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Microbiological Profile of Childhood Pneumonias in Hyderabad, India

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A B S T R A C T

The present study was undertaken from 30/6/11 – 14/8/12 to know the Bacterial, and Mycological profile profile and drug susceptibility patterns in children suffering from pneumonias. Sputa (expectorated and induced) and Pleural fluid were collected from 113 cases and processed by conventional methods. Antibiotic sensitivity testing of these isolates was done as per CLSI guidelines. Bacterial pathogens were isolated in 44.25% of cases from both Sputum and Pleural fluid. *Klebsiella pneumoniae* was the predominant isolate 28% followed by *Pseudomonas aeruginosa* 24% and *Staphylococcus aureus* 22%. 72% of *Staphylococcus aureus* strains were Methicillin Resistant. *Candida albicans* was the most common isolated fungi 6.2% followed by *Non Albicans Candida* from sputum samples. Pleural fluid cultures for fungi were negative. Respiratory tract infections are a significant public health problem in developing countries. Timely detection followed by expeditious identification of pathogens and determination of susceptibility to antimicrobial agents can have a great diagnostic and prognostic importance.

Introduction

Lower respiratory tract infections are a persistent and pervasive public health problem, placing a considerable strain on the health budget and are generally more serious than upper respiratory tract infections. They cause a greater burden of disease worldwide than human immunodeficiency virus infection, malaria, cancer, or heart attacks (1).

Pneumonia is a form of acute Lower Respiratory Tract Infection that affects the lungs and has being the single largest cause of death in children. Worldwide, 35-40% mortality among children aged less than 5 years is attributed to respiratory tract infections accounting for 2.04 million deaths/year. In India, more than 4 lakh deaths every year are due to pneumonia accounting for 13%-16% of all deaths in the pediatric hospital admissions (2, 3).

Clinicians use a wide range of disease definitions, such as tracheitis, acute bronchitis, bronchiolitis and pneumonia, depending on the symptoms and signs and anatomic structure involved, aspiration pneumonia, obstruction pneumonia and ventilator-associated pneumonia based on

pathogenesis and community acquired pneumonia and hospital-acquired pneumonia based on the location where infection was acquired.

Nevertheless, pneumonia is considered as the most serious condition and is almost always treated with antibiotics.

LRTI is caused by several infectious agents, including viruses, bacteria and fungi (2). Traditionally, the infections of the upper respiratory tract are thought to be predominantly of viral origin and the infections of the lower respiratory tract of bacterial origin (4).

But viruses are also known to cause pneumonia, e.g. the *Influenza-virus* and the novel *Coronavirus* that causes severe acute respiratory syndrome (4).

Bacterial pneumonia is usually the result of *Streptococcus pneumoniae* or *Haemophilus influenzae*, especially type b, and rarely *Staphylococcus aureus* or other streptococci. *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* cause atypical pneumonias (5).

Among the vast diversity of respiratory pathogens, fungi account for only a small portion of community-acquired and nosocomial pneumonias.

However, fungal respiratory infections generate concern in the expanding population of immunosuppressed patients (6).

Opportunistic fungal organisms (e.g., *Candida* species, *Aspergillus* species, *Mucor* species) tend to cause pneumonia in patients with congenital or acquired defects in the host immune defenses.

The present study was undertaken to note the prevalence of various Bacterial and Fungal Pathogens causing LRTI in children and an attempt was also made to examine *P. jirovecii* in induced sputum and for *Nocardia* spp.

Materials and Methods

As there is a paucity of studies in the Indian setting to find out incriminating bacterial agents in children suffering from lower respiratory tract infections, this study was undertaken to determine the prevalence of Bacterial and Fungal etiologic agents of lower respiratory tract infections and their antimicrobial susceptibility pattern in children.

Place of study

In a tertiary care hospital, Hyderabad.

Duration

The present study was undertaken for a period of one year i.e. from 30/6/11 – 14/8/12.

Study group comprises of children clinically diagnosed as cases of Pneumonias belonging to age group 0 – 18 years.

Specimen

Early morning expectorated sputum from two consecutive days was collected in sterile containers from all patients included in the study.

Induced sputum samples were collected in addition to expectorated sputum in those children who had difficulty in expectorating sputum and in those with underlying immunocompromised disease. Induction of sputum was done using a Nebulizer and 3% hypertonic saline. Induced sputum is watery and was accepted. Sputum was considered unsuitable if it had a final score of 0 or less by Bartlett's scoring system.

Pleural fluid was also collected and processed from few cases.

Processing

Specimen was first observed macroscopically whether, Mucoid, Purulent, Mucopurulent or any Blood tinge.

Culture

Sputa and pleural fluids were inoculated on the following culture media and processed.

Blood agar

Chocolate agar

MacConkey agar

Sabouraud's dextrose agar with and without antibiotics. – For fungi.

All the bacterial isolates obtained were identified conventionally by standard biochemical tests.

Two of the gram negative non fermenting bacilli identified conventionally were also confirmed as *S. maltophilia* and *B. cepacia* by VITEK 2. (BIOMERIEUX)

Control strains used

ATCC *S. aureus*: ATCC 12228, ATCC *E. coli*: ATCC 25922

Antibiotic sensitivity testing

The Antibiograms of bacterial isolates were determined by Kirby –Bauer's Disk Diffusion method and the Zone diameters were interpreted as per NCCLS guidelines

Results and Discussion

The total number of children suffering from LRTI included in the study during the one year period was 113.

Among a total of 11(22%) isolates of *Staphylococcus aureus* 8 (72.72%) were Methicillin resistant.

One isolate of *Streptococcus pyogenes* and four isolates of *Streptococcus pneumonia* were found to be sensitive to all the antibiotics tested.

One isolate of *Enterobacter* spp was obtained which was resistant to Penicillins and Cephalosporins and was sensitive to Carbapenams and Cotrimoxazole.

All the induced sputum samples which were stained by Giemsa technique were negative for trophozoites and cysts of *Pneumocystis jirovecii.*

All the sputa and pleural fluid samples stained by modified Zeihl Neelsen's staining were negative for *Nocardia* spp.

Acute lower respiratory infections are the most common cause of infectious diseases in children worldwide. Despite effective vaccines and nutritional and environmental interventions, they constitute a major cause of mortality in children aged less than five years, leading to an estimated 1.9 million deaths annually (7). WHO recognized respiratory diseases as the second important cause of death for children fewer than five years in 2010 and also these children with signs of pneumonia are more likely to have a bacterial etiology (7).

In the present study bacteria as an etiologic agent for lower respiratory tract infections were identified in 55 (48.6%) cases. Culture positive rate is higher in the present study possibly because it was done in a tertiary care hospital.

In the present study majority of the bacterial pathogens were isolated in children less than

five years of age. Similar findings were observed by Akiyoshi Nariai *et al.*, in a study who found that Bacterial pathogens were isolated with high frequency in children less than 7 years of age (13).

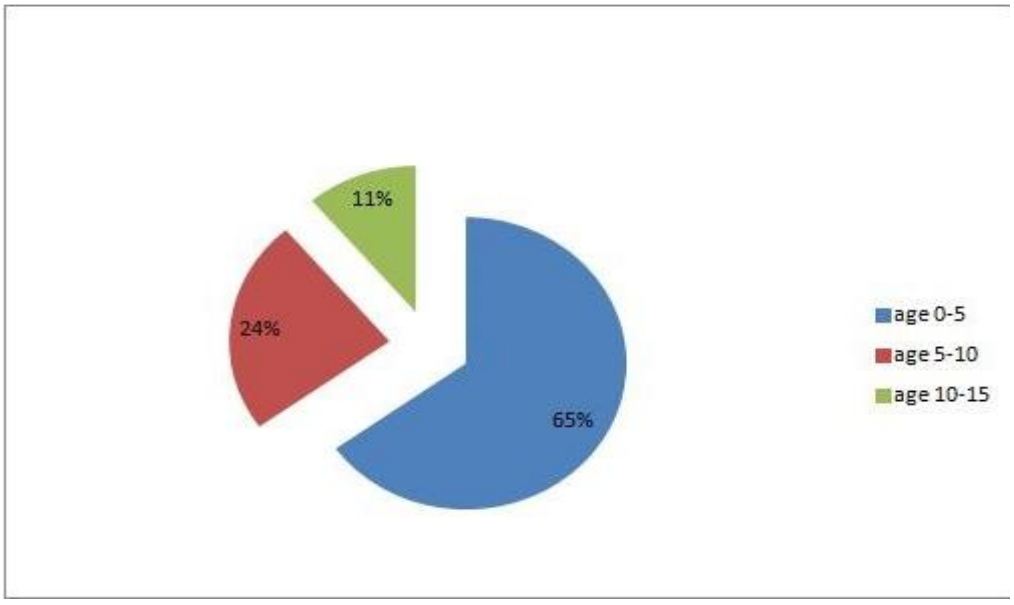
48.6% was the culture positive rate obtained. *Klebsiella pneumoniae* was found to be the most predominant organism causing LRTI in studies done by Okesola *et al.*, (2008), Juhitaneja *et al.*, (2009) (32.2%) and Dong *et al.*, (2006) (9.9%). This correlated well with the present study, among the Gram negative bacteria, *Klebsiella pneumoniae* (25.45%) was the most common pathogen isolated.

Incidence of *Pseudomonas aeruginosa* as an etiologic agent for LRTI was observed to be variable in various studies. HUA Chun-Zhen reported an incidence of (6.4%) and 9.1% incidence was reported in a study done by LüBo, Mo WeiXiong *et al.*, (9, 11). However in the present study 22% of the isolates were *Pseudomonas aeruginosa*. Chun- Yi- Lee *et al.*, observed a high isolation rate of 35.4% (21).

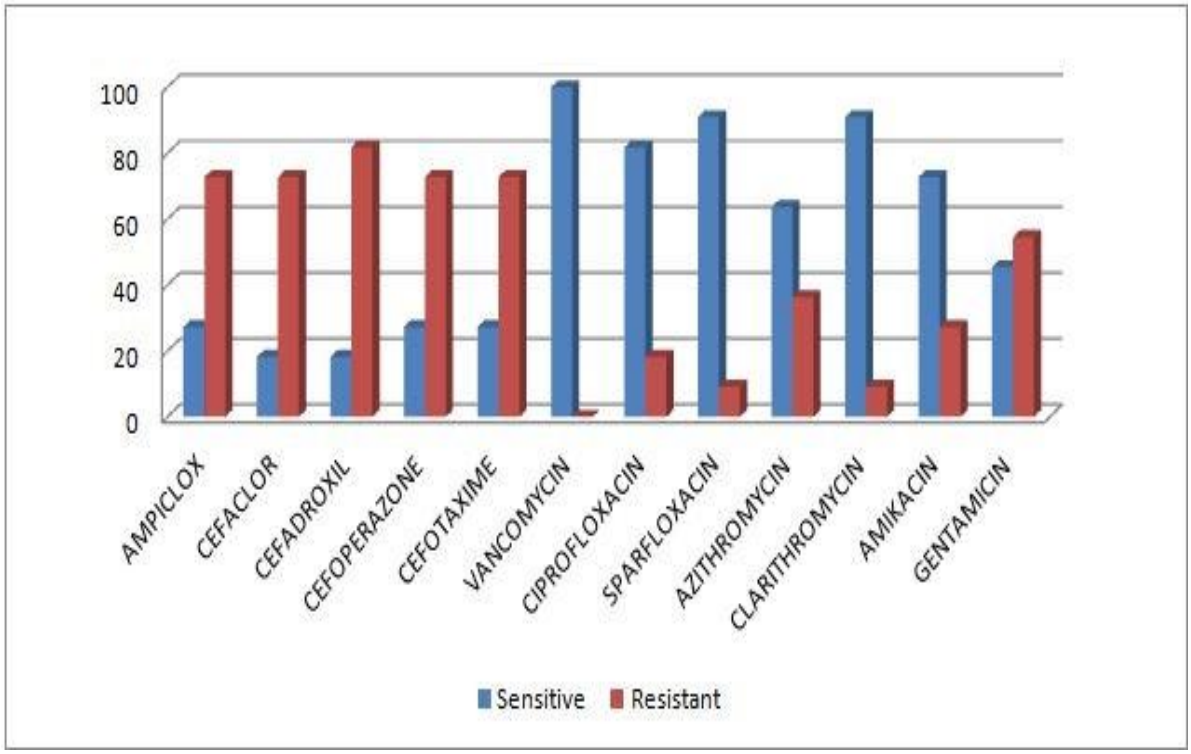
The respiratory system is the most common site for infection by Acinetobacter in immunocompromised patients and hospitalized patients, proving the statement, 2% of isolates of *Acinetobacter* spp were isolated from majority of the inpatients.

M. catarrhalis was isolated in 4% of cases. It is generally considered a commensal in the upper respiratory tract of adults, and its isolation from sputum is often reported as "normal flora of the oropharynx". However, Chong Chia YIN *et al.*, reported this organism as a pathogen causing lower respiratory tract infections in children (22). Repeat sampling was necessary to prove and confirm the organism as the etiologic agent of the respiratory infection.

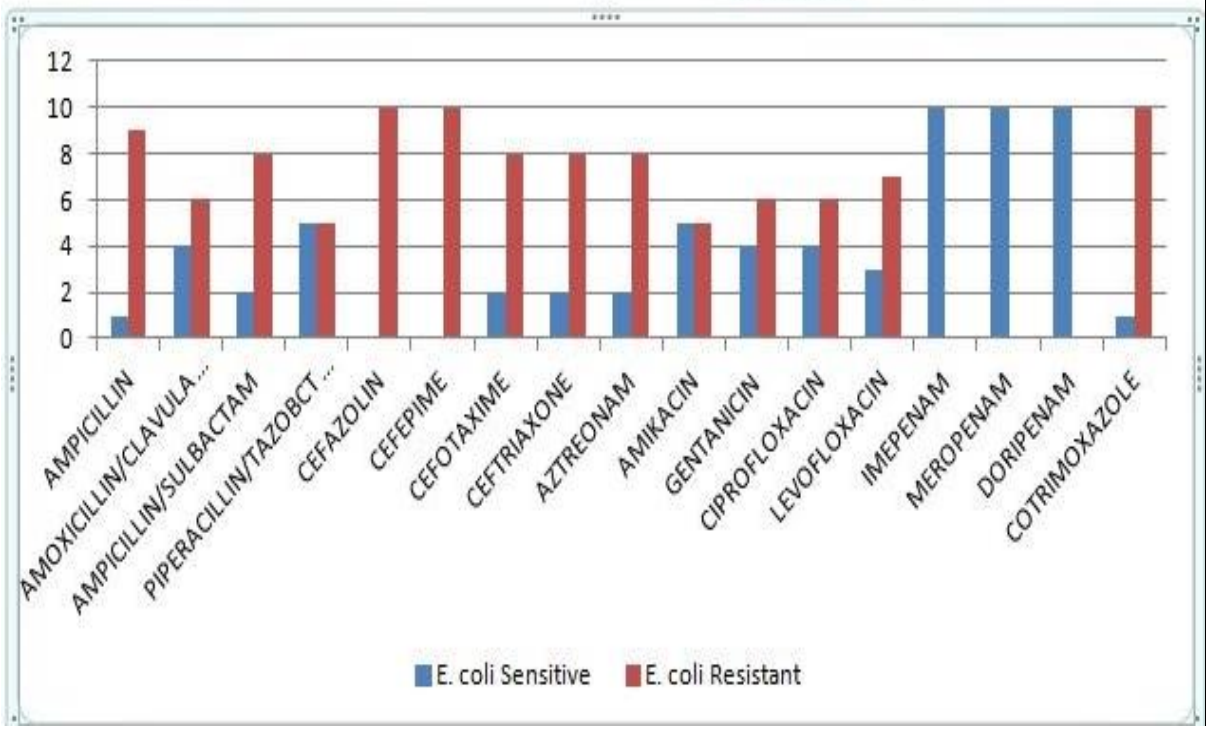
Age disribution of cases in years: n=113



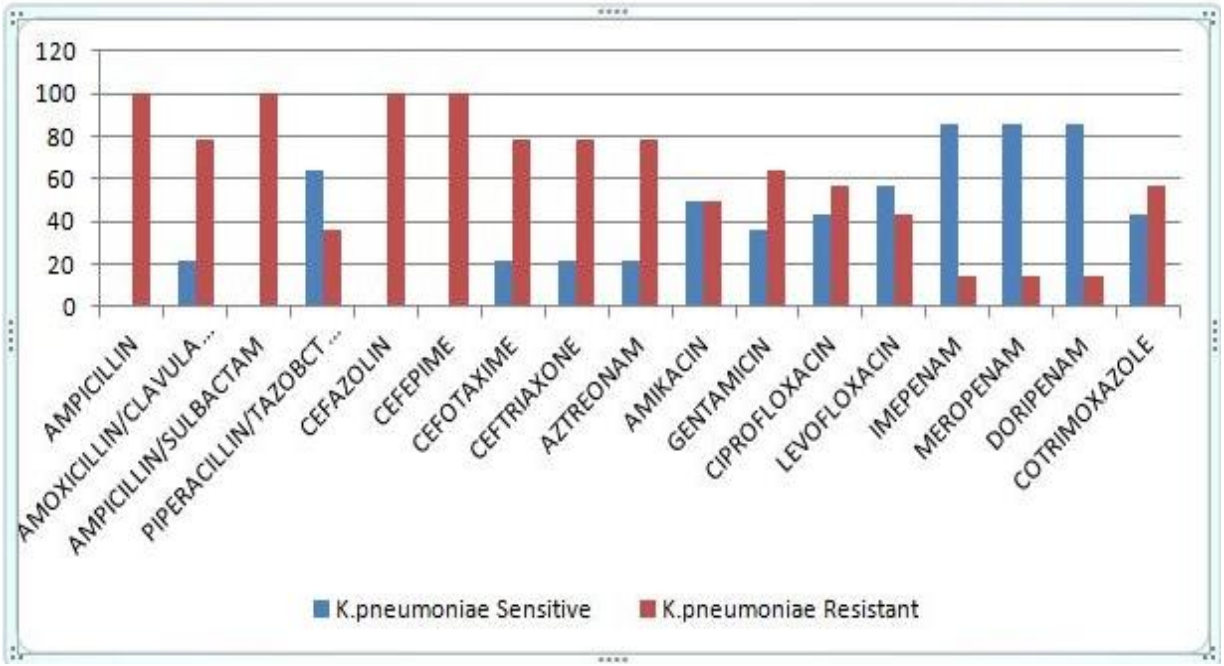
Antibiotic Susceptibility pattern of Staphylococcus aureus



Antibiotic sensitivity pattern of Eschericia coli



Antibiotic sensitivity pattern of Klebsiella pneumonia



Antibiotic sensitivity pattern of Pseudomonas aeruginosa

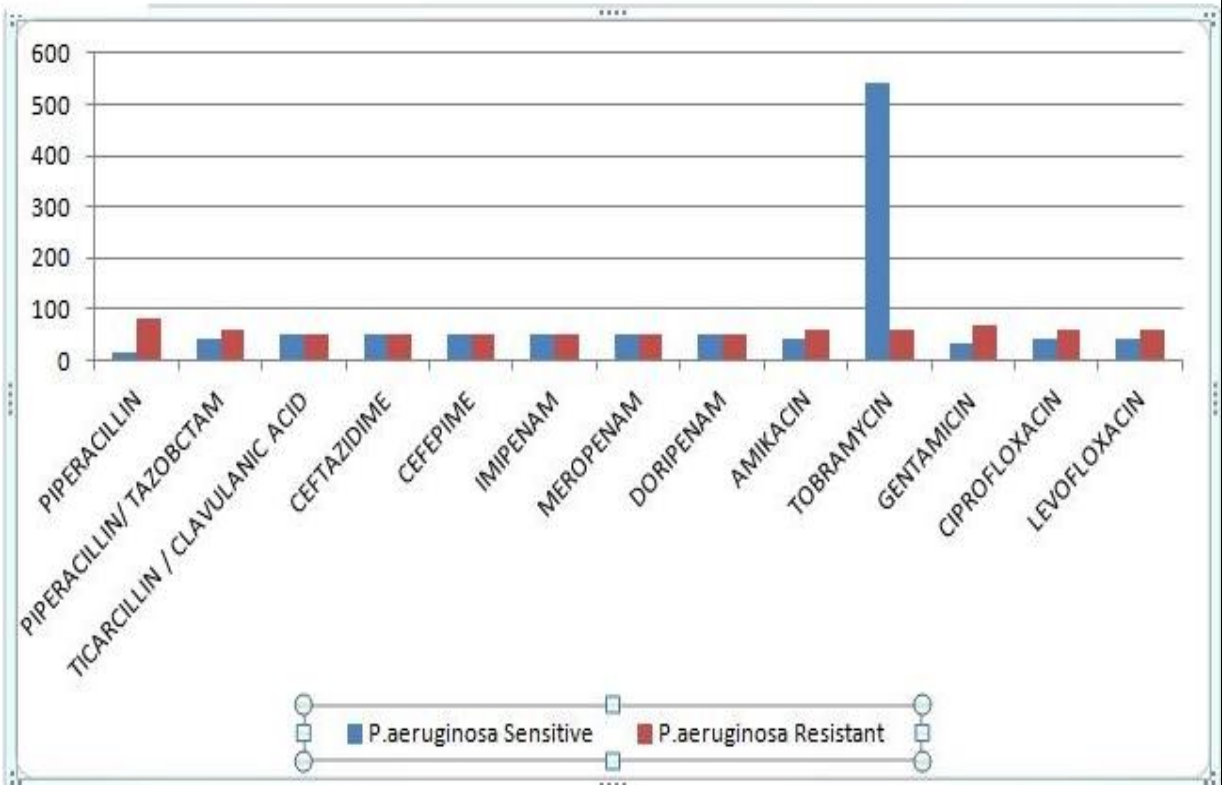


Table 1-Percentage of cases from which bacteria were isolated:

n =113

Culture	Number	%
Sterile	63	55.75%
Pathogens isolated	50	44.25%

Table 2 - Bacterial isolates obtained

Bacterial isolate	No. of isolates Sputum	No. of isolates Pleural fluid	Total	%
Klebsiella pneumoniae	10	4	14	28
Pseudomonas aeruginosa	7	5	12	24
Staphylococcus aureus	4	7	11	22
Escherichia coli	3	1	4	8
Moraxella spp.	2	0	2	4
Streptococcus pneumoniae	1	1	2	4
Streptococcus pyogenes	1	0	1	2
Enterobacter spp	0	1	1	2
Acinetobacter spp	0	1	1	2
Burkholderia cepacia	0	1	1	2
Klebsiellapneumoniae+ Stenotrophomonas maltophilia	1	0	1	2
TOTAL	**29**	**21**	**50**	**100**

Table 3 Methicillin Resistance among *Staphylococcus aureus*

n=11

Staphylococcus aureus	No.of isolates	Percentage
Methicillin resistant Staphylococcus aureus	8	72.72%
Methicillin sensitive staphylococcus aureus	3	27.28%

Table -4 Sensitivity patterns of Burkholderia *cepacia and Stenotrophomonas maltophila*

ANTIBIOTIC	*Burkholderia cepacia(1)*		*Stenotrophomonas maltophilia(1)*	
	S	**R**	**S**	**R**
Ticarcillin / clavulanic acid	100	0	100	0
Ceftazidime	100	0	100	0
Meropenam	100	0	Not tested	Not tested
Levofloxacin	100	0	100	0
Cotrimoxazole	100	0	100	0
Chloramphenicol	100	0	100	0

Table 5- Fungal isolates

Fungal isolates	Sputum	%	Pleural fluid	%	Total	%
C.albicans	7	13.21	0	0	7	6.2
Non albicans candida	2	3.77	0	0	2	1.77
Mixed	1	1.89	0	0	1	0.88
Pneumocystis jirovecii	0	0	0	0	0	0
Nocardia spp.	0	0	0	0	0	0
No fungal growth	43	81.13	60	0	103	91.15

Table 6 – Comparison of bacteria isolated in present and various studies

Studies done	Bacteria isolated	Gram negative bacteria	Gram positive bacteria
Wang Y et al in (8).	26 %	69%	20.65%
Hua Chun-Zhen et al (9).	25%	--	--
Hanna Nohynek et al (10).	25%	--	--
Lübo, Mo Weixiong et al (11).	25.1%	35.6%	64.4%
Shailaja et al (12).	44.3%	--	--
Present study	48.6%	75%	25%

Table – 7 Comparison of split up of bacterial isolates obtained in the present and various studies

	S.aureus	*S.pneumoniae*	*K.pneumoniae*	*P.aeruginosa*	*E.coli*	*Acinetobacter*
D Narayanappa, et al(14)	15(30%) 27% -MRSA	4(8%)	--	--	--	--
Vikramjeet Dutta, et al (15)	(16%), MRSA -0	(54%)	8%	6%	6%	--
Batmunkh Nyambat et al (16)	n=126	n=83	n=35	n=37		n=34
A.M. Lingayat et al (17)	45.45%	22.7%	--	18%	--	--
Wang Y et al (8)	9.7%	--	10.8%	17%	10.7%	9.03%
Present study	20%	4%	25.45%	22%	7%	11%

Table 8- incidence of MRSA in various studies

	MSSA %	MRSA %
Present study	36.7	63.3
AlexanderKallen (27)	58.5	41.5
Study in US in 2001 -2002 (28)	22	78
D Narayanappa, et al (14)	73.4	26.6%
Vikramjeet Duttaet al (15)	100%	0%

Table 9- Fungal isolates in various studies.

Author	**% isolated**	**Predominant isolate**
B.V. Navaneeth et al (29)	8.9%	--
Shailaja et al (12)	12.8 %	--
Parvez Anwar Khan et al (30)	8.7%	*Candida , Aspergillus*
Jithendra Kandati,et al (31)	34%	*Pneumocystis jirovecii, Candida ,Aspergillus , Cryptococcus*
Present study	8.85%	*Candida*

Microscopic Examination: The following staining techniques were performed

Gram's stain	To assess adequacy of sample. (Bartlett's grading) For capsulated gram positive diplococci in sputum and gram positive and gram negative organisms in pleural fluid.
Giemsa stain	For *Pneumocystis jirovecii* in nebulised sputum.
KOH mount	For fungal elements.

The etiological diagnosis of LRTI is frequently confounded by the presence of commensal flora, as well as that of potentially pathogenic organisms in the oropharynx.

The increasing trend of antibiotic resistance in respiratory bacterial pathogens poses a challenge for empiric treatment with conventional agents. Infections with drug resistant organisms lead to longer hospital stays, increased mortality, and greater costs of hospitalization.

Inappropriate initial antibiotic therapy is a potentially modifiable factor that has been associated with increased mortality in patients with serious infections. Hence antibiotic sensitivity testing help the clinician to select empirical antibiotics based on Gram stain, culture findings and susceptibility to various antimicrobials.

Hua *et al.,* (2006) showed Antibiotic susceptibility tests showed that rates of ESBL (extended spectrum beta lactamase) -positive *Klebsiella pneumoniae* and *Eschericia coli* were 42.6% and 4.5%, respectively.

Juhi Taneja *et al.,* in their study showed ESBL production rate was as follows: *Klebsiella pneumoniae* (46.1%), *Eschericia coli* (57.1%) and *Pseudomonas aeruginosa* (75%) (19). 9 isolates of *Klebsiella* and 3 isolates of *Escherichia coli* were ESBL producers in our study.

Chun- Yi- Lee *et al.,* observed that *Staphylococcus aureus* was the most common Gram positive organism isolated from children suffering from LRTI (21). This correlated well with the present study where *Staphylococcus aureus* (22%) was the most common Gram positive bacteria isolated.

The rate of admission with a diagnosis of empyema increased over the last decade, most notably in children aged 1-4 years. In addition, the identification of *Streptococcus pneumoniae* as the primary pathogen though has been reported in various parts of the world its isolation rate have stabilized over the last decade, the rate of bacterial resistance, specifically methicillin resistant *Staphylococcus aureus*, has predominated.

After the universal use of the pneumococcal conjugate vaccine, 3 major changes have occurred 1) the number of patients admitted with empyema has decreased 2) the prevalence of *S. pneumoniae* has decreased and 3) *Staphylococcus aureus* has become the most common pathogen majority of those being methicillin resistant.

Bacteria from pleural fluid was isolated in 35% of cases in our study and when compared with various other studies it was found that Lochindarat *et al.,* found 18.3% pleural fluid culture positive cases, Karen D. Schultz *et al.,* in 2004 and Ghosal, *et al.,* in 1996 observed an isolation rate of 32 % and 26.5% respectively (24, 25, 26).

11.67% was the isolation rate of *Staphylococcus aureus* from pleural fluid in the present study. This was similar to study done by Karen D. Schultz, *et al.,* who observed that *Staphylococcus aureus* was the

most common pathogen isolated (18%) in pleural fluid samples (25). Both the isolates of *Streptococcus pneumoniae* were sensitive to Ampicillin. This correlated with Juhitaneja *et al.*, in 2009 reported that all the *Streptococcus pneumoniae* isolates were sensitive to Ampicillin (19).

Fungal Pneumonia in children is a rare condition, and is often seen in individuals with compromised immune system like AIDS. The most common fungal agents that cause pneumonia in children are *Histoplasma capsulatum, Cryptococcus neoformans, Pneumocystis jiroveci, Blastomyces and Coccidioides immitis. Pneumocystis jiroveci pneumonia* (PCP) in particular is a common, serious infection among HIV-infected children and is associated with high mortality.

In the present study out of 113 sputum and pleural fluid samples processed, 91.15% showed no fungal growth.

However in the present study though 12 children presented with underlying immunocompromised disease, and in all these patients induced sputa were collected in addition to expectorated sputum samples, no Trophozoite or cysts of *Pneumocystis jirovecii* observed. Sputum and pleural fluid samples which were stained by Modified acid fast

Techniques using 1% sulphuric acid were negative for *Nocardia spp.*

No attempt was made to for viral cultures due to lack of facilities. No attempt was made for anti-fungal sensitivity testing in the present study.

The prevention of repeated infections and the early detection and management of chronic lung disease is critical to the long-term respiratory and overall health of children.

The present study helps to know the prevalence of pathogens causing respiratory tract infections and also helps the clinician in guiding antibiotic therapy which is very essential particularly in children to prevent them from landing in more serious complications.

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