BACTERIAL CAUSES OF LOWER RESPIRATORY TRACT INFECTIONS IN PATIENTS ATTENDING CENTRAL REFERRAL HOSPITAL, GANGTOK WITH REFERENCE TO ANTIBIOTIC RESISTANCE PATTERN

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HOW TO CITE THIS ARTICLE:
Priyanka Paul Biswas, Tukaram Prabhu K. "Bacterial causes of lower respiratory tract infections in patients attending central referral hospital, Gangtok with reference to antibiotic resistance pattern". Journal of Evolution of Medical and Dental Sciences 2013; Vol. 2, Issue 42, October 21; Page: 8126-8135.

ABSTRACT: BACKGROUND: There is inadequate information from India on various lower respiratory tract pathogens and their resistance pattern in hospital settings. The present study was undertaken to see the bacteriological profile and the antibiotic resistance pattern of the isolates causing LRTI from this geographic region. **OBJECTIVE:** To identify and characterize bacterial pathogens causing community acquired and hospital acquired infections with reference to antibiogram pattern. **METHODS:** A total of 137 samples from patients suffering from lower respiratory tract infections were studied. All the sputum samples were subjected to gram staining, culture. Various organisms were identified by standard methods. The Kirby –Bauer method was employed to perform the antibiotic sensitivity on Mueller Hinton agar [MHA]. For Streptococcus pneumoniae blood agar was used. MHA with 4% NaCl was used to detect methicillin resistant Staphylococcus aureus [MRSA]. **RESULTS:** Growth of pathogens was obtained in 66.4% of sputum samples in case of inpatients and in 33.5% outpatients. Klebsiella pneumonia [15.3%] was the predominant isolate among the inpatients whereas Streptococcus pneumonia [8.5%] was the most common pathogen isolated from outpatients. Haemophilus influenzae was not isolated. Quinolone was found to be most effective antibiotic against gram negative organisms. A single isolate of Moraxella catarrhalis was isolated from a case of MDR-TB. **CONCLUSION:** Culture and susceptibility reports should be encouraged before therapy to combat the problem of emergence of MDR, ESBL and MRSA strains and to subside the economic burden due to increase in cost according to the consequence of development of antibiotic resistant microbial strains.

KEY WORDS: Lower respiratory tract infections [LRTI] community acquired infection [CAI], hospital acquired infection [HAI]

INTRODUCTION: Globally, community acquired respiratory tract infections account for a large proportion of antibiotic prescriptions and visit to family practitioners. [1] It has been found that in India acute respiratory tract infection is responsible for one million deaths. Also there is inadequate information from India on various lower respiratory tract bacterial pathogens and resistance pattern in hospital settings.[2] Infection of lower respiratory tract are responsible for 6% of general practitioners consultations and from 4.4% of hospital admission. [3] One complication is the patient's expectation & belief that such infections require antibiotic treatment. The patient's desire influences a physician to prescribe even when doctors see no clinical indication. They also found that this desire may be for a prescription based medicine, not necessarily an antibiotic. [4] A common reason for a prescription is the mistaken belief that antibiotics reduce re-attendance.

Informing patients about the natural history and course of lower respiratory tract infections, on the other hand, has been shown to reduce reconsultations. [5] Doctors themselves may have

misinterpreted the evidence and there are studies which show that practitioners are more likely to prescribe antibiotics when discharge or phlegm is purulent. [6] This belief is incorporated into patient's perception. Diagnostic uncertainty and the variable relationship between clinical findings and subsequent diagnosis further influences prescribing habits. [7]

For the purpose of guiding a prognosis, definition of lower respiratory tract infections has been used on the following questions, the answer to which will allow appropriate management and planning- has the patient been previously well or is there underlying chronic respiratory or other disease, has there been any development or deterioration in either of the following symptoms – dyspnoea or sputum purulence, are there any new signs audible in the chest to suggest pneumonia [8].

To the beat of our knowledge, no study regarding the prevalence of community and hospital acquired bacterial pathogens causing LRTI has been carried out in Sikkim. The main objective of the present study was to identify and characterize the pathogens from the sputum samples and determine their antibiotic susceptibility pattern and to see the seasonal, age, sex variation of the pathogens in association with LRTIs.

MATERIAL AND METHODS:

Study Population: A total 137 early morning, expectorated sputum samples were collected from patients attending Central Referral Hospital attached to Sikkim Manipal Institute of Medical Sciences [SMIMS]. These patients were clinically diagnosed to have increases cough, purulence or volume of expectorations and increased severity of dyspnoea. Those patients who were already taking treatment in the hospital were excluded from the study. Any sample that was thin, watery and with no purulent matter was considered unsuitable for further processing. In such case a repeat sample was asked for.

Isolation & identification: Sputum samples were inoculated on 10% sheep Blood agar [BA], Chocolate agar [CA] and Mac Conkey agar, MAC, [HiMedia, Mumbai, India] and Sabouraud dextrose agar, SDA, [HiMedia, Mumbai, India]. For identification of Streptococcus pneumoniae, an optochin disc with 5µgm content [HiMedia, Mumbai, India] was placed on the primary sputum culture done on a BA plate and incubated aerobically. A second BA plate was streaked with Staphylococcus aureus to facilitate the growth of H. influenzae. A third BA plate and CA plates were incubated at 37^0C in a candle jar and Mac Conkey agar plates were incubated at the same temperature in ambient air. Plates were read after 18-24 hours to look for the presence or absence of typical colonies of different bacteria. Direct smear examination of the purulent part of sputum sample was made and examined under oil immersion after the process of gram staining.

The gram positive and gram negative bacteria were initially identified on the basis of colony morphology, gram stain morphology and motility test. A slide coagulase test followed by tube coagulase test was done for gram positive bacteria that were catalase test positive and produced golden yellow pigment on blood agar. Catalase negative colonies that showed the presence of capsule on nigrosin stain and were optochin sensitive was further confirmed by bile solubility test. This test is done directly on the culture plate by touching a colony with a loopful of 2% sodium deoxycholate reagent [p H 7.0], incubating the plate at 35 - 37^0C for 30 minutes and examined for lysis of the colony. [9] Appearance of cream coloured pasty colonies on SDA was confirmed to be

budding yeast cells by gram staining. Further, nigrosin staining of these colonies showed the absence of capsules. Identification to species level was done on the basis of carbohydrate fermentation using 1% solution of glucose, lactose, maltose, sucrose, tetrazolium reduction test and germ tube test. [10]

Identification to species level of the gram negative bacteria were done on the basis of oxidative fermentative tests, triple sugar iron test, nitrate reduction test, carbohydrate fermentation using 1% solution of following sugars: glucose, lactose, mannitol, sucrose, indolae test, methyl red test, Vokes Proskauer test, citrate utilization test, urease test, phenylalanine deaminase test and amino acid decarboxylase and dihydrolase test. [11]

Antimicrobial susceptibility testing:

Antibiotic susceptibility test was done by Kirby Bauer disc diffusion method on Muller Hinton agar [MHA]. For *Streptococcus pneumonia* blood agar was used. For detection of MRSA strains, MHA [HiMedia, Mumbai, India] supplemented with 4% NaCl was used. Inoculum was prepared and adjusted to 0.5 Mc Farland's turbidity standard. Antibiotic disc were obtained from the Hi Media Laboratories [Mumbai] [12] The concentration of each antimicrobial agent (in µg) tested per disc was

a) For Staphylococcus aureus
   oxacillin [1µg], gentamicin [10µg], cefazolin [30µg], amoxicillin [10µm], tetracycline [30µg], ciprofloxacin [5µg], clindamycin [2µg], cefuroxime [30µg], azithromycin [15µg], vancomycin [30µg]
b) For Streptococcus pneumoniae
   Penicillin [10 units], tetracycline [30µg], cefotaxime [30µg], amikacin [30µg], erythromycin [15µg]
c) For Pseudomonas aeruginosa
   gentamicin [10µg], amikacin [30µg], piperacillin [100µg], ciprofloxacin [5µg], cephotaxime [30µg], imipenem [10µg], ceftazidime [30µg], netilmicin [30µg] and tobramycin [10µg] and
d) For other gram negative bacteria
   amoxicillin [10µg], amoxicillin/clavulanic acid [10/10µg], amikacin [30µg], gentamicin [10µg], cefuroxime [30µg], cefotaxime [30µm], ciprofloxacin [5µg], imipenem [10µg]

Statistical analysis: Statistical analysis of data was done using an online application [www.physics.stats.edu/contingency/ NROW_NCOLUMN_form.html] to do the chi-quare test and find the P value.

RESULTS: A total of 137 sputum samples from patients suffering from LRTI were studied out of which 39 [28.4%] samples showed the growth of respiratory tract pathogens whereas 98 [71.5%] showed normal upper respiratory/ oral flora. Out of the 39 sputum culture positive samples, 17 [43.5%] samples showed growth of pure gram negative bacilli, 13 [33.3%] showed growth of pure gram positive cocci, 4 [10.2%] samples showed mixed growth of gram positive cocci and gram negative bacilli each while 2 [5.1%] samples showed mixed growth of gram negative bacilli each.

[Table 1] Thus a total of 45[32.8%] of positive isolates including gram positive & gram negative bacteria were found in the study.

Direct sputum smear examination showed the presence of – 88.8% from 8/9 samples of sputum as gram positive diplococcic [capsulated] along with plenty of pus cells; 88.2% from 15/17 samples as gram negative bacilli with moderate to plenty pus cells; 76.9% from 10/13 samples as gram positive cocci predominantly in clusters along with pus cells and 100% from 3/3 samples as budding yeast cells with pseudo hyphae. In 89.0% of the cases [122 out of 137cases] gram staining findings were correlating with culture findings. It was observed that purulent or mucopurulent sputum gave better results than mucosalivary sputum.

The patients in this study were between 4 – 88 years. Sex-wise analysis showed that 81 were males and 56 were females. Most of the positive cases were found in patients belonging to 22 – 27 years age group among males and 58 – 63 years among females. There is also an increase in the number of positive cases with increase in age in the females. Table 2 shows the relationship between the age, sex and type of organism isolated. Streptococcus pneumonia was a common isolate among the age group 16 -21 years whereas Klebsiella pneumoniae & Pseudomonas pneumonia were more common in age group, 22 – 27 years. Staphylococcus aureus was equally distributed in the age group 16 -21 years & middle age group [28 – 39] years.

Klebsiella pneumonia [33.3%] was the predominant isolate followed by Streptococcus pneumoniae [20%], Staphylococcus aureus [17.7%], Pseudomonas aeruginosa [13.3%], Candida albicans [6.6%], Acinetobacter calcoaceticus [4.4%], Moraxella catarrhalis and Citrobacter koseri each by [2.2%]. Out of the 137 patients, 91 [66.6%] were inpatients. The predominant isolate among the inpatients was Klebsiella pneumonia [15.3%] followed by Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumonia, Candida albicans, Moraxella catarrhalis, Acinetobacter calcoaceticus and Citrobacter koseri. Out of the 46 [33.5%] outpatients Streptococcus pneumonia was the predominant isolate. This was followed by 2 species of Pseudomonas aeruginosa and 1 each of Klebsiella pneumonia, Acinetobacter calcoaceticus, and Staphylococcus aureus. [Table 3]

Table 4 shows the predominant symptoms in the patients with sputum culture positive and negative findings. Amongst the sputum culture positive cases the predominant symptoms were fever and cough [with mucopurulent discharge] which was seen in 89.7% and 87.1% of the patients as compared to 47.9% and 52.0% in sputum culture negative cases [i.e. showing growth of commensals of upper respiratory tract]. This findings were found to be highly significant [P = 0.000]. Chest pain & hemoptysis was present in 48.7% & 14.7% of sputum culture positive cases as compared to 32.6% & 18.3% of sputum culture negative cases. These findings were statistically insignificant.

Our results showed high incidence of Klebsiella pneumoniae & Streptococcus pneumonia being isolated between November & February whereas the incidence of Pseudomonas aeruginosa & Staphylococcus aureus was irregular with high number in May & July.

Antibiogram of Staphylococcus aureus showed that 75% of the isolate were sensitive to gentamicin, cefazolin, ciprofloxacin and clindamycin whereas 37.5% strains were MRSA. Klebsiella pneumonia was found to show 80.8% resistance to cefuroxime & 53.3% & 66.6% resistance to gentamicin & amikacin. Acinetobacter calcoaceticus was found to show 100% sensitivity to co-trimoxazole & ciprofloxacin but at the same time was 100% resistance to ampicillin, gentamicin, cefuroxime & cefotaxime each. Pseudomonas aeruginosa showed 83.3% sensitivity to netilimicin,

tobramycin and ceftazidime & ciprofloxacin. Streptococcus pneumonia was found to be sensitive to all the antibiotics used. [Table 5]

DISCUSSION: The commonest LRTI are acute bronchitis, acute trachea bronchitis, chronic bronchitis and pneumonia. According to recent WHO report on the epidemiology of top ten infectious disease, LRTI tops the list in the developing countries and it becomes fourth in developed countries. [13] This study shows only 28.4% growth positive cases. Culture positivity depends on nature of sputum samples, transportation time and the number of organism present in the sample. Other studies have reported 21.8% of the sputum culture positive findings. [13] Some authors on the other hand, have reported growth of pathogenic organisms in 72% of cases. [14]

The National nosocomial infections study consistently reports aerobic gram negative bacilli causes more than 60% of nosocomial pneumonia. [2] In the present study it was found that 55.5% of the isolates that caused LRTI were gram negative bacilli and 37.9% were gram positive cocci whereas in other studies, 88.4% of the isolates were gram negative bacilli with remarkably low isolation of gram positive cocci [2.6%]. [2] The nosocomial fungus [Candida albicans] was isolated in 6.6% of cases. It has been reported that there was simultaneous increase in Candida species in causing LRTI as a result of advance in medical and surgical management. [15]

Gram staining though viewed as a matter of controversy, but has remained a time honored method for sputum samples. If gram staining shows > 25 pus cells and < 10 epithelial cells/low power field, sample is considered adequate for culture. [16] In our study gram staining findings were in correlation with the culture findings in 89.0% of the cases.

It is interesting to note that majority of the pathogens were isolated from males and females of the age range 40 – 50 years, while the least frequent were from patients of the age range 4 – 15 years. Similar findings were reported by other authors where majority of the gram negative bacilli were isolated from adults [32.7%] and elderly [24.3%] patients while the least frequent isolates were from paediatric patients. [17] It has been reported that nosocomial viral infections are more common in the younger age group thereby highlighting the study of viral agents in the younger age group. [17] In North American study prevalence was higher in 0 -4 years [12 – 18/1000] and in the Finnish study it was higher in 2 – 5 years old [i.e. 36/ 1000] and in those aged over 70 years [34/1000]. A more recent study from Mayo Clinic has also shown a rate of pneumonia of 30/1000 for patient aged 65. [18]

The microorganisms isolated most frequently from patients with nosocomial pneumonia were Klebsiella pneumoniae [15.3%], Staphylococcus aureus [7.6%], Streptococcus pneumoniae [5.4%], Pseudomonas aeruginosa [4.3%], and Candida albicans [3.2%] while Streptococcus pneumonia [8.6%], Pseudomonas aeruginosa [4.3%], Staphylococcus aureus [2.1%], Acinetobacter calcoaceticus [2.1%] and Klebsiella pneumonia [2.1%] were isolated most often from patients with community acquired pneumonia. There was significant difference [P = 0.019] in the distribution of Klebsiella pneumoniae in inpatients as compared to the outpatients. Studies elsewhere have found that Streptococcus pneumonia [4.6%], Staphylococcus aureus [10.0%], Haemophilus influenzae [8.0%] caused community acquired pneumonia while Pseudomonas aeruginosa [17.0%], Klebsiella pneumoniae [11.0%] and Staphylococcus aureus [10.0%] were isolated from patients with nosocomial pneumonia. [19] The most frequent mixed infection in our study was between Staphylococcus aureus and Klebsiella pneumonia. Different observation was seen in other studies

where the most frequent mixed infection was between Klebsiella species and Pseudomonas species. Identification of polymicrobial infection is very important for treatment strategies.

Moraxella cataharrhalis is generally considered a commensal in the upper respiratory tract of adults, and its isolation from sputum was reported as normal flora of the oropharynx. This appears to be a misconception as reported elsewhere that this organism is the second most common isolate from patients suffering with LRTI. In the present study a single isolate of Moraxella cataharrhalis was isolated from a female from bronchial asthma with MDR-TB. [20] Studies have reported that Moraxella cataharrhalis was found in lower respiratory tract of patients with chronic bronchitis where the bacteria induced histamine release leading to harmful effects on the airways important for precipitation and exacerbation of chronic bronchitis. [21]

Tuberculosis ranks the most common infections seen in the developing countries. [22] In the present study, out of 137 samples 6 [4.3%] of the patients had been reported to suffer from pulmonary tuberculosis.

A seasonal variation is also identified in the incidence of LRTI in our study. During the winters the number of cases caused by Klebsiella pneumonia & Streptococcus pneumonia started to markup. A seasonal variation was also identified in the incidence of LRTI in UK & India. [13]

Antibiotic resistance is inevitable. Although least resistance of 25.0% to cefazolin, clindamycin, ciprofloxacin and gentamicin was noted in case of Staphylococcus aureus, a highest of 50.0% resistance was shown to amoxicillin and tetracycline. 37.5% strains were found to be MRSA. Klebsiella pneumonia was highly susceptible to ciprofloxacin and tetracycline [each 66.6%], but showed high resistance to cefuroxime & amoxycillin [each 80.0%], & co-trimoxazole [66.6%]. The only isolate of Citrobacter koseri was found to be resistant to all the drugs. In another study, non fermenting gram negative bacilli [NFGNB] showed 32% mean resistance to amikacin. [19] Acinetobacter calcoaceticus was found to be sensitive to co-trimoxazole, ciprofloxacin, but was resistant to ampicillin, gentamicin, cefuroxime and cefotaxime. Similar findings were shown by other authors where multi-drug resistance pattern in Acinetobacter calcoaceticus was 89.19%. [23] This may be due to high chance of acquisition of antibiotic resistance genes and the ability of Acinetobacter species to multiply in the hospital environment. In some studies resistant strains have occurred more frequently in sputum isolates, suggesting emergence of resistance at sites more likely to have poor penetration, and thus sub-inhibitory level of aminoglycosides. [19] Streptococcus pneumoniae was found to be highly sensitive to amikacin, penicillin, erythromycin, tetracycline and cefotaxime. Ciprofloxacin has poor activity against Streptococcus pneumonia; hence it should not used empirically for treating community acquired pneumonia. Frequency of Streptococcus pneumonia exhibiting low sensitivity to penicillin decreased to 34.7% from 46% in 1998. [2] Netilmicin, tobramycin & ciprofloxacin was found to be a better drug against Pseudomonas aeruginosa than piperacillin. Similar findings were reported by other authors. [2] Inspite of the use of media like chocolate agar, H. influenzae was not grown which may be due to inadvertent use of antibiotics in the chronic sufferers which might have eradicated this organism. Similar findings were seen in other Indian studies. [16]

Gram negative organisms were the most common cause of LRTI in our study. Antibiogram suggests that the most common antibiotics that were used as the first choice to treat the LRTI were found to be less effective. Thus this study highlights the need for development of novel drugs. Twenty-five million prescriptions for antibiotics are written each year by general practitioners to

treat respiratory tract infections caused by viruses. This leads to unparallel level of multi-drug resistance among invasive pathogens such as Streptococcus pneumonia. However practical guidelines for a rational approach to the evaluation and treatment of patients with LRTI should be instituted in hospitals in an effort to decrease the overuse of antibiotics and thus prevent the spread of multidrug resistance among invasive pathogens.

The antibiogram pattern in our study, suggested that the gram negative bacteria were highly susceptible to ciprofloxacin. Thus we would like to conclude that the treatment of lower airways infections caused by this pathogen could be done with ciprofloxacin in Sikkim.

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Type of isolate	No. of sputum culture positive samples	[%]
Pure gram positive cocci [GPC]	13	33.3
Pure gram negative bacilli [GNB]	17	43.5
Mixed GPC + GNB	4	10.2
Mixed GNB	2	5.1
Candida albicans	3	7.6
TOTAL	39	100

Table 1: Isolation pattern of different organisms from various samples

Age	K. pneumonia N =15			S. pneumonia N = 9			S. aureus N = 8			P. aeruginosa N = 6			Others N =4			Candida species N =3		
	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
10 – 15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16 – 21	3	0	3	2	1	3	0	0	0	1	0	1	2	0	2	0	0	0
22 – 27	8	0	8	0	1	1	0	0	0	1	0	1	1	0	1	1	0	1
28 – 33	0	0	0	0	1	1	2	0	2	0	0	0	0	0	0	0	0	0
34 – 39	0	0	0	2	0	2	0	2	2	0	0	0	0	0	0	0	0	0
40 – 45	1	1	2	0	1	1	0	1	1	2	0	2	0	0	0	0	0	0
46 – 51	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
52 – 57	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
58 – 63	0	0	0	0	0	0	2	0	2	0	2	2	0	1	1	0	0	0
64 – 69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70- 75	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
76 – 82	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
83 - 88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2

Table 2: Relationship between the age, sex and the type of organism isolated.

Total no. of inpatients	No. [%] 91 [66.4]	Total no. of outpatients	No. [%] 46 [33.5]
Klebsiella pneumonia	14 [15.3]	Streptococcus pneumoniae	4 [8.5]
Staphylococcus aureus	7 [7.6]	Pseudomonas aeruginosa	2 [4.3]
Streptococcus pneumoniae	5 [5.4]	Klebsiella pneumonia	1 [2.1]
Pseudomonas aeruginosa	4 [4.3]	Acinetobacter calcoaceticus	1[2.1]
Candida albicans	3 [3.2]	Staphylococcus aureus	1 [2.1]
Moraxella catarrhalis	1 [1.0]	Candida albicans	0
Acinetobacter calcoaceticus	1 [1.0]	Moraxella catarrhalis	0
Citrobacter koseri	1 [1.0]	Citrobacter koseri	0

Table 3: Total distribution of pathogens in the inpatients and outpatients:

Clinical symptoms	Sputum culture positive cases N = 39	Sputum culture negative cases N = 98	P value
Fever	35	47	0.000
Cough [mucopurulent discharge]	34	28	0.000
Chest pain	19	32	0.079
Hemoptysis	5	18	0.433

Table 4: Predominant clinical symptoms in total population

Antibiotics	S.aureus N = 8	K.pneumonia N = 15	P.aeruginosa N = 6	S.pneumonia N = 9	A.calcoaciticus N =2	C.koseri N =1
	No. of resistant isolates [%]					
Oxacillin	3 [37.5]	-	-	-	-	-
Gentamicin	2 [25]	8 [53.3]	2 [33.3]	-	2 [100]	1 [100]
Amikacin	-	10 [66.6]	3 [50.0]	1 [11.1]	2 [100]	1 [100]
Cefazolin	2 [25]	-	-	-	-	-
Penicillin	-	-	-	1 [11.1]	-	-
Piperacillin	-	-	4 [66.6]	-	-	-
Tetracycline	4 [50.0]	5 [33.3]	-	1 [11.1]	1 [50.0]	1 [100]
Co-trimoxazole	-	10 [66.6]	-	-	0	1 [100]
Clindamycin	2 [25]	-	-	-	-	-
Ciprofloxacin	2 [25]	5 [33.3]	1 [16.6]	-	0	1 [100]
Amoxicillin	4 [50.0]	12 [80.0]		-	1 [50]	1 [100]
Amoxy/calv	-	6 [40.0]		-	0	1 [100]
Cefuroxime	3 [37.5]	12 [80.0]		-	2 [100]	1 [100]
Cephotaxime	-	9 [60.0]	3 [50.0]	1 [11.1]	2 [100]	1 [100]
Ceftazidime	-	-	1 [16.6]	-	-	-
Imipenem	-	8 [53.3]	2 [33.3]	-	0	1 [100]
Carbenicillin	-	-		-	-	-
Netillimicin		-	1 [16.6]	-	-	-
Tobramycin	-	-	1 [16.6]	-	-	-
Erythromycin	3 [37.5]	-	-	1 [11.1]	-	-
Azithromycin	3 [37.5]	-	-	-	-	-

Table 5: Resistance pattern of the pathogens isolated

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Date of Submission: 28/09/2013.
Date of Peer Review: 29/09/2013.
Date of Acceptance: 08/10/2013.
Date of Publishing: 18/10/2013