Original Article

Bacteriological Profile and Antibiotic Susceptibility Patterns of Clinical Isolates of Broncho Alveolar Lavage Fluid (BAL) in Patients with Lower Respiratory Tract Infections (LRTIs)

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Background : Lower Respiratory Tract Infections (LRTIs) are among the most common infectious diseases of humans worldwide. LRTIs produce between 5 and 10% of all deaths reported to the Center for Disease Control and Prevention (CDC) via the 122 Cities Mortality Reporting System. Broncho Alveolar Lavage (BAL) of a lung sub segment samples a large area of alveolar surface and sensitive tool in diagnosing pulmonary infections.

Aims and Objectives : The present study aimed to isolate and identify the bacteria from BAL samples to know the incidence of different conditions in LRTIs.

Materials and Methods : Prospectively BAL samples were collected under aseptic precautions based on protocol from clinically suspected cases of LRTIs who attended the Pulmonology Department ASRAM Hospital and microbiologically processed using standard microbial methods.

Results : Out of 150 cases of LRTIs, incidence of pneumonia was high 61(40.6%). Among 150 cases 120 were bacterial culture positives and among them Gram-positive isolate *Staphylococcus aureus* 40(26.6%) was predominant and among Gram-negative isolate the common isolate was *Klebsiella Pneumoniae* 20(13.3%). In the antimicrobial susceptibility testing, Gram-positive isolates showed maximum sensitivity to Cefotaxime (95%), Ceftriaxone (95%), Cefoxitin (90%), Vancomycin (100%), Amoxicillin (100%). The Methicillin resistant *Staphylococcus aureus* incidence was 10% in the current study. The Gram-negative isolates showed maximum sensitivity of 90% to Cefoperazone/ Sulbactam, 80% sensitive each to Imipenem and Ciprofloxacin and at the same time Pseudomonas species showed 100% sensitive to Piperacillin/ Tazobactam, Carbenicillin, Tobramycin.

Conclusions : Early diagnosis and proper choice of antimicrobials is crucial for management of LRTI cases to reduce morbidity and mortality in the present clinical scenario.

[*J Indian Med Assoc* 2024; **122(4):** 56-60]

***Key words* : BAL, Bacterial Isolate, *Staphylococcus aureus*, Antibiotic Sensitivity.**

Infections of the respiratory tract are an unrelenting and insidious health concern that puts an immense strain on society. Consultation and hospitalization are common causes. Lower Respiratory Tract Infections (LRTIs) are the world's most prevalent human infectious diseases and are responsible for highest morbidity and mortality, including in India. Infections of the lower respiratory tract cause a variety of diseases, from acute bronchitis to cases of pneumonia[1]. For all patients with infectious diseases visiting Outpatient clinics in tertiary care hospitals, LRTIs are responsible for 6 percent and 4.4 percent of hospital admissions[2]. They account for 3%-5% deaths in adult up to the age of 60 years[3]. According to WHO (2004) figures, chronic respiratory diseases account for 4 million deaths annually in India, leading to 5% of global deaths[1]. The burden of chronic respiratory diseases was estimated to account for 4% of the global burden and 8.3% of the burden of chronic diseases in 2005, calculated in Disability-adjusted Life Years (DALY)[1]. In less than 50 percent of patients with pneumonia, sputum culture produces diagnosis[4]. In hospitals with bronchoscopy facilities, samples of Broncho Alveolar Lavage (BAL) can be collected and the relevant bacterial pathogens isolated to direct therapy[1]. Lower Respiratory Tract Infection management is a challenge in terms of reasonable antimicrobial use, especially with regard to a wide range of antimicrobial agents. In addition, the advent of resistance to a wide variety of antibiotics has drawn attention to the need for improved testing

Editor's Comment :

- The emergence of resistant strains poses a major threat to the patients globally, Broncho Alveolar Lavage (BAL) has improved sensitivity and specificity in the diagnosis of pulmonary infections to strat initial empiric therapy, to reduce drug resistance and reduce morbidity and mortality.

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Received on : 24/12/2021

Accepted on : 14/04/2023

techniques and the development of new drugs to allow for much more precise therapy. This knowledge will enable doctors to understand the bacterial pathogens among the different isolates, along with the pattern of antibiotic sensitivity.

The present study was aimed to isolate and identify the bacteria from BAL specimens and to observe the antimicrobial susceptibility testing pattern which will help to control the incident rate of Lower Respiratory Tract Infection so as to reduce the morbidity and mortality of the lower respiratory tract infected patients. The study also helps to know the incidence of different conditions in the LRTIs.

Materials and Methods

This present study was a prospective study conducted in the Department of Microbiology, Alluri Sita Rama Raju Academy of Medical Sciences (ASRAM), Eluru, Andhra Pradesh, India. The total number of 150 bronchial wash (BAL) samples were collected with aseptic precautions based on protocol from clinically suspected cases of LRTIs from both Outpatients (OP) and Inpatients (IP) who attended the Pulmonology Department, GEMS Hospital, Srikakulam from April, 2021 to October 2021, ie, for a period of 7 months. Exclusion criteria includes age younger than 15 years of age, use of anti-platelet drugs, use of anti-vitamin K medications, coagulation failure, opposition from patient, bronchospasm.

The protocol was approved by the ethics review committee of the hospital. Review of clinical records patient demographics (gender, age and smoking status) and clinical characteristics (co morbidities, symptoms and physical signs) were obtained and reviewed. The bronchoscopy was performed by the physician by fiber optic flexible bronchoscopy and transported to microbiology lab immediately. Samples were carefully observed for consistency, specific color and odour. All the samples were cultured on MacConkey agar, sheep blood agar and chocolate agar. Plates were incubated at 37°C overnight. The next day, the growth on the petridishes was observed and the bacterial isolates were identified according to standard protocol (as shown in Figs 1-7). For aerobic bacterial isolates, identification was done by culture 10^4 CFU/ml. Sensitivity to various antibiotics was assessed using the disk-diffusion method by Kirby-Bauer disc diffusion method.The procedure was performed according to CLSI guidelines (CLSI Catalogue, 2016).

Results

Out of 150 cases of LRTI majority of cases 65(43.33%) belong to age group ie, 45 to 59 years of age and 112 (74.67%) were males. Majority of cases 116 (77.3%) belong to rural area. Out of 150 cases of LRTIs, incidence of pneumonia was high 61(40.6%).

Fig 1 — Bacterial growth on different culture media (Nutrient Agar, Blood Agar, Mac Conkey Agar)

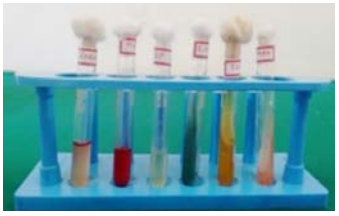

Fig 2 — Bio-chemical Reactions of *Escherichia coli*. Indole +ve, MR +ve, VP –ve, Citrate –ve, TSI A/A with Gas, Urease –ve

Fig 3 — Bio-chemical reactions of *klebsiellapneumoniae* from left to right – sugar fermentation of glucose, lactose, sucrose, mannitol with the production of acid and abundant Gas, nitrate reduction, indole –ve, MR -Ve, VP +ve, citrate +ve,urease +ve, TSI – K/A with gas

Fig 4 — Identification of *pseudomonas* spp

Fig 6 — Identification of *Streptococcus Pneumoniae*

Fig 5 — Growth And Identification of *Staphylococcus Aureus*

Fig 7 — Positive Bile Esculin Test for *Enterococcus spp*

Smoking was found to be the most Common risk factors among all cases. Among different groups of lower respiratory tract cases incidence of bacterial culture positives was highest in pneumonia cases 48(40%) followed by COPD 32 (26.6%). Among 150 cases 120 were bacterial culture positives, predominance isolate was *Staphylococcus aureus* 40 (26.6%), followed by *Streptococcus Pneumoniae* 24 (16.0%), *Klebsiella Pneumoniae* 20(13.3%), Pseudomonas species 15 (12.5%), Enterococcus species 11 (7.3%), *Escherichia coli* 10(6.6%) (Table 1). Antimicrobial resistance among Gram-positive and Gram-negative organisms in isolates from BAL fluid was shown in Tables 2 & 3 and Figs 8&9 respectively.

Table 1 — *Spectrum of bacterial isolates from BAL fluid*

Types of organism isolated	Male n= 85	Female n=65	Total n=150
Staphylococcus aureus	28	12	40(26.6%)
Streptococcus pneumonia	12	12	24(16.0%)
Enterococcus spp.	5	6	11(7.3%)
Klebsiella Pneumoniae	14	6	20(13.3%)
Pseudomonas species	10	5	15(12.5%)
Escherichia coli	7	3	10(6.6%)

Table 2 — *Antimicrobial sensitivity pattern of Gram-positive isolates from BAL fluid*

Enterococcus species (n = 11)	*Streptococcus pneumonia (n= 24)*	*Staphylococcus aureus (n =40)*		Name of organisms isolate
NT	NT	39(90%)	S	Cefoxitin
NT	NT	4(10%)	R	
NT	NT	35(87.5%)	S	Azithromycin
NT	NT	5(12.5%)	R	
9(81.8%)	NT	23(57.5%)	S	Ciprofloxacin
2(18.18%)	NT	17(42.5%)	R	
NT	21(87.5%)	38(95%)	S	Ceftriaxone
NT	3(12.5%)	2(5%)	R	
NT	20(83.3%)	38(95%)	S	Cefotaxime
NT	4(16.6%)	2(5%)	R	
NT	23(95.8%)	NT	S	Amoxyclav
NT	11(4.1%)	NT	R	
10(90%)	NT	36(90%)	S	Amikacin
1(9%)	NT	4(10%)	R	
8(72.7%)	22	NT	S	Pencillin
3(72.27%)	2(8.3%)	NT	R	
NT	18(75%)	NT	S	Trimethoprim/
NT	6(25%)	NT	R	sulfamethaoxazole
11(100%)	NT	NT	S	Amoxycillin
0(0%)	NT	NT	R	
11(100%)	NT	NT	S	Vancomycin
0(0%)	NT	NT	R	
7(63%)	19(79.1%)	NT	S	Erythromycin
4(36%)	5(20.8%)	NT	R	

Table 3 — *Gram-negative organism's antibiotic sensitivity and resistance pattern*

Pseudomonas (n=15)	E. Coli. (n=10)	Klebsiella Pneumoniae (n=20)		Name of organisms isolated
NT	8(80%)	13(65%)	S	Cefotaxime
NT	2(20%)	7(35%)	R	
13(86.6%)	7(70%)	15(75%)	S	Gentamycin
2(13.3%)	3(30%)	5(25%)	R	
NT	8(80%)	16(80%)	S	Ciprofloxacin
NT	2(20%)	4(20%)	R	
14(93.3%)	9(90%)	16(80%)	S	Imipenem
1(6.6%)	1(10%)	4(20%)	R	
NT	9(90%)	18(90%)	S	Cefoperazone/
NT	1(10%)	2(10%)	R	Sulbactam
15(100%)	NT	NT	S	Carbenicillin
0(0%)	NT	NT	R	
15(100%)	NT	NT	S	Tobramycin
0(0%)	NT	NT	R	
13(86.6%)	8(80%)	16(80%)	S	Ceftazidime
2(13.3%)	2(20%)	4(20%)	R	
15(100%)	NT	NT	S	Piperacillin/Tazobactam
0(0%)	NT	NT	R	

Discussion

Due to their prevalence and economic effects and a significant cause of mortality and morbidity worldwide, Lower Respiratory Tract Infections are a public health problem in both developed and developing countries. This research was conducted to test infectious bacterial agents in patients with LRTIs and to determine their susceptibility to various antibiotics. In this study, majority of cases 65(43.33%) belong to age group 45-59 years with male predominance which correlated with the study conducted by Tripathi Purti, *et al* (2014)[5] and Dey, *et al* (2007)[6] and reason might be due to age related physiological changes, reduced immunity, malnutrition. Majority of the cases belong to rural area compare to urban area which is similar to Agnihotram, *et al* (2005)[7] and might be due to lack of knowledge of the occurrence of infections of the lower respiratory tract with their lifestyle in unhealthy areas, overcrowding, indoor pollution exposure, soil-cooking fuels, poor sanitation conditions. The present study yielded positive bacterial BAL cultures in 80% of the cases of LRTIs, incidence of bacterial culture positives was highest 48(40%) in pneumonia cases followed by

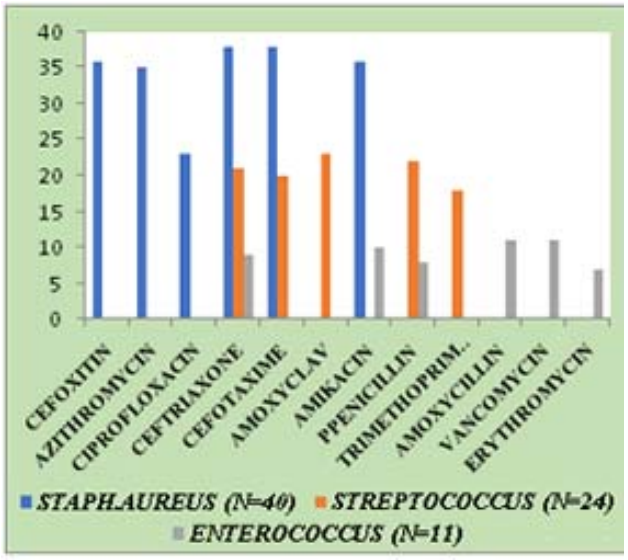

Fig 8 — Antimicrobial sensitivity pattern of Gram-positive Organisms

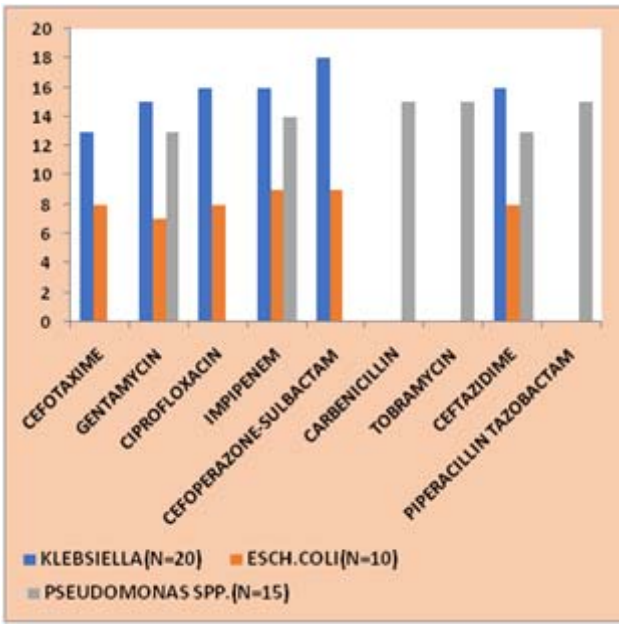

Fig 9 — Antimicrobial sensitivity pattern of Gram-negative Organisms

COPD 32 (26.6%) which was similar to Goto, *et al* (2006)[8] (50.8%) in pneumonia. Out of 120 bacterial isolates among Gram-positive bacteria, *Staphylococcus aureus* was highest 40(26.6%) followed by *Streptococcus Pneumoniae* 24(16.0%) and Enterococcus species 11(9.1%). Other studies Bajpai, *et al* (2013)[9] (3.55%), Goto, *et al* (2006)[8] (20.9%), Moisoiu, *et al* (2007)[10] (54.1%), Abdul Kashmet, *et al* (2014)[11] (20.6%) also showed *Staphylococcus aureus* as most common isolated organism, reason might be due to multidrug resistant *Staphylococcus aureus*, high rate of carriers for Staphylococcus among hospital staffs and nosocomial infections. But it is dissimilar to study done by Latabaswanna and Pradnyashankar (2015)[12] where *Streptococcus pneumoniae* is the dominant isolate. Among Gram-negative organisms, the isolation of *Klebsiella pneumoniae* was high 20(16.6%) followed by Pseudomonas Species 15(12.5%), *E coli* 10(6.6%) but other studies Bajpai, *et al* (2013)[9], Latabaswanna and Pradnyashankar, *et al* (2015)[12] showed Pseudomonas Species as most common which is dissimilar to this study. Another study Abdul Kashmet, *et al* (2014)[11] showed equal number of Klebsiella species and Pseudomonas species. Klebsiella is a part of normal flora of the mouth and most widely associated with pneumonia in a hospitalized patients and elderly. Hence, its predominance may be related to more elderly population in our study. Among Gram-positive antibiogram *Staphylococcus aureus* more sensitive to cefotaxime (95%), ceftriaxone (95%), cefoxitin (90%) followed by amikacin (90%) Azithromycin (87.5%) and low sensitivity rate was seen in ciprofloxacin (57.5%). In this study, methicillin resistant *Staphylococcus aureus* was 10% similar to study done by Kitara Anywar, *et al* (2011) [13], Maciel, *et al* (2012)[14], Masood and Nousheen, *et al* (2010)[15], Manikandan and Amsath, *et al* (2013)[16], but not similar to study done by Falcone, *et al* (2002)[17] where methicillin resistance was 30%. In this study *Streptococcus Pneumoniae* showed more sensitivity to Amoxycillin/Clavulanate (95.8%), Penicillin (91.6%), Cefuroxime (87.5%), cefotaxime (83.3%) highest resistance was seen in Trimethoprim/ sulfamethoxazole (25%) similar to Tribuddharat, *et al*. (2008)[18], Naaber, *et al* (2006)[19]. Enterococcus species showed 100% sensitive to Amoxicillin and Vancomycin dissimilar to study done by Oncu, *et al*[20] (2004), where Enterococcus showed maximum resistance to Vancomycin. Among Gram-negative antibiogram *Klebsiella Pneumoniae* showed 90% Cefoperazone/ Sulbactam, 80% sensitive each to Imipenem and Ciprofloxacin,75% sensitive to Gentamycin similar to study done by Ravi Chitra, *et al* (2014)[21], Archana Singh, *et al* (2011) [22] which shows high sensitive to Quinolones, Aminoglycosides, Amikacin, Gentamycin. *Escherchia coli* isolates showed maximum sensitivity to Imipenem, Cefoperazone-Sulbactam, Cefotaxime similar to Kibert, *et al* (2011)[23], Kmar, *et al* (2015)[24] but dissimilar to Chaudhary, *et al* (2012)[25] which shows low sensitive to Imipenem, Cefoperazone/Sulbactam, Gentamycin.in this study Pseudomonas species showed 100% sensitive to Piperacillin/Tazobactam,

Carbenicillin, Tobramycin, 93.3% sensitive to Imipenem which was similar to study done by VireJaviya, *et al* (2008) [26], Rakesh Kumar, *et al* (2015)[27] but dissimilar to Latabaswanna and Pradnyashankar, *et al* (2015)[12] showing maximum resistance to Piperacillin/Tazobactam.

Conclusion

So based on the observations in this study it was customary for pulmonologist that all the clinically suspected LRTI cases attending the Department of Pulmunology should be tested with patients BAL specimens by Bronchoscopy and those specimen should be processed in the Microbiology Department by various microbiological diagnostic tests for the confirmation of LRTI as observed in this study so as to start early specific treatment with specific antibiotics to treat LRTI cases to reduce morbidity and mortality.

Conflict of Interest : The authors declared no conflict of interest

Sources of Support: The work was not supported by any funds

Institutional Review Board Statement : This study was reviewed and approved by The Ethics Committee of ASRAM hospital, Eluru, Andhra Pradesh, India where the study was conducted

References

1 George B, Ronald, light W, Richward, Matthau A, Michael, *et al* — Chest medicine: essential of Pulmunory and critical care medicine. 3rd edition. Elsevier publications. 2007; 423-27.

2 Hosker RSH, Jones GM, Hikey — Review: management of community acquired lower respiratory tract infection. *BMJ* 1994; **308:** 701-5.

3 Mandell SF, Dowell JG, Caroll DR, *et al* — Antibiotics and respiratory illness. *BMJ* 1974; **III:** 125-9.

4 Arancibia F, Ewig S, Martinez JA, Ruiz M, Bauer T, Marcos MA, *et al* — Antimicrobial treatment failures in patients with community-acquired pneumonia: Causes and prognostic implications. *Am J Respir Crit Care Med* 2000; **162:** 154-60.

5 Tripathiputi C, Kiran D — Lower respiratory tract infections: current etiological trends and antibiogram. *J Pharm Biomed Sci* 2014; **4(3):** 249-55.

6 Dey A — Incidence of multi drug resistant organisms causing ventilator associated pneumonia in a tertiary care hospital: A nine months prespective study. *Ann Thoracic Med* 2007; **2(2):** 52-7.

7 Agnihotram VR — Respiratory Disease Burden In Rural India: A Review From multiple data sources 2005; 357.

8 Goto H, Takeda — Susceptibility of bacterial isolates from patients with lower respiratory tract diseases to antibiotics. *Jpn J Antibiotics* 2006; **59(5):** 353-8.

9 Bajpai T, Shrivastav G — Microbiological profile of lower respiratory tract infections in neurological intensive care unit of a tertiary care center from central India. *J Basic Clin Phram* 2013; **4(3):** 51-5.

10 Moisoiu A, Dorobat OM — Incidence and resistance patterns of pathogens from lower respiratory tract infections (LRTI). 2007; **56(1);** 7-15.

11 Mannan AM, Kashem AM — Microbiological profile of lower respiratory tract infections in intensive care unit of a tertiary care cente of Dhaka, Bangladesh. *Crit Care J* 2014; **2(2):** 53-6.

12 Latabaswannagalate, Gajbhiye PS — Microbiological profile and antibiogram pattern of lower respiratory tract infection. 2015; **3(4):** 1-6.

13 Kitara LD, Anywar AD — Antibiotic susceptibility of *Staphylococcus aureus* in suppurative lesions in Lacor hospital, Uganda. *Afr Health Sci* 2011; **11 suppl 1:** S34-9.

14 Maciel MA, Caraciolo FB — Antimicrobial resistance profile of *Staphylococcus aureus* isolates obtained from skin and soft tissues infections of outpatients from a university hospital in Recife–PE, Brazil. *An Bras Dermatol* 2012; **87(6):** 857-61.

15 Syed HM, Nousheenaslam — In vitro suspectibity test of different isolates against Ceftriaxone. *Oman Med J* 2010; **25(3):** 199-202.

16 Mnikandan C, Amsath A — Antibiotic susceptibility of bacterial strains isolated from wound infection patients in Pattukkottai, Tamilnaidu, India. 2013; **2(6):** 195-203.

17 Falcone M, Carfagna P — *Staphylococcus aureus* sepsis in hospitalized non neutropic patients: retrospective clinical and microbiological analysis. *Ann Ital Med Int* 2002; **17(3):** 166-72.

18 Srifuengfung S, Tribuddhart C — Antimicrobial susceptibility of *Streptococcus Pneumoniae* isolated from patients with respiratory tract infections in Thailand. *Southeast Asian J Trop Med Public Health* 2008; **39(3):** 461-6.

19 Naaber AP — Antimicrobial susceptibility of common pathogens from community acquired lower respiratory tract infections in Estonia 2006; **18(6):** 603-9.

20 Oncu S, Punar M — Susceptibility patterns of Enterococci causing infections. *Thokou Journal Med* 2004; **202:** 23-9.

21 Ravichitra KN, Kumari HP — Isolation and antibiotic sensitivity of *Klebsiellae Pneumoniae* from pus, sputum and urine samples 2014; **3(3):** 115-9.

22 Singh AS, Vardhanbatra H — Prevalence of antimicrobial drug resistance of *Klebsiellae Pneumoniae* in India. *International Journal of Bioscience, Biochemistry and Bioinformatics* 2011; **1(3):** 1-6

23 Kibert M, Abera B — Antimicrobial susceptibility patterns of *E Coli* from clinical sources in Northeast Ethiopia. *Afr Health Sci* 2011; **11(supp l1):** s40-s45.

24 Kumar D, Singh AK — Antimicrobial susceptibility pattern of extended spectrum beta-lactamase producing *Klebsiella pneumoniae* clinical isolates in an Indian Tertiary Care Hospital 2015; **41(31):** 153-9.

25 Chaudhary M, Payasi A — Prospective study for antimicrobial susceptibility of *EscherchiaCcoli* isolated from various clinical specimens in India. *J Microb Biochem Technol* 2012; 157-60.

26 Vire A, Javiya, Somsurva B, Ghatak — Antibiotic susceptibility patterns of *Pseudomonas Aeruginosa* at a tertiaty care hospital in Gujrat, India. *Indian J Pharmacol* 2008; **40(5):** 230-4.

27 Kumar R, Srivastav P — Detection and antimicrobial susceptibility pattern of *Pseudomonas Aeruginosa* isolates in various clinical samples with special references to Metallo Beta Lactamase from a tertiary care hospital in Jaipur, India. *Nat J Med Res* 2014; **4(2):** 128-13.