

Original Article

NOSOCOMIAL INFECTION BY NON-FERMENTING GRAM NEGATIVE BACILLI IN TERTIARY CARE HOSPITAL: SCREENING AND CURE

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ABSTRACT

Objective: The purpose of the present study was to identify the Non-Fermenting Gram Negative Bacteria from the tertiary care hospital.

Methods: The conventional method was used i.e. Antibiotic susceptibility test which was performed by Kirby-Bauer disc diffusion method and analysis of microbes was done by Vitek-2.

Results: 172 samples were identified as Non-Fermenting Gram-Negative Bacilli from 1526 respiratory samples. *Acinetobacter baumannii* was the predominant isolate accounting 103 (59.88%) specimens followed by *Pseudomonas aeruginosa* 33.13%. Other isolates were *Stenotrophomonas maltophilia* 5.23% and *Burkholderia cepacia* 1.74%. From the 172 samples, Endotracheal Aspirate was found to be the most infected amongst all the other samples. *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were the most common NFGNB isolated in our study from patients of Respiratory Tract Infections.

Conclusion: Both *Acinetobacter baumannii* and *Pseudomonas aeruginosa* showed good sensitivity to colistin, amikacin, cefoperazone while in most cases carbapenem is found to be highly resistant. Colistin along with amikacin and cefoperazone should be used against this pathogen for treatment.

Keywords: Nosocomial infection, Gram negative bacilli, Tertiary care hospital, Lower respiratory tract infection, Non-fermenting bacilli

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INTRODUCTION

Lower Respiratory Tract Infection (LRTI) is the fastest leading causes of the morbidity and mortality in the world. It isn't a single disease but a group of specific infection each with a different epidemiology, pathogenesis, clinical presentation and outcome. The etiology and symptomatology of these diseases vary with age, gender, season, the type of population at risk, etc. These are commonly the first infections to occur after birth and pneumonia is too often the final illness to occur before death [1]. Infections of the Lower respiratory tract are caused in 4.4% of all hospital admissions and 6% of all another medical practitioner. They are a cause for 3 to 5% of deaths in adults. The problem is much greater in developing countries where pneumonia is the most common cause of hospital attendance in adults [2]. In India, acute lower respiratory tract infection (ARI) is causes one million deaths. There is inadequate information from developing countries like India on various lower respiratory tract bacterial pathogens and their resistance patterns in hospitals. In addition, the emergence of resistance as a major problem has drawn attention to a need for better diagnostic techniques and newer drugs to allow more specific therapy [3]. The etiologies of respiratory infections play a significant role in the decision making, as they concern the choice of antibiotics, isolation and hospitalization measures. A variety of organisms are usually implicated in their etiologies; the most common ones is gram negative bacteria, followed by gram positive organisms [4]. The dramatic rise in the antimicrobial resistance among the respiratory pathogens may be due to the prophylactic administration of antibacterial therapy even before the availability of the culture results [5]. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are aerobic gram-negative bacilli that are glucose fermenting bacteria present everywhere in the environment. These bacteria are emerging opportunistic organisms causing a wide variety of nosocomial infections such as wound infection; ventilators associated pneumonia, bloodstream infection, intensive care unit infection and urinary tract infection. In a hospital environment, these non-fermenting gram-negative bacilli affect seriously ill patients by interfering with the treatment and may also lead to

septicaemia. The cause of infection could be the hospital environment i.e. mainly device related infections which are often resistant to disinfectants. Devices have the potential to spread the infection from patient-to-patient via fomites or hands of medical personnel along with that they spread from soap cakes for hand washing in hospitals or through the dust on the baby weighing machines [6]. These microorganisms have the ability to grow in a wide range of environmental conditions so can grow in poor nutritional conditions, even in distilled water, extreme temperatures, etc. The water supply in hospitals may be an important source for colonization and infection in susceptible patients [7]. The rapid acquisition of a wide variety of antibiotic resistance genes, as well as their ability to survive in various harsh environments that has caused difficulties in the control and eradication of the pathogen [8]. This extreme rapid development of resistance has caused serious therapeutic problems worldwide [8, 9]. Resistance to β -lactams in *P. aeruginosa* and *A. baumannii* is most commonly associated with the production of high levels of naturally produced cephalosporinase (Amp C) [10]. The major reason of multi-drug resistance is due to the mutations in their outer membrane porins resulting in reduced permeability to antimicrobials and overexpression of multidrug efflux pumps [10]. Carbapenems are often agents of last resort. Thus, the emergence of carbapenem resistance in *P. aeruginosa* and *A. baumannii* is of particular concern. Carbapenem resistance is primarily caused by two mechanisms, either by reducing intracellular concentration or by hydrolysis of the drug [11]. Reduced intracellular carbapenem concentration is the most common mechanism of imipenem resistance due to the loss of or reduced expression of the outer membrane porin, Opr D. Different mechanisms may have implications with respect to cross-resistance, combination therapy, and the emergence of resistance. Penicillin-binding proteins all may contribute to carbapenem resistance.

The purpose of present study was to identify Non-Fermenting Gram Negative Bacteria from tertiary care hospital. This study was done by using a standard conventional method that is antibacterial susceptibility test, and another is VITEK method.

MATERIALS AND METHODS

Source of sample collection

1526 samples from the clinically suspected cases of respiratory infections were collected from both the sexes and from all age groups that were admitted in the wards, ICU and from the outpatient department. The respiratory samples which were received in the Microbiology Laboratory it includes Sputum, Endotracheal Aspirate, Tracheal Aspirate, Bronchoalveolar Lavage. Tracheal or endotracheal suctioning may be used to collect secretions for detection of the etiological agent of a lower respiratory tract infection. Aseptic conditions were strictly maintained throughout the examinations.

Identification and isolation of pure bacteria isolate

Respiratory tract samples were plated on Blood agar, Chocolate agar and Mac Conky agar. They were allowed to incubate at 37 °C for 24 h. The numbers of bacterial isolates were counted. Colony morphology, hemolytic pattern, Gram reaction and microscopic features were used as primary identification criteria. Biochemical tests were performed for identification for gram-negative bacteria [12].

Antibiotic susceptibility testing

Antibiotic susceptibility test of the bacterial isolates was performed according to the criteria of Clinical and Laboratory Standards Institute [13] using the Kirby-Bauer disc diffusion method on Muller-Hinton Agar (MHA). Isolates were grown in peptone water at 37 °C and turbidity were matched with 0.5 McFarland standards. The lawn culture was done on Mueller-Hinton agar plate, and antibiotic discs were placed. The plates were incubated at 37 °C overnight, and the zones of inhibition were observed according to CLSI guidelines. In Kirby-Bauer Method, tips of 4-5 colonies of the isolated organism were picked up with straight wire, suspended in 2 ml sterile saline. The density of the suspension to be inoculated was measured by comparison with a turbidity standards, Mac Farland standard 0.5/turbidometer. Plates were dried for about 15-30 min in the incubator. Using a sterile swab, the inoculums were inoculated into the MHA plate by streaking in three different directions. After 15 min antibiotics were placed on the inoculated plates and allowed to incubate overnight 37 °C. Number of discs to be put in one plate. Inhibition zone was observed against antibiotics and expressed in sensitive (S), resistance (R) or intermediate (I). A significant decrease of antibiotic susceptibility was observed.

RESULTS

Clinico Microbiological data from the "Tertiary Care Setup" has shown that 172 strains of NFGNB accounting for an isolation rate are 11.27%. The clinical specimen included Endotracheal Secretion, Tracheal Secretion, Sputum and Bronco-alveolar Lavage has shown isolation rate of 67.44%, 15.11%, 9.30% and 8.72% respectively. *Acinetobacter baumannii* was the predominant isolate accounting for the isolation rate of 59.88% and specimens followed by *Pseudomonas aeruginosa* that has shown isolation rate of 33.13%. Other isolates from various samples were *Stenotrophomonas maltophilia* with an isolation rate of 5.23% and *Burkholderi acepaciaas* 1.74% as shown in fig 1, 2 and 3. Gender wise observation revealed that males were more susceptible to the infection as compared to females. Data as shown in fig. 4 says isolation rate of 69.76% of the patients accounted for males, and 30.20% were females and out of total 62.79 % patients recovered from the infection so were discharged from the hospital. Whereas 37.21% patients died because of the infection. These isolates were found to be maximum during the stay of patient 0-10 d i.e. 39.53% patients followed by duration of stay of more than 30 d i.e. 22.09% patients were infected with NFGNB. While the duration of 10-20 d 20.34% were suffering from the infection. The duration of 20 to 30 d has shown 18.02% got the infection as shown in fig. 5. The majority of the patients i.e. 35.46% were found to be adult aged between 45-60 y. While patients from age groups 0-15 and 60-75 y were similar i.e. 33 patients each group accounting for 19.18%. Patients with age groups 15-30 y and >75 y account 7.5% patients each. Whereas patients aged between 30-45 y account 11.04% patients as shown in fig. 6.

Biochemical tests

Catalase, Coagulase, Oxidase, Citrate, and Indole test were observed, and the tests were found to be negative for the samples.

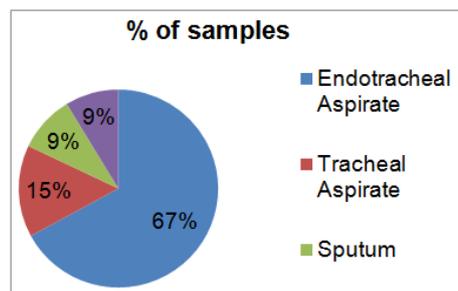


Fig. 1: Percentages of received respiratory samples

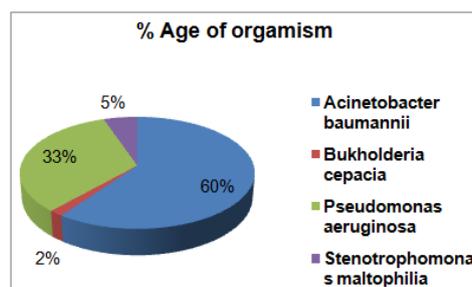


Fig. 2: Percentage of occurrence of the microbial isolates respectively

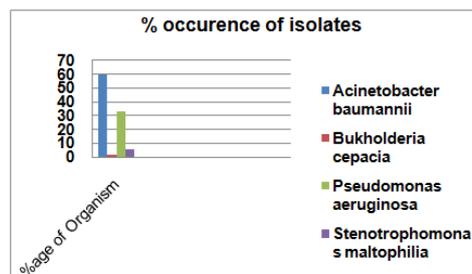


Fig. 3: Graphical representation of percent occurrence of isolates

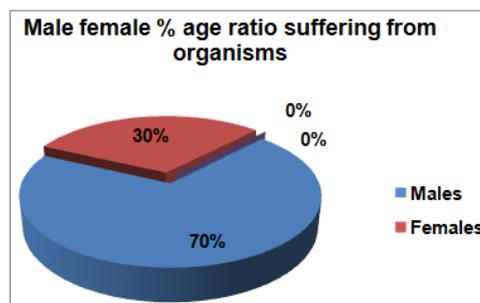


Fig. 4: Percentage of infected males and females

Disc diffusion method

A significant decrease of antibiotic susceptibility was observed. The most active antibiotics against *Acinetobacter baumannii* were

colistin accounting almost 98% sensitive followed by amikacin 60% sensitive. While, *Pseudomonas aeruginosa* was found to be susceptible to colistin 100%, amikacin 70% and cefoperazone 50%. In some specimens carbapenems (imipenem and meropenem) has shown susceptibility while in most of the cases was found to be resistant. A few specimens were found to be multidrug resistant. The other method was also considering i.e. VITEK-2 which was used for both identifications as well as susceptibility toward antibiotics. A bacterial suspension was adjusted to a McFarland standard of 0.5 in 2.5 ml of a 0.45% sodium chloride solution with a VITEK 2 DensiChek instrument. Data were analyzed using VITEK-2 database version 4.01, which allowed us for organism identification in the kinetic mode after 2 h of incubation.

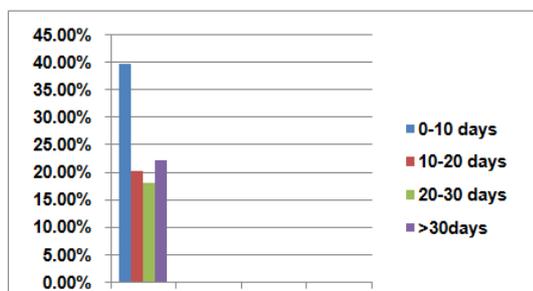


Fig. 5: Percentage of infection as per duration of stay

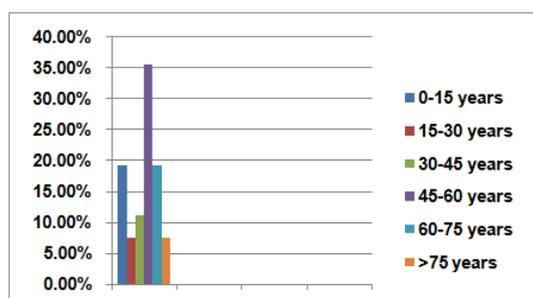


Fig. 6: Percentage of patients in different age groups

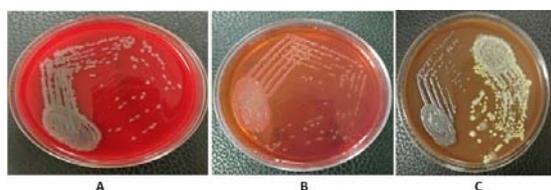


Fig. 7: Quadrant streaking of NFGNB isolated on blood agar (A), macConkey agar (B) and chocolate agar (C)

DISCUSSION

Non-Fermenting Gram-Negative Bacilli were considered to be as a contaminant in the past, but new emerges of the strain has shown an important impact on the health care facilities [14]. In the present study, the most common isolate was *Acinetobacter baumannii*. Whereas in previous studies similar results had been reported [15]. *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are known to be the common nosocomial pathogens [14] by many scientific observers. *Acinetobacter baumannii* and *Pseudomonas aeruginosa* account for 93.02% of the isolates in the present study. A similar result has been reported from Kolar that 87% of all isolates comprises of *Pseudomonas* and *Acinetobacter species* [16]. Out of 172 respiratory samples, Endotracheal Secretion has shown maximum infection at the rate of isolation i.e. 67.44% whereas Treacheal Secretion has shown 15.11% isolation rate. Sputum and Bronco-

alveolar Lavage has shown isolation rate of 9.30% and 8.72% respectively. Similarly from the previous study of Gujarat, out of 268 samples, 101 and 167 samples were tracheal secretion and sputum samples respectively yielded NFGNB [17]. Similarly, in the previous study from Kolar states, it was observed that 24 respiratory tract samples i.e. 13 sputum samples, and 11 endotracheal aspirate samples yielded NFGNB [16]. In the present study, 69.76% of the patients were males while 30.20% were females. Similar trends could be seen from the previous study of Eastern India, where 55% patients were males and 45% were females [15]. While a study from Rotak has revealed male to female ratio was 1:1 [18]. In the present study, 62.79% patients recovered and were discharged while 37.21% died. NFGNB are either free-living saprophyte where they were found to be reported as emerging pathogen in human. NFGNB were found to be present in large numbers, so were likely to release large endotoxin content and may have disastrous effect on the host. The cause of infection could be the hospital environment, mainly device related infections which are often resistant to disinfectants and have the potential to spread from patient-to-patient [19-26]. In our present study majority of the patients i.e. 35.46% were an adult aged between 45-60 y. This was found to be similar to a study of Eastern India where 72% of the patients were adults aged above 45 y [14]. Intake of steroids and other immune suppressive therapies could be the cause of low immunity thus a cause of infection in this age group. The maximum duration of stay of the patient was 0-10 d i.e. 39.53% patients followed by duration of stay of more than 30 d i.e. 22.09% patients were infected with NFGNB. While, when the duration of stay was 10-20 d 20.34% were suffering from the infection and when the duration was 20 to 30 d 18.02% got the infection. Similar results have been found in a study of Madras where the duration of stay was 1-3 d, 4-7 d and >7 d and NFGNB were isolated from 24%, 20% and 47% patients respectively [27]. The real cause of the infection was yet not clear but may be due to the destruction of respiratory epithelium by other microbes such as viruses allowed increased adherence of bacteria to the infected cells by viruses. The antibiotic susceptibility pattern in the present study by colistin has shown the sensitivity of 98%, whereas amikacin has shown 60% and Cefoperazone 22%. Many strains have shown multidrug resistance. In some cases, Carbapenems has shown sensitivity but in the majority of the cases was found to be resistant. Gram-negative bacteria produce ESBL enzymes that are produced by *Enterobacteriaceae* family that hydrolysis beta-lactam rings produced by beta-lactam antibiotics that yield them inactive. These have become a worldwide problem leading to high mortality rates and increased duration of stay [28]. ESBLs inactivate broad-spectrum cephalosporins and beta-lactamases, and they are associated with beta-lactam resistance and frequent resistance to fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole [28, 29]. This could be because of decreased outer membrane permeability, increased efflux systems, and inhibitors produced by the microbes. This was in concordance with the report of Utrakhand where carbapenem resistance had been reported [30]. This was also found to be observed in a similar study of Kolar, where antibiotic sensitivity pattern was mentioned [16]. It was found from few previous studies that they aimed to find the ratio of Gram-negative bacilli due to antibiotic resistance to the patient compared to the patient-to-patient transmission had been conducted in non-outbreak settings, with a significant [31]. It was also provided evidence that some strains of *Klebsiella* are transmitted in ICU patients [32]. Connection to a ventilator, history of invasive procedures, steroids, and immunosuppressive therapy and underlying disease accounted for a significant decrease of antibiotic activity [17].

CONCLUSION

We, authors, would like to conclude from the above study that NFGNB which are regarded as a contaminant, are important pathogenic bacteria cause a wide range of nosocomial infections. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the most common NFGNB isolates from our present study. So this study requires an urgent need to identify & restrict these isolates and antibiotic susceptibility test for appropriate treatment. Prevalence of NFGNB varies between communities, hospitals in the same community and among different patient populations in the same

hospital. It is important for clinicians to be updated with the occurrence and antibiotic susceptibility pattern of the pathogens. These pathogens have a great potential to survive in a hospital environment so improved antibiotic susceptibility and infection control measures will be required to prevent or slow down their emergence and spread. Standard infection control practices should be used for MDR Gram-negative infections. Hand hygiene is essential when one comes into contact with patients, their secretions, and the environment. Patients should be isolated, and the use of gowns and gloves is recommended. Continuous awareness is needed to maintain good housekeeping, equipment decontamination and strict attention to hand washing. Emphasis must be laid on multi drug resistant species of NFGNB for further treatment and prevention of the disease.

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CONFLICT OF INTERESTS

Declared none

REFERENCES

- Mishra SK, Kattel HP, Acharya J, Shah NP, Shah AS, Sherch JB, Rijal BP, et al. Recent trend of bacterial aetiology of lower respiratory tract infections in a tertiary care center of Nepal. *Int J Infection Microbiol* 2012;1:3-82.
- Panda S, Premanadini B, Ramani TV. Lower respiratory tract infection-bacteriological profile and anti-biogram pattern. *Int J Curr Res Rev* 2012;4:149-55.
- Srivastav P, Kumar P, Nirwan PS, Sharma M. Bacteriological profile and antibiogram pattern of lower respiratory tract infections in a tertiary care hospital in Northern, India. *Int J Pharm Res Bio-Sci* 2013;2:225-33.
- Goel N, Chaudhary U, Aggarwal R, Bala K. Antibiotic sensitivity pattern of gram-negative bacilli isolated from the lower respiratory tract of ventilated patients in the intensive care unit. *Indian Soc Critical Care Med* 2009;13:148-515.
- Olugbue V, Onuoha S. Prevalence and antibiotic sensitivity of bacterial agents involved in lower respiratory tract infections. *Int J Biol Chem Sci* 2011;5:774-81.
- Fiorillo L, Zucker M, Sawyer D, Lin AN. The pseudomonas hot-foot syndrome. *N Engl J Med* 2001;345:335-8.
- Blanc DS, Nahimana I, Petignat C. Faucets as a reservoir of endemic *Pseudomonas aeruginosa* colonization/infections in intensive care units. *Intensive Care Med* 2004;30:1964-8.
- Thuong M, Arvaniti K, Ruimy R. Epidemiology of *Pseudomonas aeruginosa* and risk factors for carriage acquisition in an intensive care unit. *J Hospital Infection* 2003;53:274-82.
- Crespo MP, Woodford N, Sinclair A. Outbreak of carbapenem-resistant *Pseudomonas aeruginosa* producing VIM-8, a novel metallo-beta-lactamase, in a tertiary care center in Cali, Colombia. *J Clin Microbiol* 2004;42:5094-101.
- Jacoby GA. Amp C β -Lactamases. *Clin Microbiol Rev* 2009;22:161-82.
- Venezia SN, Ben-Ami R, Carmeli Y. Update on *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infections in the healthcare setting. *Curr Opin Infect Dis* 2005;18:306-13.
- Cappuccino JG, Sherman N. Techniques for isolation of pure culture. In: *Microbiology: a laboratory manual*. (VI Ed.) Pearson Education, Inc. Singapore. Collee, JG, Duguid JP, Frasee AG, Marmion BP. Publishers by Pearson Education (Singapore) Pte. Ltd.; 2002. p. 63-6, 149-85, 428-31.
- Murray PR. Editor-in-chief. *Manual of clinical microbiology*. 7th. ed. Washington, D.C.: ASM Press; 1999.
- Kalidas RIT, Falguni N, Hirak JR, Maity PK. Prevalence and susceptibility profiles of nonfermenting gram-negative bacilli infection in a tertiary care hospital of eastern India. *Indian J Clin Practice* 2013;24:4305-91.
- Kaur A, Gupta V, Chhina D. Prevalence of metallo- β -lactamase-producing (MBL) acinetobacter species in a tertiary care hospital. *Iran J Microbiol* 2014;6:22-5.
- Malini A, Deepa EK, Gokul BN, Prasad SR. Non-fermenting gram-negative bacilli infections in a tertiary care hospital in Kolar, Karnataka. *J Lab Physicians* 2009;1:62-6.
- Patel PH, Pethani JD, Rathod SD, Chauhan B, Shah PD. Prevalence of non-fermenting gram-negative bacilli infection in tertiary care hospital in ahmedabad, gujarat. *Indian J Basic Appl Med Res* 2013;2:608-13.
- Gagneja D, Goel N, Aggarwal R, Chaudhary U. Changing trend of antimicrobial resistance among gram-negative bacilli isolated from lower respiratory tract ICU patients: A 5-year study. *Indian J Crit Care Med* 2011;3:164-7.
- Bertrand X. Endemic city, molecular diversity and colonisation routes of *Pseudomonas aeruginosa* in intensive care units. *Intensive Care Med* 2001;27:1263-8.
- Gardam MA. Is surveillance for multidrug-resistant *Enterobacteriaceae* an effective infection control strategy in the absence of an outbreak? *J Infect Dis* 2002;186:1754-60.
- Harris AD, McGregor JC, Furuno JP. What infection control interventions should be undertaken to control multidrug-resistant Gram-negative bacteria? *Clin Infect Dis* 2006;43:57-61.
- Harris AD. Patient-to-patient transmission is important in extended-spectrum-beta-lactamase-producing *Klebsiella pneumoniae* acquisition. *Clin Infect Dis* 2007;45:1347-50.
- Johnson JK. The role of patient-to-patient transmission in the acquisition of imipenem-resistant *Pseudomonas aeruginosa* colonization in the intensive care unit. *J Infect Dis* 2009;200:900-5.
- Michelin A, Henderson DK. Infection controls guidelines for prevention of health care-associated transmission of hepatitis B and C viruses. *Clin Liver Dis* 2010;14:119-36.
- Olson B, Weinstein RA, Nathan C, Chamberlin W, Kabins SA. Epidemiology of endemic *Pseudomonas aeruginosa*: why infection control efforts have failed. *J Infect Dis* 1984;150:808-16.
- Ortega B, Groeneveld AB, Schultsz C. Endemic multidrug-resistant *Pseudomonas aeruginosa* in critically ill patients. *Infect Control Hospital Epidemiol* 2004;25:825-31.
- Paramasivan CN, Sivadasan K, Datta M, Vallishayee RS, Prabhakar R. Non-fermenting gram-negative bacilli associated with acute respiratory infections in children in madras. *Oxford University Press* 1987;33:343-5.
- Juyal D, Prakash R, Shanakarayan, Sharma M, Negi V, Sharma N. Prevalence of non-fermenting gram-negative bacilli and their *in-vitro* susceptibility pattern in a tertiary care hospital of Uttarakhand: a study from foothills of Himalayas. *Saudi J Microbiol* 2013;2:108-12.
- Harris AD. How important is patient-to-patient transmission in extended-spectrum beta-lactamase *Escherichia coli* acquisition? *Am J Infect Control* 2007;35:97-101.
- Giske CG, Monnet DL, Cars O, Carmeli Y. Clinical and economic impact of common multidrug-resistant Gram-negative bacilli. *Antimicrob Agents Chemother* 2008;52:813-21.
- Nicasio AM, Kuti JL, Nicolau DP. The current state of multidrug-resistant Gram-negative bacilli in North America. *Pharmacotherapy* 2008;28:235-49.
- Lautenbach E, Patel JB, Bilker WB, Edelstein PH, Fishman NO. Extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for infection and impact of resistance on outcomes. *Clin Infect Dis* 2001;32:1162-71.