the antibiotic susceptibility test of the causative agent to prevent the spread of the pathogen within the community, which ultimately leads to complications [2]. However, in recent years, there has been a dramatic increase in antibiotic resistance among respiratory pathogens due to various mechanisms [4]. The consequence of increased drug resistance which is a difficult task since bacterial infection of lower respiratory tract is a major cause of death among infectious disease [5].

This study was conducted to determine the prevalence of lower respiratory tract infections in patients attending SRG hospital and Jhalawar Medical College, Jhalawar, as well as to know the current antibiotic resistance pattern and their mechanism necessary for the prescription of appropriate therapy and prevention of spreading the antibiotic resistance within the community.

MATERIALS AND METHODS

Study Population

A total of 200 samples, 11-80 years of age, were obtained from the outpatient and inpatient Department of Medicine and Chest & TB, SRG hospital and Jhalawar Medical College, Jhalawar were included in the study from June 2012 to June 2013.

Exclusion criteria

Patients with pulmonary tuberculosis, old H/o Tuberculosis, congestive heart failure, pulmonary infarction, and AIDS were excluded from the study.

Sample collection and processing

Two sets of sputum samples were collected after proper instructions regarding collection of sputum sample for which informed consent from patients were taken who were presenting with LRTI (fever, H/o cough, productive sputum, chest pain, anorexia, headache and weight loss) for bacteriological culture into a wide-mouthed sterile containers, transported to the laboratory and processed within 2 hours. The samples were subjected to the following investigations:-

1. Microscopy of gram stained smears was done to examine the character of exudates, number & type of organisms, > 25 polymorphonuclear leucocytes and < 10 epithelial cells per low power field, if justified were included in the study.

2. Culture of sputum was done on Blood agar, chocolate agar (incubated in candle jar at 37°C) and MacConkey agar. Any bacteria showing heavy growth on culture or a moderate or light growth along with Gram stain report compatible with the culture results were considered to be the causative agents.
3. Identification of bacterial isolates was done by the relevant biochemical tests.
4. Antibiotic sensitivity test was done by modified Kirby Bauer's method as per CLSI guidelines [6].
    - For Gram positive organisms to Ampicillin 10mcg, Ampicillin+Sulbactam-10mcg, Amoxyclav-10mcg, Amikacin-30mcg, cephoxitin-30mcg, ceftriaxone-30 mcg, Ciprofloxacin-5mcg, chloramphenical-30mcg, erythromycin-15mcg, gentamycin-10mcg, ofloxacin-5mcg, tetracycline-30mcg, Vancomycin-30mcg.
    - For Gram negative organisms to Ampicillin + Sulbactam-10mcg, Amoxyclav- 10mcg, Amikacin-30mcg, Cefotaxime-30mcg, cefotaxime + clavulanic acid-30/10mcg, ceftazidime-30mcg, ceftazidime + clavulanic acid-30/10mcg, ceftriaxone-30mcg, Ciprofloxacin-5mcg, chloramphenical-30mcg, erythromycin-15mcg, gentamycin-10mcg, imipenam-10mcg, netilmycin-30mcg, ofloxacin-5mcg, tetracycline-30mcg.
5. For detection of Methicillin resistance cephoxitin resistance was taken as an indicator [7].
6. For detection of extended spectrum beta lactamase (ESBL) production cefotaxime + clavulanic acid and ceftazidime + clavulanic acid should be considered only when there is > 5 mm increase in the zone diameter of these drugs were measured as compared to cefotaxime and ceftazime disc alone.

RESULTS

A total of 200 samples were obtained from the outpatient and inpatient Department of Medicine and Chest & TB of which 66% were male and 34% were female [Table/Fig-1].

Seventy seven percent of samples were culture positive for both single pathogen and mixed infection of which 56.5% were male and 20.5% were female as males are more at risk for LRTI. [Table/Fig-2-4].

Mixed infection is mainly found in admitted patients as they have more chances of nosocomial infection due to stay of patient, also more chances of contact with nosocomial pathogens in the hospital setting.

Klebsiella pneumoniae was the most prevalent pathogen (71/193), followed by Coagulase positive Staphylococci (43/193) [Table/Fig-5].

	No. of Cases	Male	Female
IPD *	102 (51%)	53 (26.5%)	49 (24.5%)
OPD **	98 (49%)	79 (39.5%)	19 (9.5%)
Total	200 (100%)	132 (66%)	68 (34%)

[Table/Fig-1]: Showing comparative data of different clinical and functional parameters. * Inpatient department, ** outpatient department

	Culture Positive			Culture negative
	Male	Female	Total	
For single pathogen	86 (43%)	29 (14.5%)	115 (57.5%)	-
For mixed infection	27 (13.5%)	12 (6%)	39 (19.5%)	-
Total	113 (56.5%)	41 (20.5%)	154 (77%)	46 (23%)

[Table/Fig-2]: Distribution of culture positive cases.

More resistant pattern was found in CONS showed 61.11% MRSA incidence compared to 41.86% in COPS, [Table/Fig-6] also regarding ESBL production *Escherichia coli* showed incidence of 36.36% as compared to other gram negative bacilli [Table/Fig-7].

Age in years	No. of cases
11-20	17
21-30	23
31-40	49
41-50	26
51-60	52
61-70	24
71-80	9
Total	200

[Table/Fig-3]: Distribution of cases according to Age.

Mixture of organism	Male	Female	Total
Coagulase Positive *Staphylococcus* + *Klebsiella* pneumoniae	12	3	15
Coagulase Positive *Staphylococcus* + *Pseudomonas aeruginosa*	4	3	7
Klebsiella pneumoniae + *Pseudomonas aeruginosa*	4	2	6
Klebsiella pneumoniae + Group A beta hemolytic *Streptococci*	3	3	6
Klebsiella pneumoniae + *Candida albicans*	2	1	3
Coagulase negative *Staphylococcus* + Group A beta hemolytic *Streptococci*	2	0	2
Total	27 (69.23%)	12 (30.77%)	39 (100%)

[Table/Fig-4]: Distribution of cases according to mixed infection.

Pseudomonas aeruginosa was the most resistant organism found based on the antibiotic susceptibility pattern while *Proteus mirabilis* was the most sensitive organism [Table/Fig-8].

DISCUSSION

The result shows that LRTI was more prevalent in males than in females. Humphrey et al., in their study of prevalence of pneumonia

Organism	Total no. isolated	IPD	OPD	Male	Female
Klebsiella pneumoniae	71	57	14	53	18
Coagulase Positive *Staphylococcus* (COPS)	43	36	7	31	12
Pseudomonas aeruginosa	23	15	8	17	6
Group A beta hemolytic *Streptococci*	19	14	5	12	7
Coagulase negative *Staphylococcus* (CONS)	18	13	5	13	5
Esch. coli	11	7	4	8	3
Proteus mirabilis	5	4	1	3	2
Candida albicans	3	3	0	3	0
Total	193 (100%)	149 (77.2%)	44 (22.8%)	140 (72.54%)	53 (27.46%)

[Table/Fig-5]: Number of organism isolated.

Organism	MRSA	Non-MRSA
Coagulase Positive *Staphylococcus*	18/43 (41.86%)	25/43 (58.14%)
Coagulase negative *Staphylococcus*	11/18 (61.11%)	7/18 (38.89%)

[Table/Fig-6]: Distribution of Methicillin resistant Staphylococci isolates.

Organism Organism	ESBL		Non- ESBL	
	Cefotaxime + Clavulanic acid	Ceftazidime + clavulanic acid	Cefotaxime + Clavulanic acid	Ceftazidime + clavulanic acid
Klebsiella pneumoniae	12/71 (16.9%)	13/71(18.3%)	59/71 (83.1%)	58/71 (81.7%)
Pseudomonas aeruginosa	4/23 (17.39%)	5/23 (21.74%)	19/23 (82.61%)	18/23 (78.26%)
Esch. coli	4/11 (36.36%)	4/11 (36.36%)	7/11 (63.64%)	7/11 (63.64%)
Proteus mirabilis	0/5 (0%)	1/5 (20%)	5/5 (100%)	4/5 (80%)

[Table/Fig-7]: Distribution of Extended spectrum β lactamase producing organism.

Antibioticdiscs	*Klebsiella pneumonia* (n = 71)		Coag + Staph (n = 43)		*Pseudomonas aeruginosa* (n = 23)		Coag – staph (n = 18)		*Esch. Coli* (n = 11)		*Proteus mirabilis* (n = 5)	
	S	R	S	R	S	R	S	R	S	R	S	R
Ampicillin	NA	-	6	37	NA	-	3	15	NA	-	NA	-
Ampicillin + Sulbactum	42	29	18	25	4	19	7	11	7	4	4	1
Augmentin	18	53	17	26	3	20	6	12	5	6	4	1
Amikacin	59	12	38	5	9	14	13	5	8	3	5	0
Cephoxitin	NA	-	25	18	NA	-	7	11	NA	-	NA	-
Cefotaxime	37	34	NA	-	8	15	NA	-	4	7	4	1
Cefotaxime + Clavulanic acid *	49	22	NA	-	12	11	NA	-	8	3	4	1
Ceftazidime	38	31	NA	-	9	14	NA	-	5	6	3	2
Ceftazidime + clavulanic acid *	51	20	NA	-	14	9	NA	-	9	2	4	1
Ceftriaxone	36	35	21	22	9	14	7	11	4	7	2	3
Ciprofloxacin	16	55	12	31	3	20	3	15	3	8	2	3
Chloramphenicol	18	53	19	24	2	21	5	13	2	9	1	4
Erythromycin	23	48	22	21	4	19	4	14	1	10	2	3
Gentamycin	29	42	17	26	7	16	7	11	3	8	3	2
Imipenam	63	8	NA	-	2	21	NA	-	2	9	5	0
Netilmycin	52	19	NA	-	8	15	NA	-	7	4	5	0
Ofloxacin	13	58	25	18	3	20	6	12	2	9	2	3
Tetracycline	46	25	27	16	6	17	11	7	5	6	3	2
Vancomycin	NA	-	43	0	NA	-	18	0	NA	-	NA	-

[Table/Fig-8]: Antibiotic susceptibility pattern.
Note: * Sensitivity of cefotaxime + clavulanic acid and ceftazidime + clavulanic acid considered only when there is > 5 mm increase in the zone diameter of these drugs as compared to cefotaxime and ceftazime disc alone (ESBL production indicator)

and lower respiratory tract infection reported a high prevalence in males than females [8]. The reason for high risk in males of LRTI as well as COPD is attributable to smoking, use of tobacco, alcohol consumption, etc., causing decreased local immunity in the respiratory tract due to defective mucociliary clearance, mucous plugging, airway collapse, respiratory muscle fatigue and the effect of medications used. Females enrolled in the study comprised largely of housewives, who, being less mobile experienced less exposure to respiratory risk factors.

Maximum number of patients (50.5%) were from 31-40 (24.5%) and 51-60 years (26%) correlated well with the study from Finland, which showed the higher incidence of LRTI in patients over the age of 50 years as the immunity is lower in young and old age group so there is more chances of infection at these age [9]. Studies from different areas reported an aetiological diagnosis between 45% to more than 80% [10] as seen in our study (77%).

Patients in the older age group are more susceptible to gram negative pneumonia because of waning immunity and pulmonary defense mechanisms, underlying chronic diseases such as malnutrition, diabetes mellitus, emphysema, uraemia etc., silent aspiration and increased exposure to antibiotics. Institutional care also makes the patients more susceptible to gram negative pneumonia. In the present study, incidence of mixed infections was 19.5% which is consistent with the fact that incidence of mixed infections does not usually exceed 30% [11]. Identification of polymicrobial infection is very important for treatment strategies. The injury to ciliary motility of epithelial cells occurs due to the entry of infective agent which opens the entry of other infectious agents to infiltrate the lower respiratory tract.

Indian studies have reported higher incidence of gram negative bacilli form several decades among culture positive pneumonia as well as in other form of LRTI [12]. *K. pneumoniae*, *P. aeruginosa* and CONS are the commonest organism causing LRTI in inpatients whereas among outpatients, *S.aureus* is the commonest organism causing LRTI in the present study.

The pathogen causing LRTI, in decreasing order, were Klebsiella pneumoniae (36.79%), Coagulase positive *staphylococci* (22.28%), *Pseudomonas aeruginosa* (11.92%), CONS (9.33%), *Esch. coli* (5.7%), *Proteus mirabilis* (2.59%) and *Candida albicans* (1.55%) in this study. This is comparable with a report from Nigeria [13] where commonest organism isolated was *K. pneumoniae* accounting for 38% of the isolates. A higher prevalence of *Klebsiella* pneumoniae was found in most studies from India.

Shailaja et al., had earlier reported *K. pneumoniae* (32.26%) as the most prevalent bacterial isolate [14]. They identified risk or susceptibility to infections with encapsulated organisms such as *S. pneumonia* and *K. pneumoniae* to be on higher side. Due to variation in age, season, the type of population at risk, and various other factors the differences in the prevalence of bacterial isolates in different studies were observed elsewhere in India [15].

The sensitivity tests indicated that the isolates were resistant to one or more antibiotics, although generally, a low percentage of the isolates were sensitive to the antibiotic tested. The result of the sensitivity test indicates that Gram-positive and Gram negative isolates showed highest sensitivity to Amikacin, ampicillin + sulbactum, cefotaxime + clavulanic acid, Ceftazidime + clavulanic acid, Ceftriaxone, Imipenam, tetracyclin and netilmycin while high resistance was also recorded for antibiotics such as Ampiciilin, augmentin, ciprofloxacin, chloramphenicol, erythromycin, ofloxacin and gentamycin. This observation poses a serious public health problem and also documented in other studies also [2,16]. The pattern of antibiotic resistance recorded in this study among *P. aeruginosa*, *K. pneumoniae* and *E. coli* isolates is correlated well with the results obtained from Gauchan et al., and Kumari et al., [2,5]. Although *P. aeruginosa* has been shown to be resistant to many antimicrobioal agents as was found in our study as various mechanism of resistance can be involved including ESBL, metallo beta lactamase (MBL) and ampC beta lactamase production, in its resistance.

More resistant pattern was found in CONS showed 61.11% MRS incidence compared to 41.86% in COPS as now a days more pathogenicity is found to be associated with CONS as they are more involved in serious infections as shown in study of Onanuga A et al., in Nigeria have reported a high prevalence of 69% [17].

In *Escherichia coli* ESBL production has an incidence of 36.36% as compared to other gram negative bacilli which showed a lower

incidence, this was mostly found in other studies as the commonest agent for ESBL production was *Escherichia coli*, but the number of *Escherichia coli* was very less in our study so it require further elaboration to confirm. There is an alarmingly high resistance rate was observed to cephalosporins, β lactam-β- lactamase inhibitors, and carbapenem against gram negative microorganisms because of various mechanism of resistance transferred to one bacteria from other bacteria.

The increase in the antibiotic non-susceptible strains of pathogens in recent years could be attributed to their indiscriminate and promiscuous use. This alarming situation developed due to widespread confusion over the aetiology of respiratory infection caused primarily by virus or bacteria which led to the emergence of resistant microorganism. Use of combined therapy & its use for long duration increase the opportunity of resistant strains for acquisition and/or amplification of their resistant mechanisms. In previous era, first line medications were effective and cheaper. With the onset of newer resistance mechanism, newer treatments are developing which are effective but proving too costly to the vast majority of cases living in poor developing countries.

LIMITATIONS

Limitations of this study were lack of availability of facilities for detection of atypical and viral pathogens. Atypical pathogens have been reported in several studies on LRTI's accounting for 10-15% of the causes of pneumonia as for the diagnosis of atypical pathogens more advanced technologies are required which is not feasible for us.

CONCLUSION

The level of antibiotic resistance, is a serious public health problem, must bring to light the need for timely and proper diagnosis of the major microbial causes of the respiratory infections, based on antibiotic susceptibility testing in order to administer appropriate therapy of the causative agent. This study revealed that there has been mildly significant change which occurs regarding aetiology of bacterial pathogens involved in LRTI. Judicious use of antibiotics must be done by the clinicians, pharmacist and others who are incorporated in drug delivery system so that we can make a check on the emergence of pathogens acquiring drug resistance to various antibiotics having a role in critical condition or emergency. Also, there is a need first to start empirical therapy and change of antibiotic must be made when we receive the antibiotic susceptibility pattern for that microorganism to hamper the development of resistance mechanism. However resistance to β lactam antibiotics requires further evaluation by more standardized method.

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