# Journal of Microbiology and Related Research




**Red Flower Publication Pvt. Ltd.**
48/41-42, DSIDC, Pocket-II, Mayur Vihar, Phase-I
Delhi - 110 091 (India)
Tel: 91-11-22754205, 45796900, Fax: 91-11-22754205
E-mail: info@rfppl.co.in
Website: www.rfppl.co.in

**Journal of Microbiology and Related Research (pISSN: 2395-6623, eISSN: 2455-832X )** is a leading source of up-to-date information in the field of microbiology. The journal is a peer-reviewed official organ of the Red Flower Publication Pvt. Ltd. It publishes papers based on original research in fundamental and applied biology of bacteria, archaea and lower eukaryotes as well as on all aspects of microbiological research. JMRR cover areas of agricultural, food, environmental, industrial, medical, pharmaceutical, veterinary and molecular microbiology.


**Subscription Information**

Institutional (1 year): INR8500/USD664

*Payment methods*

*Bank draft / cashier & order / check / cheque / demand draft / money order* should be in the name of **Red Flower Publication Pvt. Ltd.** payable at **Delhi**.

*International Bank transfer / bank wire / electronic funds transfer / money remittance / money wire / telegraphic transfer / telex*

1. **Complete Bank Account No.** 604320110000467
2. **Beneficiary Name (As per Bank Pass Book):** Red Flower Publication Pvt. Ltd.
3. **Address:** 41/48, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi – 110 091(India)
4. **Bank & Branch Name:** Bank of India; Mayur Vihar
5. **Bank Address & Phone Number:** 13/14, Sri Balaji Shop,Pocket II, Mayur Vihar Phase- I, New Delhi - 110091 (India); Tel: 22750372, 22753401. **Email:** mayurvihar.newdelhi@bankofindia.co.in
6. **MICR Code:** 110013045
7. **Branch Code:** 6043
8. **IFSC Code:** BKID0006043 (used for RTGS and NEFT transactions)
9. **Swift Code:** BKIDINBBDOS
10. **Beneficiary Contact No. & E-mail ID:** 91-11-22754205, 45796900, E-mail: sales@rfppl.co.in

***Online*** You can now renew online using our RFPPL renewal website. Visit http://rfppl.co.in/subscribe.php?mid=7 and enter the required information and than you will be able to pay online.

Send all Orders to: **Red Flower Publication Pvt. Ltd.,** 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi – 110 091(India). Phone: 91-11-22754205, 45796900, Fax: 91-11-22754205, E-mail: sales@rfppl.co.in, Website: www.rfppl.co.in

Journal of Microbiology and Related Research

JMRR

July - December 2018
Volume 4, Number 2

# *Contents*

https://journals.indexcopernicus.com/search/form?search=Journal%20of%20Microbiology%20and%20Related%20Research

INDEX COPERNICUS INTERNATIONAL

ICI World of Journals | ICI Journals Master List | Contact

Login/ Register

Journal title: Journal of Microbiology and Related Research
ISSN: 2455-832X, 2395-6623
GICID: n/a
Country / Language: IN / EN
Publisher: Red Flower Publication Private Limited

| | |
|---|---|
| Citation: | N/A |
| MNiSW 2016: | N/D |
| ICV 2017: | E/P |
| ICV 2016: | 76.35 |

Journal of Microbiology and Related Research
Volume 4 Number 2, July - December 2018
DOI: http://dx.doi.org/10.21088/jmrr.2395.6623.4218.1

# Prevalence of Bacterial Agents Causing Lower Respiratory Tract Infections in Patients Attending Gujarat Adani Institute of Medical Science, Bhuj, Kutch, Gujarat: A Cross-Sectional Study

**Krupali Kothari[1], Jigar Gusani[2]**

**Author Affiliation**
[1]Assistant Professor, Department of Microbiology, Gujarat Adani Institute of Medical Sciences, Bhuj, Gujarat 370001, India. [2]Associate Professor, Department of Microbiology, Dr. N.D Desai Faculty of Medical Science and Research, Nadiad, Gujarat 387001, India.

**Corresponding Author**
**Jigar Gusani,**
Associate Professor, Department of Microbiology, Dr. N.D Desai Faculty of Medical Science and Research, Nadiad, Gujarat 387001, India.

**E-mail:** researchguide86@gmail.com

**Received on** 26.10.2018,
**Accepted on** 14.11.2018

**Abstract**

*Aim:* The Current research was performed to find out the prevalence of bacterial agents responsible for LRTI and to find out the associated risk factors. *Material and Methods:* Present cross sectional study was performed in the department of Microbiology, Gujarat Adani Institute of Medical Science, Bhuj, Kutch, Gujarat. Total 200 samples including expectorated sputum and Endotracheal tube (ET) aspirates were collected from both OPD and IPD patients with clinically diagnosed Lower Respiratory Tract Infection. Expectorated sputum was collected into a sterile container with a screw cap that is tightly secured following proper instructions given to the patient. ET aspirates were transferred to a sterile screw cap container with the cap tightly secured before transport. Analysis was done using SPSS version 15 (SPSS Inc. Chicago, IL, USA) Windows software program. *Results:* Among the 200 samples processed, sputum and ET aspirates were 190 and 10 respectively. Out of these 38.5% samples acquiesced noteworthy development and rest of 61.5% demonstrated either no growth or modest growth which was measured as no growth. Gender and age wise allocation showed maximum number, (35.25%) of culturally confirmed LRTI cases were in the 61 – 71 years of age group. Klebsiella pneumoniae (55%) was the predominant pathogen, followed by Pseudomonas aeruginosa (20%), Acinetobacter spp (10%), Citrobacter freundii (8%), Staphylococcus aureus (2%), Streptococcus pneumoniae (3%). *Conclusion:* LRTIs are frequently analyzed clinically, but etiological analysis could be completed by culturing different samples from patients which will assist clinician to set up precise treatment.

**Keywords:** Kutch; Lower Respiratory Tract Infections; Risk Factors; Sputum.

## Introduction

Lower respiratory tract infections (LRTIs) are leading cause of illness and death in children and adults across the world. Acute LRTI comprise pneumonia, and other infections disturbing the airways such as acute bronchitis and bronchiolitis, influenza and whooping cough [1]. LRTIs are accountable for 4.4% of all hospital admission and 6% of all general practitioner consultation [2]. multiplicity of organisms are frequently caught up in etiology of LRTI. Gram positive bacteria like Staphylococcus aureus, Streptococcus pneumoniae etc. and Gram negative bacteria like Klebsiella spp., Pseudomonas spp., Hemophillus influenzae, Acinetobacter spp., have been recovered from LRTIs [3].

Clinically LRTI is defined as an acute ill health usually for a period of 1-3 wks, presenting with symptoms of cough, expectoration, dyspnoea, wheeze & chest pain/discomfort [4]. Various predisposing factors which may lead to LRTI are smoking, alcohol, immunosuppressive conditions. Diabetes mellitus, COPD, Bronchial asthma etc. [5]. The present study was performed to know the prevalence of bacterial agents causing LRTI and to find out the associated risk factors.

## Material and Methods

Present cross sectional study was conducted in the department of Microbiology, Gujarat Adani Institute of Medical Science, Bhuj, Kutch, Gujarat. Total 200 lower respiratory tract samples including expectorated sputum and Endotracheal tube (ET) aspirates were collected from both OPD and admitted patients with clinically diagnosed LRTI after taking detailed history of the patient. Expectorated sputum was collected into a sterile container with a screw cap that is tightly secured following proper instructions given to the patient. ET aspirates were transferred to a sterile screw cap container with the cap tightly secured before transport. Following compilation of the sample, it was transported to Bacteriology section [6].

There are mainly three steps in the Procedure:

1. Direct microscopy by Gram stain [7].

2. Criteria for assessing quality sputum sample:

3.Culture & isolation [8,9]

*Statistical analysis*

The data was coded and entered into Microsoft Excel spreadsheet. Analysis was done using SPSS version 15 (SPSS Inc. Chicago, IL, USA) Windows software program. The variables were assessed for normality using the Kolmogorov-Smirnov test. Descriptive statistics were calculated.

## Results

Among the 200 samples processed, sputum and ET aspirates were 190 and 10 respectively. Out of these 38.5% samples yielded significant growth and rest of 61.5% showed either no growth or commensal growth which was considered as no growth. Sputum culture positive cases were 36.9% and ET aspirates culture positive cases were 67.01.%. Age and gender wise distribution showed highest number, (35.25%) of culturally confirmed LRTI cases were in the 61–71 years of age group. Overall 71.28% cases were found in Male, whereas in female it was 28.72%. Single gram negative bacilli was isolated in 90.10% cases, while single gram positive cocci was isolated in 6.12% cases.

From 98 culture positive samples a total of 100 isolates were recovered, out of which Klebsiella pneumoniae (55%) was the predominant pathogen, followed by Pseudomonas aeruginosa (20%), Acinetobacter spp (10%), Citrobacter freundii (8%), Staphylococcus aureus (2%), Streptococcus pneumoniae (3%) and 1% each of Enterobacter spp., Edwardsiella spp., Escherichia coli. On further analysis of sample wise distribution of bacterial isolates, Klebsiella pneumoniae was found to be predominant in both sputum and ET aspirates samples [Table 1].

Thirty five percentage % culturally confirmed LRTI cases were associated with either single risk factor or multiple comorbidities i.e more than one risk factor. Out of which Multiple comorbidities (14.01%) were predominant followed by Hypertension (9.20%), Diabetes (4.9%), Smoking (4.12%), COPD (2.12%) and Alcohol (1.10%)

**Table 1:** Distribution of Bacterial isolates from Sputum and ET aspirates

| Bacteria | Sputum(N) | ET aspirates (N) | Total (%) |
|---|---|---|---|
| Klebsiella pneumoniae | 52 | 3 | 55 (55) |
| Pseudomonas aeruginosa | 16 | 4 | 20 (20) |
| Acinetobacter spp. | 9 | 1 | 10 (10) |
| Citrobacter freundii | 7 | 1 | 8 (8) |
| Staphylococcus aureus | 2 | Nil | 2 (2) |
| Streptococcus pneumoniae | 3 | Nil | 3 (3) |
| Enterobacter spp | 1 | Nil | 1 (1) |
| Edwardsiella spp. | 1 | Nil | 1 (1) |
| Escherichia coli | 1 | Nil | 1 (1) |

## Discussion

Two Hundred samples were collected from patients with LRTI within a period of 3.5 years. Overall culture confirmed cases were found to be 38.5%. Previous studies from various places reported culture positivity rate ranges from 21.5% to 83% [2,10]. Mishra S et al. 2012 [11] too demonstrated superior positivity in ET aspirates than sputum samples. In this present study LRTI cases are more widespread in Males compared to females which are analogous to findings of other researches done by Panda S. et al. 2012 [2] and Akingbade OA. et al. 2012 [12]. sixty one to seventy year age group exhibits uppermost percentage of LRTI cases which was in agreement with study done by Tripathi P. et al. 2014 and Panda S et al. 2012 [2,3].

In this study, Klebsiella pneumonia was found to be predominant isolated organism followed by Pseudomonas aeruginosa, Acinetobacter spp, Citrobacter freundii. Finding of this study was similar to studies done by Tripathi P. et al. 2014,

Shrivastava P. et al. 2013, Biswas P. et al. 2013 [3,13,14]. Percentage of Staphylococcus aureus and Streptococcus pneumoniae were 2% and 3% correspondingly. Mannur S et al. 2015 reported incidence of Staphylococcus aureus as 5%, while incidence of Streptococcus pneumonia were 8.5% and 8.6% respectively in the studies done by Biswas P et al. 2013 and Mishra S et al. 2012 [5,13,11]. small figure of gram positive cocci in present research may be due to hospital based study and complexity in separating such a fragile organisms like Streptococcus pneumonia. The risk factors connected with LRTI cases are Smoking, Alcohol, COPD, Diabetes, Hypertension and multiple co morbidities.

## Conclusion

LRTIs are mostly diagnosed clinically, but etiological diagnosis could be done by culturing various samples from patients which will help clinician to start specific therapy. Therefore regular surveillance is necessary in our hospital as there is a probability of changing trends of etiological agents and associated predisposing factors. Short duration might be the limitation of the study which could not determine the exact prevalence as well as the risk factors of LRTI.

## References


1. Europeanlung.org/Acute-Lower – Respiratory Tract Infections.
2. Panda S, Prema Nandini B, Ramani TV. Lower respiratory tract infection bacteriological profile and antibiogram pattern. Int J Cur Res Rev 2012;4(21):149-55.
3. Tripathi Purti C, Dhote K. Lower Respiratory Tract Infection: Current Etiological Trends and Antibiogram. J Pharm Biomed Sci. 2014;04(3): 249– 55.
4. Woodhead M, Blasi F, Ewig S, Huchon G, Ieven M, Schaberg T et al. Guidelines for the management of adult lower respiratory tract infections. EurRespir J, 2005;26:1138-80.
5. Mannur S, BR A, et al. Study of risk factors, chest X- ray findings, Aetiological Agents and Their sensitivity pattern among patients with lower respiratory tract infection. Int J Pharm Bio Sci. 2015;6(3): 336–41.
6. Henry D. Isenberg. Clinical Microbiology Procedure Handbook. Second Edition (2007). 3.11.2: Lower respiratory tract cultures.
7. Henry D. Isenberg. Clinical Microbiology Procedure Handbook. Second Edition (2007). 3.2.1: Gram stain.
8. Colle JG, Dugoid JP,Fraser AG, Marimion BP, Simmons A, Laboratory strategy in the diagnosis of infective syndrome. In: Colle JG, Dugoid JP,Fraser AG,Marimion BP, Simmons A (Editors). Mackie and Mc Cartney Practical Medical Microbiology. 14th edition. Churchill Livingstone. Inc: London; 1996.pp.53–94.
9. Bailey & Scott's Diagnostic Microbiology, 12th edition, Chapter 13: Overview of Bacterial Identification Methods and strategies.
10. Taura D. W., Hassan A., Yayo A. M. and Takalmawa H. Bacterial isolates of the respiratory tract infection and their current sensitivity pattern among patients attending Aminu Kano Teaching Hospital Kano-Nigeria. Int. Res. J. Microbiol. 2013;4(9):226-31.
11. Mishra SK, Kattel HP, Acharya J, et al. Recent trend of bacterial aetiology of lowerrespiratory tract infections in a tertiary care centre of Nepal. Int J Infect Microbiol 2012;1(1):38.
12. Akingbade OA, Ogiogwa JI, Okerentugba PO, Innocent-Adiele HC, Onoh CC, Nwanze JC, Okonko IO. Prevalence and Antibiotic Susceptibility Pattern of Bacterial Agents Involved In Lower Respiratory Tract Infections in Abeokuta, Ogun State, Nigeria. Rep Opinion 2012;4(5):25-30. (ISSN: 1553-9873). http://www.sciencepub.net/report.
13. Biswas P, Tukaram P. Bacterial causes of lower respiratory tract infection in patientsattending central referral hospital, Gangtok with reference to antibiotic resistance pattern.J of Evolution of Medicine & Dental Sciences. 2013;2(42):8126-35.
14. Srivastava P, Kumar P, Nirwan P, Sharma M. Bacteriological profile and antibiogrampattern of lower respiratory tract infections in a tertiary care hospital in northern India.International journal of pharmaceutical research and bioscience. 2013;2(3): 225–33.

*Revised Rates for 2018 (Institutional)*

| Title of the Journal | Frequency | India(INR) Print Only | India(INR) Online Only | Outside India(USD) Print Only | Outside India(USD) Online Only |
|---|---|---|---|---|---|
| Community and Public Health Nursing | Triannual | 5500 | 5000 | 430 | 391 |
| Dermatology International | Semiannual | 5500 | 5000 | 430 | 391 |
| Gastroenterology International | Semiannual | 6000 | 5500 | 469 | 430 |
| Indian Journal of Agriculture Business | Semiannual | 5500 | 5000 | 413 | 375 |
| Indian Journal of Anatomy | Bi-monthly | 8500 | 8000 | 664 | 625 |
| Indian Journal of Ancient Medicine and Yoga | Quarterly | 8000 | 7500 | 625 | 586 |
| Indian Journal of Anesthesia and Analgesia | Monthly | 7500 | 7000 | 586 | 547 |
| Indian Journal of Biology | Semiannual | 5500 | 5000 | 430 | 391 |
| Indian Journal of Cancer Education and Research | Semiannual | 9000 | 8500 | 703 | 664 |
| Indian Journal of Communicable Diseases | Semiannual | 8500 | 8000 | 664 | 625 |
| Indian Journal of Dental Education | Quarterly | 5500 | 5000 | 430 | 391 |
| Indian Journal of Diabetes and Endocrinology | Semiannual | 8000 | 7500 | 597 | 560 |
| Indian Journal of Emergency Medicine | Quarterly | 12500 | 12000 | 977 | 938 |
| Indian Journal of Forensic Medicine and Pathology | Quarterly | 16000 | 15500 | 1250 | 1211 |
| Indian Journal of Forensic Odontology | Semiannual | 5500 | 5000 | 430 | 391 |
| Indian Journal of Genetics and Molecular Research | Semiannual | 7000 | 6500 | 547 | 508 |
| Indian Journal of Hospital Administration | Semiannual | 7000 | 6500 | 547 | 508 |
| Indian Journal of Hospital Infection | Semiannual | 12500 | 12000 | 938 | 901 |
| Indian Journal of Law and Human Behavior | Semiannual | 6000 | 5500 | 469 | 430 |
| Indian Journal of Legal Medicine | Semiannual | 8500 | 8000 | 607 | 550 |
| Indian Journal of Library and Information Science | Triannual | 9500 | 9000 | 742 | 703 |
| Indian Journal of Maternal-Fetal & Neonatal Medicine | Semiannual | 9500 | 9000 | 742 | 703 |
| Indian Journal of Medical & Health Sciences | Semiannual | 7000 | 6500 | 547 | 508 |
| Indian Journal of Obstetrics and Gynecology | Bi-monthly | 9500 | 9000 | 742 | 703 |
| Indian Journal of Pathology: Research and Practice | Monthly | 12000 | 11500 | 938 | 898 |
| Indian Journal of Plant and Soil | Semiannual | 6500 | 6000 | 508 | 469 |
| Indian Journal of Preventive Medicine | Semiannual | 7000 | 6500 | 547 | 508 |
| Indian Journal of Research in Anthropology | Semiannual | 12500 | 12000 | 977 | 938 |
| Indian Journal of Surgical Nursing | Triannual | 5500 | 5000 | 430 | 391 |
| Indian Journal of Trauma and Emergency Pediatrics | Quarterly | 9500 | 9000 | 742 | 703 |
| Indian Journal of Waste Management | Semiannual | 9500 | 8500 | 742 | 664 |
| International Journal of Food, Nutrition & Dietetics | Triannual | 5500 | 5000 | 430 | 391 |
| International Journal of Neurology and Neurosurgery | Quarterly | 10500 | 10000 | 820 | 781 |
| International Journal of Pediatric Nursing | Triannual | 5500 | 5000 | 430 | 391 |
| International Journal of Political Science | Semiannual | 6000 | 5500 | 450 | 413 |
| International Journal of Practical Nursing | Triannual | 5500 | 5000 | 430 | 391 |
| International Physiology | Triannual | 7500 | 7000 | 586 | 547 |
| Journal of Animal Feed Science and Technology | Semiannual | 7800 | 7300 | 609 | 570 |
| Journal of Cardiovascular Medicine and Surgery | Quarterly | 10000 | 9500 | 781 | 742 |
| Journal of Forensic Chemistry and Toxicology | Semiannual | 9500 | 9000 | 742 | 703 |
| Journal of Global Medical Education and Research | Semiannual | 5900 | 5500 | 440 | 410 |
| Journal of Global Public Health | Semiannual | 12000 | 11500 | 896 | 858 |
| Journal of Microbiology and Related Research | Semiannual | 8500 | 8000 | 664 | 625 |
| Journal of Nurse Midwifery and Maternal Health | Triannual | 5500 | 5000 | 430 | 391 |
| Journal of Orthopedic Education | Triannual | 5500 | 5000 | 430 | 391 |
| Journal of Pharmaceutical and Medicinal Chemistry | Semiannual | 16500 | 16000 | 1289 | 1250 |
| Journal of Plastic Surgery and Transplantation | Semiannual | 26400 | 25900 | 2063 | 2023 |
| Journal of Practical Biochemistry and Biophysics | Semiannual | 7000 | 6500 | 547 | 508 |
| Journal of Psychiatric Nursing | Triannual | 5500 | 5000 | 430 | 391 |
| Journal of Social Welfare and Management | Triannual | 7500 | 7000 | 586 | 547 |
| Medical Drugs and Devices Research | Semiannual | 2000 | 1800 | 156.25 | 140.63 |
| New Indian Journal of Surgery | Bi-monthly | 8000 | 7500 | 625 | 586 |
| Ophthalmology and Allied Sciences | Triannual | 6000 | 5500 | 469 | 430 |
| Otolaryngology International | Semiannual | 5500 | 5000 | 430 | 391 |
| Pediatric Education and Research | Triannual | 7500 | 7000 | 586 | 547 |
| Physiotherapy and Occupational Therapy Journal | Quarterly | 9000 | 8500 | 703 | 664 |
| RFP Indian Journal of Medical Psychiatry | Semiannual | 8000 | 7500 | 625 | 586 |
| RFP Journal of Gerontology and Geriatric Nursing | Semiannual | 5500 | 5000 | 430 | 391 |
| Urology, Nephrology and Andrology International | Semiannual | 7500 | 7000 | 586 | 547 |

**Terms of Supply:**

1. Agency discount 10%. Issues will be sent directly to the end user, otherwise foreign rates will be charged.
2. All back volumes of all journals are available at current rates.
3. All Journals are available free online with print order within the subscription period.
4. All legal disputes subject to Delhi jurisdiction.
5. Cancellations are not accepted orders once processed.
6. Demand draft / cheque should be issued in favour of "**Red Flower Publication Pvt. Ltd.**" payable at **Delhi**
7. Full pre-payment is required. It can be done through online (http://rfppl.co.in/subscribe.php?mid=7).
8. No claims will be entertained if not reported within 6 months of the publishing date.
9. Orders and payments are to be sent to our office address as given above.
10. Postage & Handling is included in the subscription rates.
11. Subscription period is accepted on calendar year basis (i.e. Jan to Dec). However orders may be placed any time throughout the year.

**Order from**

Red Flower Publication Pvt. Ltd., 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi - 110 091 (India),
**Mobile: 8130750089, Phone: 91-11-45796900, 22754205, 22756995 E-mail: sales@rfppl.co.in, Website: www.rfppl.co.in**

Journal of Microbiology and Related Research
Volume 4 Number 2, July - December 2018
DOI: http://dx.doi.org/10.21088/jmrr.2395.6623.4218.2

# Prevalence of Non Fermenting Gram Negative Bacilli Infections in a Tertiary Care Hospital

**Shakthi R.[1], Venkatesha D.[2]**

**Author Affiliation**
[1]Assistant Professor [2]Professor and Head, Department of Microbiology, Adichunchanagiri Institute of Medical Sciences, B.G. Nagara, Mandya, Karnataka 571448, India

**Corresponding Author**
**Shakthi R.,**
Assistant Professor, Department of Microbiology, Adichunchanagiri Institute of Medical Sciences, B.G. Nagara, Mandya, Karnataka 571448, India
**E-mail:** shakthi.rals@yahoo.com

**Received on** 11.07.2018,
**Accepted on** 30.07.2018

**Abstract**

*Background and Objective:* Non fermenting Gram Negative bacilli (NFGNB) are saprophytic in nature and have emerged as important healthcare associated pathogens. NFGNB group which are known to cause infections are *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Burkholderia cepacia complex* and *Stenotrophomonas maltophila*. This study was undertaken to identify the various non fermenters isolated from patients admitted to our hospital, to assess their clinical significance and type of healthcare associated infections they caused and to know their antimicrobial sensitivity pattern. *Methodology:* A total of 200 non fermenter isolates from various clinical specimens received in department of Microbiology, Adichunchanagiri Institute of Medical Sciences, B.G. Nagara formed the study group. Samples were inoculated on blood agar, MacConkey agar, chocolate agar and incubated at 37°C for 18-24 hours. The clinical isolates were identified using the conventional methods and susceptibility testing was done by Kirby-Bauer disc diffusion method as per CLSI guidelines. *Results:* 200 NFGNB were isolated from various clinical specimens. *Pseudomonas aeruginosa* was the most common isolate accounting for 140 (70%) followed by *Acinetobacter baumanii* 32 (16%) and other nonfermenters 28 (14%). *P.aeruginosa* showed sensitivity to imipenem (94.2%), ceftazidime (70.7%), amikacin (69.2%), tobramycin and ticarcillin (62.8%). *A.baumannii* showed 100% sensitivity to imipenem and 53.1% sensitivity to amikacin and gentamicin. *Conclusion: P.aeruginosa* and *A.baumannii* were the common NFGNB isolated in our study from patients of urinary tract infections, surgical site infections, bacteremia and ventilator associated pneumonia. Thus NFGNB are emerging as important opportunistic pathogens and are resistant to commonly used antimicrobials. Therefore early diagnosis and institution of empirical therapy based on antibiotic sensitivity data of the institute would decrease mortality and improve patient management.

**Keywords:** Non Fermenting Gram Negative Bacilli (NFGNB); Pseudomonas; Acinetobacter.

## Introduction

Non Fermenting Gram Negative Bacilli (NFGNB) are a group of aerobic, non-sporing, bacilli/ coccobacilli which are either incapable of utilizing carbohydrates as a source of energy or degrade them via oxidative, rather than fermentative pathway. This group includes numerous organisms but the ones which are known to cause nosocomial infections are *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, *Burkholderia cepacia complex* (BCC) and *Stenotrophomonas maltophila* [1-5]. NFGNB are known to account for about 15% of all bacterial isolates from a clinical microbiology laboratory [6]. They can be recovered from hospital environment and are often resistant to disinfectants and have the potential to spread from patient to patient

via fomites or the hands of medical personnel. In recent years, due to the liberal and empirical use of antibiotics, NFGNB have emerged as important healthcare-associated pathogens. They have been incriminated in infections such as septicaemia, meningitis, pneumonia, urinary tract infections (UTI) and surgical site infections (SSI) [7]. NFGNB are innately resistant to many antibiotics and are known to produce extended spectrum ß-lactamases and metallo ß-lactamases. The antimicrobial resistance exhibited by the NFGNB facilitates colonization and super infection in antibiotic treated patients [2-7].

The aim of the present study was to isolate and identify various non fermenters isolates from patients admitted to our hospital and to assess their antimicrobial susceptibility pattern.

## Materials and methods

The present study was carried out in the department of Microbiology, Adichunchanagiri Institute of Medical Sciences, B.G.Nagara for a period of one and half years. Ethical committee clearance was taken from the institution. A total of 200 non fermenter isolates were isolated from various clinical samples like urine, pus, blood, sputum, endotracheal aspiration collected from patients. The samples were inoculated on blood agar, MacConkey agar, chocolate agar and incubated at 37°C for 18-24 hours. The organisms isolated were identified using standard procedures. All the organisms that grew on Triple Sugar Iron agar and produced an alkaline reaction were provisionally considered to be NFGNB and identified further by using a standard protocol for identification [1]. The characters assessed are morphology on Gram's stain, motility, pigment production, oxidase test, OF test (Hugh-Leifson's medium) for glucose, lactose, sucrose, maltose, mannitol and xylose, growth on 10% lactose agar, lysine and arginine decarboxylase test and gelatin liquefaction test.

The antibiotic susceptibility test was performed by Kirby-Bauer disc diffusion method using commercially available discs (Hi-media). The different antimicrobials tested were Imipenem (10µg), Meropenem (10µg), Piperacillin–Tazobactum (100/10µg), Netilmycin (30µg), Ticarcillin (75µg), Amikacin (30µg), Gentamicin (10µg), Tobramycin (10µg), Ciprofloxacin (5µg), Ofloxacin (5µg), Levofloxacin (5µg), Ceftazidime (30µg), Ceftriaxone (30µg), Cefepime (30µg) and Trimethoprim/sulfamethoxazole. The results were interpreted as per the CLSI guidelines [8]. *E.coli* ATCC 25922 and *P.aeruginosa* ATCC 27853 were used as the control strains.

## Results

A total of 200 non fermenting Gram negative bacilli were isolated from various clinical specimens like urine, pus, blood, sputum, endotracheal aspiration and body fluids collected from out-patients and in-patients admitted in the hospital. Of these 200 strains of NFGNB, 120 (60%) were from males and 80 (40%) were from females. Most of them belonged to the age group 41-60 years (65, 32.5%), followed by patients of more than 60 years of age (50,25%) as shown in Table 1.

Out of 200 clinical samples, majority of the isolates were from pus (117) followed by sputum (24), urine (23), blood (22), endotracheal aspiration (9) and body fluid samples (5). The NFGNB isolated from various clinical samples are shown in table 2

**Table 1:** Age and Gender Wise Distribution of Clinical Isolates of NFGNB

| Age group (years) | Male (no.) | Female (no.) | Total (no.)% |
|---|---|---|---|
| <20 | 25 | 20 | 45 (22.5%) |
| 21-40 | 20 | 20 | 40 (20%) |
| 41-60 | 35 | 30 | 65 (32.5%) |
| >60 | 40 | 10 | 50 (25%) |
| Total | 120 | 80 | 200 (100%) |

**Table 2:** NFGNB isolated from various clinical samples

| Organisms | Pus | Sputum | Urine | Blood | ET aspirates | Fluid aspirates |
|---|---|---|---|---|---|---|
| *Pseudomonas aeruginosa* | 75 | 15 | 20 | 18 | 7 | 5 |
| *Acinetobacter baumannii* | 24 | 3 | 2 | 1 | 2 | 0 |
| Other NFGNB | 18 | 6 | 1 | 3 | 0 | 0 |
| Total | 117(58.5%) | 24(12%) | 23(11.5%) | 22(11%) | 9(4.5%) | 5(2.5%) |

**Table 3:** NFGNB Isolated

| Organisms isolated | Number | Percentage (%) |
|---|---|---|
| *Pseudomonas aeruginosa* | 140 | 70% |
| *Acinetobacter baumannii* | 32 | 16% |
| Other NFGNB | 28 | 14% |

**Table 4:** Antimicrobial Susceptibility Patterns of various NFGNB

| Antibiotic | No / % of sensitive *pseudomonas* isolates | No / % of sensitive *Acinetobacter* isolates | No / % of sensitive other NFGNB isolates | Total |
|---|---|---|---|---|
| Ceftazidime | 99 (70.7%) | 10 (31.2%) | 5 (17.8%) | 114 |
| Ceftriaxone | 84 (60%) | 11 (34.3%) | 4 (14.2%) | 99 |
| Cefetoxime | 82 (58.5%) | 11 (34.3%) | 2 (7.1%) | 95 |
| Cefipime | 87 (62.1%) | 12 (37.5%) | 3 (10.7%) | 102 |
| Meropenem | 128 (91.4%) | 31 (96.8%) | 16 (57.1%) | 175 |
| Imepenem | 132 (94.2%) | 32 (100%) | 17 (60.7%) | 181 |
| Piperacillin\tazobactam | 79 (56.4%) | 23 (71.8%) | 15 (53.5%) | 117 |
| Netilmicin | 80 (57.1%) | 15 (46.8%) | 8 (28.5%) | 103 |
| Ticarcillin | 88 (62.8%) | 16 (50%) | 5 (17.8%) | 109 |
| Ciprofloxacin | 62 (44.2%) | 14 (43.7%) | 6 (21.4%) | 82 |
| Ofloxacin | 60 (42.8%) | 15 (46.8%) | 5 (17.8%) | 80 |
| Levofloxacin | 65 (46.4%) | 15 (46.8%) | 6 (21.4%) | 86 |
| Amikacin | 97 (69.2%) | 17 (53.1%) | 5 (17.8%) | 119 |
| Gentamicin | 80 (57.1%) | 17 (53.1%) | 5 (17.8%) | 102 |
| Tobramicin | 88 (62.8%) | 16 (50%) | 4 (14.2%) | 108 |
| Trimethoprim/ Sulfamethoxazole | 50 (35.7%) | 11 (34.3%) | 3 (10.7%) | 64 |

*The* most common isolate was *Pseudomonas aeruginosa* 140 (70%), followed by *Acinetobacter baumannii* 32 (16%) and other NFGNB 28 (14%) The majority of the non fermenters were isolated from pus and sputum samples. The numbers and percentage of *Pseudomonas aeruginosa, Acinetobacter baumannii* and other NFGNB isolated from different specimens are shown in table 3

In the present study, most of the isolates of *P. aeruginosa* were sensitive to imipenem (94.2%), ceftazidime (70.7%), amikacin (69.2%), tobramycin and ticarcillin (62.8%). *A.baumannii* showed 100% sensitivity to imipenem followed by 71.8% to Piperacillin and 53.1% to amikacin and gentamicin. Sensitivity pattern of the NFGNB isolated is shown in table 4

## Discussion

NFGNB are emerging as important opportunistic pathogens and are resistant to commonly used antimicrobials. In recent years, a considerable increase in the prevalence of multidrug resistance among non fermenting Gram negative bacilli has been noticed with high morbidity and mortality, hence performing antibiotic susceptibility testing on a regular as well as a periodic basis is necessary.

In this study, a total of 200 (13.5%) isolates of NFGNB were isolated from various clinical samples received from out-patients and in-patients admitted in the hospital and their antimicrobial susceptibility patterns were determined. Majority of isolates were from male and older age group 41-60 years (65, 32.5%), followed by patients of more than 60 years of age (50,25%). This may be due to decreased immunity, prolonged hospitalization and other associated co-morbidities in these age group.

In the present study, the most common NFGNB isolate was *Pseudomonas aeruginosa* (70%) correlating with the study of Arora et al., (72.83%), Malini et al., (64.4%) and Vijaya D et al., (78.94%) [9-11]. The second commonest isolate was *Acinetobacter baumannii* 32%, which is correlating with the study of Juyal D et al., (29.27%), Malini D et al.,(25.3%) and in contrary Arora D et al., reported 8.4% [9,10,12].

Majority of *Pseudomonas aeruginosa* (64.1%) were isolated from pus specimens, which is comparable with the study conducted by Dipak Bhargava et al., (43.9%), where as Hariom Sharan et al.,reported 26.32%[13.14].

In the present study, *Pseudomonas aeruginosa* was highly sensitive to Imipenem (94.2%), which correlates with the study of Kamalraj et al., (88.4%) and Vikas Jain et al., (78.57%). *Acinetobacter baumannii* showed high sensitivity to imipenem (100%) which is comparable with the study of Vikas Jain et al. [15,16].

## Conclusion

In the present study, NFGNB have emerged as an important pathogen and shows resistance to commonly used antimicrobial drugs. To minimize the drug resistance, importance should be given to proper identification of organism and regular antibiotic susceptibility monitoring is essential, which helps and guides the physicians to prescribe the right combinations of anti-microbial drugs for proper management and prevention of the emergence of MDR.

## References

1. Winn W Jr, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, et al., editors. In: Koneman›s Color Atlas and textbook of Diagnostic Microbiology. 6th ed. USA: Lippincott Williams and Wilkins Company; Nonfermenting Gram negative bacilli; 2006.pp.305–91.
2. Johanson WG Jr, Higuchi JH, Chaudhuri TR; Bacterial adherence to epithelial cells in bacillary colonization of the respiratory tract. Am Rev Respir Dis 1980;121:55–63.
3. Buck AC, Cooke EM; The fate of ingested Pseudomonas aeruginosa in normal persons. J Med Microbiol 1969;2:521–5.
4. Rebecca Reik, Theodore Spilker, John J; LiPuma: Distribution of Burkholderia cepacia Complex Species among Isolates Recovered from Persons with or without Cystic Fibrosis. Journal of Clinical Microbiology 2005;43(6):2926–8.
5. Richet H, Escande MC, Marie JP. Epidemic Pseudomonas aeruginosa serotype 016 bacteremia in hematology-oncology patients. J Clin Microbiol 1989;27:1992–6.
6. Rubin SJ, Granato PA, Wasilauskas BL. Glucose nonfermenting Gram negative bacteria. In: Lennette EH, Balows A, Hausler WJ Jr, Shadomy HJ, editors. Manual of Clinical Microbiology. 4th ed. Washington, D.C: American Society for Microbiology; 1985.pp.330–49.
7. Gales AC, Jones RN, Forward KR, Linares J, Sader HS, Verhoef J. Emerging importance of multidrug-resistant *Acinetobacter* species and *Stenotrophomonas maltophilia* as pathogens in seriously ill patients: Geographic patterns, Epidemiological features, and trends in the SENTRY antimicrobial surveillance program (1997-1999) Clin Infect Dis. 2001;32:104–13.
8. Clinical and Laboratory Standard Institute. Performance standards for antimicrobial susceptibility testing: 23rd informational supplement 2013.
9. Arora D, Jindal N, Kumar R. Emerging antibiotic resistance in Pseudomonas a challenge. International Journal Pharmacy Pharmaceutical Sciences. 2011;3(2):82-84.
10. Malini A, Deepak EK, Gokul BN, Prasad SR. Non-fermenting gram negative bacilli infections in a tertiary care hospital in Kolar Karnataka. J Lab Physicians. 2009;1(2):62-66.
11. Vijaya D, Kamala, Bavani S, Veena M. Prevalence of non-fermenters in clinical specimens. Indian J. Med. Sci. 2000;54:87-91.
12. Juyal D, Prakash R, Shankarnarayan SA, Sharma M, Negi V, Sharma N. Prevalence of non fermenting gram negative bacilli and there in vitro susceptibility pattern in a tertiary care hospital of Uttarakhand. A study from foothills of Himalayas. Saudi Journal for Health Sciences. 2013;2(2):108-12.
13. Dipak Bhargava, Sanjay Kar and Mukesh Saha. Prevalence of Non Fermentative Gram Negative Bacilli Infection in Tertiary Care Hospital in Birgunj, Nepal Int.J.Curr.Microbiol. App.Sci 2015;4(7): 301-07.
14. Hariom Sharan, Neeraj Katare, Aparna Pandey, Ganesh Shivmurti Bhatambare, Trupti Bajpai. Emergence of Hospital Acquired Carbapenem Resistant Non Fermenters in Teaching Institute. Journal of Clinical and Diagnostic Research. 2016;10(12):20-23.
15. Kamalraj M, Sivashankari S, Thamarai S, Apurba Shankar Sastry. Study on Non fermenting gram negative bacilli from various clinical samples in a tertiary care hospital. Int J Bio. Med Res. 2015;6(4):5230-35.
16. Vikas Jain, V.K. Ramnani and Navinchandra Kaore. Antimicrobial Susceptibility Pattern amongst Aerobic Bacteriological Isolates in Infected Wounds of Patients Attending Tertiary Care Hospital in Central India. Int.J.Curr.Microbiol.App.Sci. 2015;4(5):711-19.

Journal of Microbiology and Related Research
Volume 4 Number 2, July - December 2018
DOI: http://dx.doi.org/10.21088/jmrr.2395.6623.4218.3

# Speciation and Antibiogram of Enterococci in a Tertiary Care Centre with Special Reference to VRE

**Shakthi R.[1], Venkatesha D.[2]**

**Author Affiliation**
[1]Assistant Professor [2]Professor and Head, Department of Microbiology, Adichunchanagiri Institute of Medical Sciences, B.G. Nagara, Mandya, Karnataka 571448, India.

**Corresponding Author**
**Shakthi R.,**
Assistant Professor, Department of Microbiology, Adichunchanagiri Institute of Medical Sciences, B.G. Nagara, Mandya, Karnataka 571448, India.

**E-mail:** shakthi.rals@yahoo.com

**Received on** 31.07.2018,
**Accepted on** 17.09.2018

**Abstract**

*Background: Enterococci* are the normal commensals of the oral cavity, gastrointestinal tract and vagina. They have emerged as serious nosocomial pathogens and its multidrug resistance as a cause for concern. This study is undertaken to isolate and speciate *Enterococci* from various clinical specimens by biochemical methods and to study the antibiogram. *Methodology:* A total of 100 isolates from the clinical specimens like urine, pus, blood and body fluids were processed in the department of Microbiology, Adichunchanagiri Institute of Medical Sciences, B.G. Nagara, for a period of one and half year. The isolates were speciated by using conventional tests and antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method. Confirmation of vancomycin susceptibility was done by the Epsilometer test (E test) *Results:* Out of 100 *Enterococcal* isolates, majority were from age group of 51-60 years (24%) and male (58%). Most common species isolated was *E.faecalis* (74%) followed by *E.faecium* (26%). All isolates were sensitive to Teicoplanin and Linezolid. Majority of the isolates were resistant to *Erythromycin* and *Ciprofloxacin.* 20 isolates were intermediately sensitive to *Vancomycin* by the Kirby Bauer disc diffusion method. All intermediately sensitive isolates to *Vancomycin* were further tested by the E test and they were found to be *Vancomycin* sensitive. *Conclusion:* There is an increase in the rate of infection and antibiotic resistance in the *Enterococcus* species. The emergence of *Vancomycin* resistant *Enterococci* (VRE) presents a serious challenge for clinicians treating the patients and the Kirby Bauer disc diffusion method is not an accurate method for detecting the VRE.

**Keywords:** *Enterococcus*; Speciation; VRE.

## Introduction

*Enterococci* are one of the emerging nosocomial infections. *Enterococci* can present as commensals forming the indigenous flora of intestinal tract, oral cavity and vagina [1]. The most frequent infections caused by them are urinary tract infections [UTIs] followed by wound infections, biliary tract infections, intra-abdominal infection, rarely septicaemia, meningitis, endocarditis, bacteraemia and pelvic infection [2].

Although many of *Enterococcus* species have been identified, only two namely *E.faecalis, E.faecium* are responsible for 95% of human infections caused by *Enterococci*. The other common species include *E.durans, E.casseliflavus, E.avium, E.gallinarum, E.hirae*, *E.mundtii*, *E.malodoratus* and *E.salitorius*.

They exhibit both intrinsic and acquired resistance to aminoglycoside and cephalosporin. The emergence of vancomycin resistant *Enterococci* (VRE) in addition to the increasing incidence of high level aminoglycoside resistance (HLAR), presents a serious challenge for clinicians treating the patients with infections due to *Enterococci*. So it is important to monitor resistance pattern for *Enterococci* in hospital regularly [2-4]. The aim of our study was to determine the prevalence of *Enterococcus* from various clinical specimens and to

determine the antibiogram with special reference to the vancomycin susceptibility.

## Methodology

A total of 100 isolates from the clinical specimens like urine, pus, blood and body fluids from the patients attending AH & RC, B.G. Nagara, were processed in the department of Microbiology, Adichunchanagiri Institute of Medical Sciences, B.G. Nagara., for a period of one and half year. The specimens were inoculated onto Blood agar, MacConkey agar and were incubated overnight at 37ºC. Presumptive identification was done by using standard protocol like gram staining, catalase test, bile esculin test, heat and salt tolerance test and α- pyrrolidonyl β- naphthylamide (PYR) test [Figure 1 & 2]. Speciation was done using sugar fermentation test, arginine hydrolysis, growth in pyruvate broth, motility and pigment production [Figure 3 & 4] [2,5].

The antimicrobial susceptibility testing was performed by the Kirby Bauer disc diffusion method by using commercially available antimicrobial discs (Himedia®) like Ampicillin (10µg), vancomycin (30µg), Erythromycin (15µg), Tetracycline (30µg), ciprofloxacin (5µg), High level gentamicin (120µg), teicoplanin (30µg) and linezolid (30µg). The results were interpreted as per the CLSI guidelines using *E.faecalis* ATCC29212 as the control strains. The MIC of *Vancomycin* was determined by the E test for all the *Enterococci* isolates which showed intermediate sensitivity by the Kirby Bauer disc diffusion method [Figure 5]. The zone of inhibition was observed in the form of an ellipse and interpreted as per the CLSI guidelines [6].


**Fig. 1:** Bile-esculin agar showing black colored *Enterococcus* colonies.

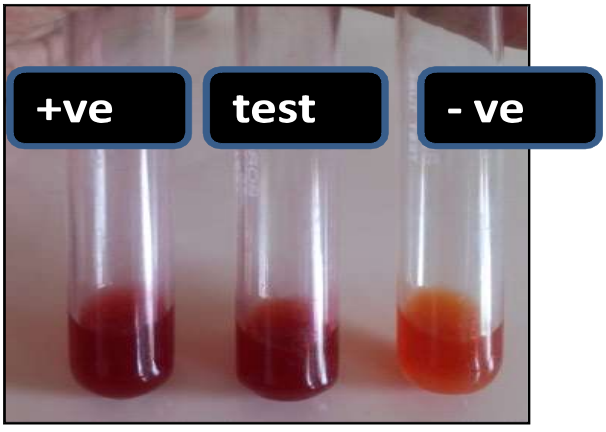


**Fig. 2:** PYR test.

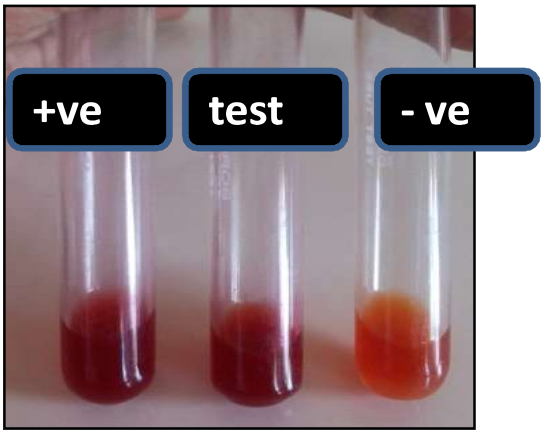


**Fig. 3:** Arginine hydrolysis by *E.faecalis.*

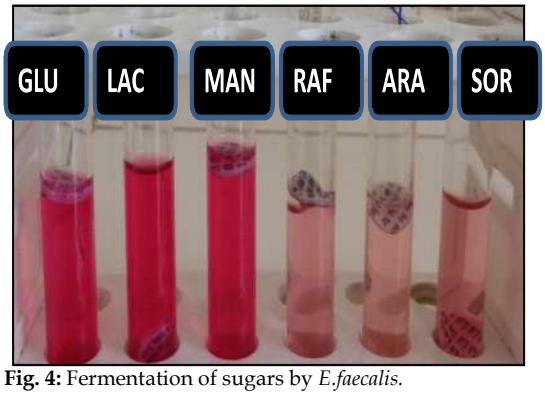


**Fig. 4:** Fermentation of sugars by *E.faecalis.*

**Fig. 5:** MIC for *Vancomycin* by E –test.

## Results

Out of 100 *Enterococcal* isolates, majority were in the age group of 51-60Years 24 (24%) and male 58 (58%). Age and gender wise distribution is shown in Table 1.

Majority of isolates were from urine sample (52%) and patients admitted in Medicine ward (40%). The common species isolated was *E.faecalis* (74%) followed by *E.faecium* (26%). All *Enterococcal* isolates were sensitive to Teicoplanin and Linezolid and Antibiotic susceptibility pattern of *Enterococci* is shown in Table 2. 20% of isolates showed an intermediate sensitivity to *Vancomycin* by the Kirby Bauer disc diffusion method. These isolates were sensitive to vancomycin; with a MIC of less than 4µg which was determined by E test [Table 3].

**Table 1:** Age and gender wise distribution

| Age in Years | Male (%) n=58 | Female (%) n=42 | Total (%) n=100 |
|---|---|---|---|
| 0-10 | 10 (17.2) | 5 (11.9) | 15 (15) |
| 11-20 | 1 (1.7) | 3 (7.14) | 4 (4) |
| 21-30 | 5 (8.6) | 7 (16.6) | 12 (12) |
| 31-40 | 4 (6.9) | 5 (11.9 ) | 9 (9) |
| 41-50 | 13 (22.4) | 5 (11.9) | 18 (18) |
| 51-60 | 16 (27.5) | 8 (19.04) | 24 (24) |
| 61-70 | 8 (13.8) | 7 (16.6) | 15 (15) |
| 71-80 | 1 (1.7) | 2 (4.8) | 3 (3) |
| Total | 58 (58) | 42 (42) | 100(100) |

**Table 2:** Antibiotic susceptibility pattern of *Enterococcal* species

| Antibiotics | *E.faecalis* (n=74) | | *E.faecium* (n=26) | |
|---|---|---|---|---|
| | Sensitive n (%) | Resistance n (%) | Sensitive n (%) | Resistance n (%) |
| Ampicillin | 60 (81) | 14 (18.9) | 7 (27) | 19 (73) |
| Teicoplanin | 74 (100) | 0 (0) | 26 (100) | 0 (0) |
| Tetracycline | 38 (51.4) | 36 (48.6) | 11 (42.3) | 15 (57.7) |
| Erythromycin | 24 (32.4) | 50 (67.6) | 2 (7.7) | 24 (92.3) |
| Ciprofloxacin | 25 (33.8) | 49 (66.2) | 3 (11.5) | 23 (88.5) |
| Linezolid | 74 (100) | 0 (0) | 26 (100) | 0 (0) |
| High level gentamicin | 60 (81) | 14 (18.9) | 7 (27) | 19 (73) |

**Table 3:** *Vancomycin* susceptibility testing

| Sensitivity pattern | Kirby Bauer disc diffusion method (%) | E-test (%) |
|---|---|---|
| Sensitive | 80 | 100 |
| Intermediate Sensitive | 20 | 0 |
| Resistant | 0 | 0 |

## Discussion

*Enterococci* are commensals of the gastrointestinal tract of human beings. Although a dozen of *Enterococcus* species have been identified, only two are responsible for the majority of human infections, i.e., *E.faecalis* and *E.faecium*. Over the past two decades they have become important nosocomial pathogens probably due to inherent resistance to antibiotics (cephalosporins), ability to adhere to indwelling medical devices and ability to survive adverse environmental conditions [7]. The emergence of VRE is a cause of concern, since they are very difficult to treat and control. Correct speciation is very important since there is variation in resistance to antibiotics by particular *Enterococcal* species [8].

In the present study, the majority of *Enterococca*l isolates were from urine sample (52%) which is correlating with the study of Shinde RS et al. (53%).

*E.faecalis* (74%) is the predominant species isolated followed by *E.faecium* and which is comparable with the study of Desai PJ et al. (49.5%), Chakraborthy A et al. (90.85%) and Toledo C et al. (82.6 %)[8-11].

Majority of *Enterococci* showed resistance to *Erythromycin* (74%) and *Ciprofloxacin* (72%), which is comparable with the study of Parameswarappa J et al [12].High level gentamicin resistance was seen in 33% of isolates, which is comparable with Shinde RS et al. (44%) and Toledo C et al. (48.6%) [9,11]. 20% of the *Enterococcus*, which showed intermediate sensitivity to vancomycin by the Kirby Bauer disc diffusion method, were further tested by E test. The isolates were found to be sensitive to *Vancomycin* by E test.

*The inaccuracy of the disk diffusion method has resulted in an unwarranted utilization of this drug as a part of the treatment regimens. Therefore, a routine MIC monitoring of important antibiotics like vancomycin has to be done, before reporting it as resistant or intermediately sensitive[13].*

## Conclusion

The *Enterococcus* species have now emerged as nosocomial pathogens. Hence, it is important to know the changing patterns of the *Enterococcus* infections and the antimicrobial susceptibility patterns of the isolates. In addition to the increasing incidence, emergence of VRE presents a serious challenge for clinicians. Thus proper isolation, identification and knowing antibiotic susceptibility pattern will help in the early identification of resistant isolates and preventing their spread.

It is the role of the microbiologists to give prompt reports by using appropriate procedures in laboratories and to prevent the emergence of resistance by taking standard precautions and formulation of antibiotic policy in the institution for the proper use of antimicrobials by physicians [1].

## References

1. Sreeja S, Babu PRS, Prathab AG. The Prevalence and the Characterization Of The *Enterococcus* Species From Various Clinical Samples In A Tertiary Care Hospital. J Clin Diag Res 2012;6(9):1486-8.
2. Winn WJ, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P et al. Streptococci, *Enterococci* and the "Streptococcus – like" bacteria. In: Koneman's color atlas and textbook of diagnostic microbiology Sixth ed. Philadelphia: Lipincott Williams and Wilkins; 2006.pp.674-745.
3. Murray BE. The Life and the Times of The *Enterococci*. Clin Microbiol Rev 1990;3:46-65.
4. Sood S, Malhotra M, Das BK, Kapil A. *Enterococcal* Infections and Antimicrobial Resistance. Indian J Med Res 2008;111-21.
5. Ross PW. Streptococci and *Enterococci*. Mackie and McCartney's Practical Medical Microbiology.14th ed. Elsiever; 2006:268-9.
6. Clinical and Laboratory Standard Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI document M100-S25. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA. 2015.
7. Qamer S, Sandoe JAT and Kerr KG. Use of Colony Morphology to Distinguish Different *Enterococcal* Strains and Species in Mixed Culture from Clinical Specimens. J Clin Microbiol 2003;41(6):2644–6.
8. Desai PJ, Pandith D, Mathur M, Gogate A. Prevalence, Identification and distribution of various species of *Enterococci* isolated from clinical samples with special reference to urinary tract infections in catheterized patients. Indian J Med Microbiol 2001;19:132-7.
9. Shinde RS, Koppikar GV, Oommen S. Characterization and antimicrobial susceptibility pattern of clinical isolates of *Enterococci* at a tertiary care hospital in Mumbai, India. Ann Trop Med Public Health 2012;5:85-8.
10. Chakraborty A, Pal NK, Sarkar S and Gupta MS. Antibiotic resistance pattern of *Enterococci* isolates from Nosocomial infections in a tertiary care hospital in Eastern India. J Nat Sci Biol Med 2015; 6(2):394-7.
11. Toledo C, Perez ME, Rocchi M, Gribaudo G, Mangiaterra S, Monterisi A. Isolation of *Enterococci* species causative of infections and sensitivity to antimicrobial drugs. Rev Agent Microbiol 2004;36(1):3-5.
12. Parameswarappa J, Basavaraj VP, Basavaraj CM. Isolation, Identification and antibiogram of *Enterococci* isolated from patients with urinary tract infection. Ann Afr Med 2013;12:176-81.
13. Karmarkar MG, Gershom SE, Mehta PR. The Enterococcal infections with a special reference to the phenotypic characterization and the drug resistance. Indian J Med Res 2004;119:22-25.

Journal of Microbiology and Related Research
Volume 4 Number 2, July - December 2018
DOI: http://dx.doi.org/10.21088/jmrr.2395.6623.4218.4

# Colistin Resistance amongst Non-Fermenters in the Hospital Setting: A Lurking Threat

**Yogita Verma[1], S Suguna Hemachander[2], Krunal Shah[3]**

**Author Affiliation**
[1]Assistant Professor [2]Professor and Head [3]Associate Professor, Department of Microbiology, Smt. B K Shah Medical Institute and Research Centre, Pipariya, Vadodara, Gujarat 391760, India.

**Corresponding Author**
**Yogita Verma,**
Assistant Professor, Department of Microbiology, Smt. B K Shah Medical Institute and Research Centre, Pipariya, Vadodara, Gujarat 391760, India.

**E-mail:** yogitaverma2004@gmail.com

**Received on** 15.10.2018,
**Accepted on** 31.10.2018

**Abstract**

*Introduction:* Non-fermenters have gained significance as etiological agents of mild to potentially life threatening healthcare associated infections in recent years. Pseudomonas and Acinetobacter are amongst the commonest non fermenters causing infections in a hospital setting. The aim of this study was to find the prevalence of colistin resistance in Pseudomonas and Acinetobacter isolates from inpatients. *Methodology:* This retrospective study was done in our hospital which is a tertiary care centre, from April 2016 to March 2017. Identification and antibiotic susceptibility testing was done by VITEK®2 system (BioMerieux, North Carolina/USA). Antibiotic susceptibility results were interpreted according to the criteria of Clinical Laboratory Standards Institute M100S (26th edition). Patient information and microbiological profile of the organism isolated was recorded. For statistical analysis, data was described in terms of fractions and percentages and percentages were used to compare the data in two sets. *Results:* The most common isolates amongst non fermenters were Acinetobacter sp.(45.93%), and Pseudomonas sp. (41.62%). 92.13% of the Acinetobacter baumannii isolates were MDR and 88.76% were resistant to carbapenems. Sixty-four percent of Acinetobacter baumannii isolates were susceptible to colistin and tigecycline only and 7.86% were resistant to colistin. In case of Pseudomonas aeruginosa, 74.67% isolates were MDR and 56% were resistant to carbapenems. Nearly 77.33% isolates were susceptible, 5.33% were intermediate 17.34% were resistant to colistin. The colistin resistant isolates of Acinetobacter baumannii and Pseudomonas aeruginosa were predominantly isolated from endotracheal aspirate (57.14%) and pus (61.53%) samples respectively. Overall 5 out of 23 (21.74%) colistin resistant isolates were resistant only to colistin and were susceptible to all other antibiotics tested. *Conclusion:* Increasing role of non fermenters as pathogens in the hospital settings is worrying. Judicious use of antibiotics is needed to curb the high antibiotic resistance amongst non-fermenters.

**Keywords:** Colistin Resistance; Acinetobacter; Pseudomonas; Non Fermenters.

## Introduction

Non fermenters are taxonomically diverse gram negative bacilli which utilise glucose oxidatively or not at all. They exist as environmental saprophytes or commensals in the human gut [1,2]. Lately, they have gained much significance as etiological agents of mild to potentially life threatening healthcare associated infections. These infections include device related infections, urinary tract infections, surgical site infections, pneumonia, bacteremia and sepsis [3,4]. *Pseudomonas and Acinetobacter* are amongst the commonest non fermenters causing infections in a hospital setting [5].

Antibiotic resistance amongst non-fermenters is an increasingly menacing situation. *Pseudomonas*

and *Acinetobacter* are increasingly becoming resistant to cephalosporins, carbapenems and other drugs commonly used for treating infections caused by them [6]. Colistin is a commonly used therapeutic drug for MDR *Pseudomonas* and *Acinetobacter* and is a drug of last resort in many such cases [7]. Due to this increased use, colistin resistance in these organisms has surfaced. High rates of colistin resistance in *Pseudomonas* and *Acinetobacter* have been reported in recent times [8].

Therefore, it is important that the antibiotic susceptibility profile of non-fermenters in a hospital is known and the antibiotic policy is updated accordingly. The aim of this study was to find the prevalence of colistin resistance in *Pseudomonas* and *Acinetobacter* isolates from inpatients.

## Materials and methods

### *Study design*

This retrospective study was done in our hospital which is a tertiary care centre in western India over a period of one year (April 2016 to March 2017). All the samples from inpatients sent for culture to the microbiology laboratory were included in the study. Samples studied were sputum, endotracheal aspirate, broncho-alveolar lavage (BAL), pus, urine, blood, CSF (cerebrospinal fluid), pleural fluid, pericardial fluid and ascitic fluid. Samples from patients of both sexes and all ages were included. Samples from patients with HIV and patients receiving immunosuppressive drugs were excluded from the study. Repeated isolates from a single patient were excluded from the final analysis. patient records were reviewed for relevant details.

### *Microbiological analysis*

*Culture*: Culture was done on Blood agar and MacConkey agar (Hi-Media, Mumbai, India) using standard technique. In case of urine, cultures showing a significant growth of $\geq 10^5$ CFU/ml were further processed. Cultures with mixed growth on the culture media were excluded.

*Identification and AST*: Identification of isolates and analysis of their antibiotic susceptibility patterns was done by automated VITEK®2 system (BioMerieux, North Carolina/USA), as per the manufacturer's instructions. Antibiotic susceptibility results were expressed as susceptible, intermediate or resistant according to the criteria of Clinical Laboratory Standards Institute M100S, 26th edition (2016) [9]. For the purpose of quality control, *Escherichia coli* (ATCC 25922) was used.

*MDR*: Isolates resistant to three or more classes of antibiotics were considered as multi drug resistant (MDR).

*CRB*: Isolates resistant to both Imipenem and Meropenem were considered as Carbepenem Resistant Bacteria(CRB).

### *Statistical analysis*

Data was described in terms of fractions and percentages. Percentages were used to compare the data in two sets.

*Ethical considerations:* The study was approved by the institutional ethics committee (SVIEC/ OW/17014)

## Results

### *Baseline characteristics*

A total of 1630 samples from inpatients were processed in the study period and 947 were culture positive. Seven hundred and eighty-four isolates had gram-negative bacterial growth, out of which 209 were non fermenters. The most common isolates amongst non fermenters were *Acinetobacter* sp. (45.93%), *Pseudomonas* sp. (41.62%) followed by *Burkholderia cepacia* group, *Sphingomonas*, *Myroides* and others [Table1].

Study population consisted of 134 (64.11%) males and 75 (35.89%) females. The mean age of patients was 42 years. Samples with non-fermenter isolation were received from ICU, Surgical wards, Orthopaedics wards, OT recovery ward, NICU, PICU, Neurosurgical ICU and respiratory medicine wards amongst others [Figure 1]. Respiratory tract samples were the most common (98/209) followed by pus samples (79/209), blood samples (15/209), urine (8/209), ascitic fluid (5/209), pleural fluid (2/209) and CSF (2/209). Out of the 98 respiratory tract samples, 55 were endotracheal aspirates, 40 were sputum samples and 2 were BAL samples. Fifty three patients were diagnosed with Ventilator associated pneumonia (VAP), 68 with surgical site infections (SSI), 14 with Blood stream Infection (BSI), 8 with UTI, and 2 with meningitis.

### *Antibiotic susceptibility pattern*

*Acinetobacter baumannii* was the most common species of *Acinetobacter* isolated. Eighty two out of 89 (92.14%) *Acinetobacter baumannii* isolates were MDR and 79/89 (88.76%) were resistant to carbapenems [Table 2]. A total of 57/89 (64%) were susceptible to colistin and tigecycline only.

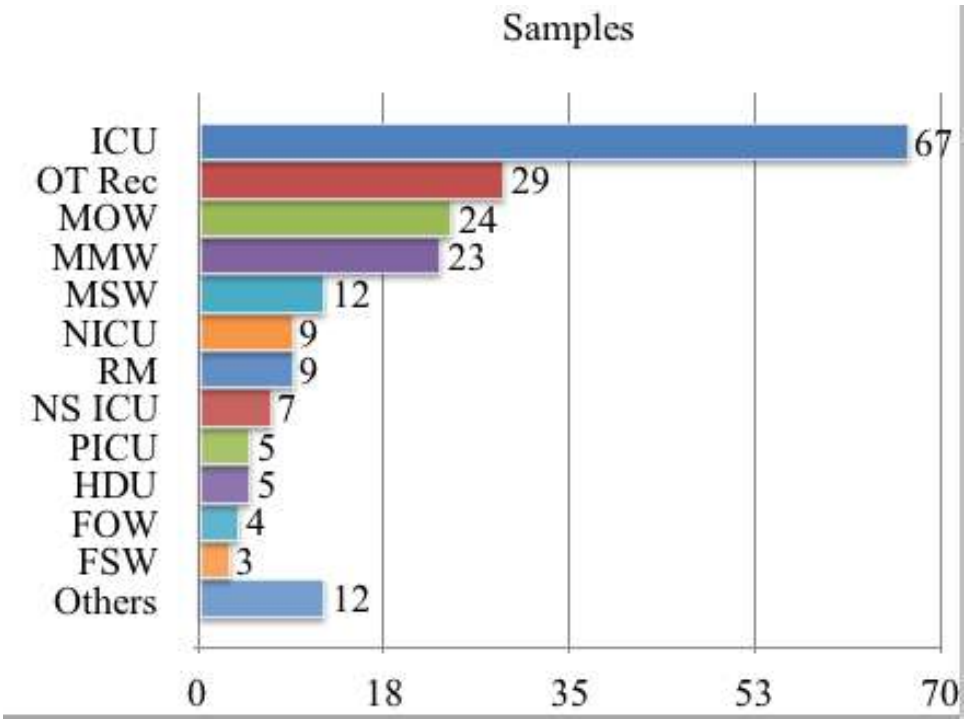


**Fig. 1:** Distribution of NFGNB isolation from wards in which patients were admitted during the study period.

ICU= Intensive care unit, OT Rec = OT recovery, MOW= Male orthopaedics ward, MMW= Male medicine ward, MSW= Male surgical ward, NICU= Neonatal intensive care unit, RM= Respiratory medicine, NS ICU= Neurosurgery intensive care unit, PICU= Paediatric intensive care unit, HDU= high dependency unit, FOW= Female orthopaedics ward, FSW= Female surgical ward.

**Table 1:** Non fermenters isolated from inpatients during the study period (n=209).

| S.No | Isolate | Total |
|---|---|---|
| 1 | Acinetobacter | |
| | Acinetobacter baumannii | 89 |
| | Acinetobacter lwoffii | 4 |
| | Acinetobacter junii | 2 |
| | Acinetobacter haemolyticus | 1 |
| 2 | Pseudomonas | |
| | Pseudomonas aeruginosa | 75 |
| | Pseudomonas flourescens | 6 |
| | Pseudomonas putida | 2 |
| | Pseudomonas mendocina | 2 |
| | Pseudomonas stutzeri | 1 |
| | Pseudomonas luteola | 1 |
| 3 | Burkholderia cepacia group | 12 |
| 4 | Sphingomonas paucimobilis | 6 |
| 5 | Myroides sp. | 3 |
| 6 | Elizabethkingia meningoseptica | 2 |
| 7 | Others | 3 |
| | | 209 |

**Table 2:** Antibiotic resistance amongst Pseudomonas and Acinetobacter(n=183)

| S.No | Isolate | Number of Isolates | MDR | CRB | Resistant to Colistin | Susceptible to Colistin |
|---|---|---|---|---|---|---|
| 1 | Acinetobacter | | | | | |
| 1(a) | Acinetobacter baumannii | 89 | 82 (92.14%) | 79 (88.76%) | 7 (7.86%) | 82 (92.14%) |
| 1(b) | Acinetobacter lwoffii | 4 | 2 (50%) | 1 (25%) | 0 (0) | 4 (100%) |
| 1(c) | Acinetobacter junii | 2 | 0 (0) | 0 (0) | 0 (0) | 2 (100%) |
| 1(d) | Acinetobacter haemolyticus | 1 | 1 (100%) | 1 (100%) | 0 (0) | 1 (100%) |
| 2 | Pseudomonas | | | | | |
| 2(a) | Pseudomonas aeruginosa | 75 | 56 (74.67%) | 42 (56%) | 13 (17.34%) | 58 (77.33%) |
| 2(b) | Pseudomonas flourescens | 6 | 3 (50%) | 3 (50%) | 2 (33.34%) | 4 (66.66%) |
| 2(c) | Pseudomonas putida | 2 | 1 (50%) | 1 (50%) | 0 (0) | 2 (100%) |
| 2(d) | Pseudomonas mendocina | 2 | 0 (0) | 0 (0) | 0 (0) | 2 (100%) |
| 2(e) | Pseudomonas stutzeri | 1 | 1 (100%) | 0 (0) | 0 (0) | 1 (100%) |
| 2(f) | Pseudomonas luteola | 1 | 0 (0) | 0 (0) | 1 (100%) | 0 (0%) |
| | Total | 183 | 146 (79.78%) | 127 (69.40%) | 23 (12.57%) | 156 (85.24%) |

MDR= Multi Drug Resistant, CRB= Carbapenem Resistant Bacteria

It was observed that 82/89 (92.14%) isolates were susceptible to colistin and 7/89 (7.86%) isolates were resistant to colistin with an MIC of 4µg/ml in 2 cases and ≥16µg/ml in 5 cases. However, amongst the 82 susceptible isolates, 5 (6.1%) had a higher MIC of 2µg/ml as compared to the rest with MIC ≤0.5µg/ml.

In case of *Pseudomonas aeruginosa*, 56/75 (74.67%) isolates were MDR and 42/75 (56%) were resistant to carbapenems [Table 2]. Eighteen of 75 (24%) isolates were susceptible to colistin only. It was observed that 58/75 (77.33%) isolates were susceptible, 4/75 (5.33%) were intermediate (MIC=4µg/ml) and 13/75 (17.34%) were resistant (MIC ≥16µg/ml) to colistin. Out of the 58 sensitive isolates, 56 had MIC≤0.5µg/ml and 4 had an MIC of 2µg/ml. One third (2/6) of *Pseudomonas flourescens* and 1/1 (100%) of *Pseudomonas luteola* isolates were resistant to colistin (MIC≥16µg/ml). All Isolates of *Burkholderia, Myroides, Elizabethkingia* and *Sphingomonas* were resistant to colistin

*Characteristics of the colistin resistant isolates*

Colistin resistant isolates of *Pseudomonas aeruginosa* were predominantly isolated from patients with SSI (8/13-61.53%), VAP(3/13-23.07%), BSI (1/13-7.7%) and UTI (1/13-7.7%). It was observed that 11/13 (84.61%) of the colistin resistant isolates of *Pseudomonas aeruginosa* were multi drug resistant and 10/13 (76.92%) were resistant to carbapenems. Highest concomitant resistance was seen to tigecycline, ciprofloxacin and TMP-SMX (92.30% each). However, 5/13 (38.46%) isolates were susceptible to cefepime [Table 3].

One colistin resistant isolate of *Pseudomonas fluorescens* was concomitantly resistant to all other antibiotics tested and another was susceptible to tigecycline and TMP-SMX only. The colistin resistant isolate of *Pseudomonas luteola* was susceptible to all other antibiotics tested.

The colistin resistant isolates of *Acinetobacter baumannii* were isolated from patients with VAP (4/7-57.14%), SSI (2/7-28.57%) and BSI (1/7-14.29%). Five out of 7 (71.42%) were multi drug resistant and 4/7 (57.14%) were resistant to carbapenems. High concomitant resistance to amoxycillin-clavulinic acid, amikacin, ciprofloxacin, aztreonam and TMP-SMX (71.42% each) was observed. However unlike *Pseudomonas aeruginosa*, 5/7 (71.42%) isolates were susceptible to tigecycline [Table 3].

Overall 5 out of 23 (21.74%) colistin resistant isolates were resistant only to colistin and were susceptible to all other antibiotics tested. These

**Table 3:** Antibiotic susceptibility patterns of colistin resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*

| S.no | Antibiotic tested | Acinetobacter baumannii(n=7) | | | Pseudomonas aeruginosa(n=13) | | |
|---|---|---|---|---|---|---|---|
| | | S | I | R | S | I | R |
| 1 | Amoxicillin-clavulinic acid | 2 | 0 | 5 | 2 | 0 | 11 |
| 2 | Piperacillin -tazobactam | 3 | 0 | 4 | 2 | 2 | 9 |
| 3 | Cefepime | 4 | 0 | 3 | 5 | 2 | 6 |
| 4 | Imipenem | 3 | 0 | 4 | 3 | 0 | 10 |
| 5 | Meropenem | 2 | 1 | 4 | 3 | 0 | 10 |
| 6 | Amikacin | 2 | 0 | 5 | 2 | 0 | 11 |
| 7 | Gentamicin | 1 | 2 | 4 | 2 | 0 | 11 |
| 8 | Ciprofloxacin | 2 | 0 | 5 | 1 | 0 | 12 |
| 9 | Tigecycline | 5 | 0 | 2 | 1 | 0 | 12 |
| 10 | Aztreonam | 2 | 0 | 5 | 3 | 0 | 10 |
| 11 | TMP-SMX | 2 | 0 | 5 | 1 | 0 | 12 |

S= Sensitive, I= Intermediate, R=Resistant.

included 2 *Acinetobacter baumannii* (MIC>16ug/ml), 2 *Pseudomonas aeruginosa* (MIC>16ug/mg) and 1 *Pseudomonas luteola* (MIC=8ug/ml) isolates. These were isolated from pus (3), blood (1) and endotracheal aspirate (1).

## Discussion

The isolation rate of non fermenters was 12.82% (209/1630) in this study. This is similar to a 12.8% isolation rate reported by Rit et al. [10]. Nearly one fourth of the gram-negative bacterial infections in hospital setting were caused by non fermenters according to this study. This is much higher as compared to recently reported results (11.6%) by Grewal et al. [11]. This is probably because their study included analysis of all the isolates including outpatients and inpatients whereas our study focussed only on inpatients. *Acinetobacter* and *Pseudomonas* were the most common non fermenters isolated. We observed that *Acinetobacter* was the most common isolate (45.93%) amongst non fermenters as opposed to *Pseudomonas* as found by studies published in 2009 and 2013 [5,10]. However in a study published from Dehradun, India, in 2017, *Acinetobacter* was the most common non fermenter isolated (63.63%) followed by *Pseudomonas*, similar to our study [12].

Our results show that 92.13% of the *Acinetobacter baumannii* isolates were MDR and 88.76% were CRB. This rate of MDR *Acinetobacter baumanii* is much higher than that reported by Grewal et al. [11] (64.71%) from India and Cai et al. [13] (72.23%) from China. This could be attributed to different patient populations studied. Other studies from India [12,14] have however reported a high resistance to carbapenems (90.5%-100%) in *Acinetobacter baumanii*, similar to our results.

Colistin resistance was found to be 7.86% in *Acinetobacter baumanii* in the present study. World over the colistin resistance in *Acinetobacter* varies from region to region and amongst different patient groups. Colistin resistance rates as less as 1.4% from Brazil to as high as 40% from Spain have been reported [15,16]. Taneja et al. [17] reported 3.5% colistin resistance in *Acinetobacter baumanii* in 2011. However, in a more recently published study (Behera et al. 2017) [18], the resistance rate was 7% in *Acinetobacter baumanii*, similar to our study results. Interestingly, Gupta et al. [14] reported 53% resistance to colistin in *Acinetobacter baumanii* isolated from ventilator associated pneumonia patients.

Three-fourth (74.67%) of *Pseudomonas aeruginosa* isolates were found to be MDR and more than half (56%) were CRB in our study. Various studies have explored susceptibility of *Pseudomonas aeruginosa* to imipenem and the resistance rates ranging from 8.2% to 90% have been reported in the past five years from different regions of India [10,19,20]. Agarwal S. et al. [12] recently published imipenem resistance rates of 52% in *Pseudomonas* and 90% in *Acinetobacter*, which resonates with our results.

Colistin resistance rates in Pseudomonas were much higher (17.34%) than *Acinetobacter* (7.86%) in the present study. Colistin resistance in *Pseudomonas aeruginosa* is also variable in reports from different Indian hospitals. Wattal et al. [6] reported 8% colistin resistance in ICU samples from north India whereas Ramesh et al. [21] reported colistin resistance of 30% from two south Indian hospitals. High resistance to carbapenems is leading to increased use of colistin

as a therapeutic agent in many hospitals including ours which in turn is leading to development of colistin resistance. There are multiple mechanisms of colistin resistance in *Pseudomonas* and *Acinetobacter* and the understanding of the same is still evolving. The most important mechanisms of colistin resistance in *Acinetobacter baumannii* are loss of LPS, modification of lipid A with phosphoethanolamine and glysosylation of Lipid A with hexosamine [22,23,25]. Mechanisms of colistin resistance in *Pseudomonas aeruginosa* are alteration of LPS composition, overexpression of outer membrane protein OprH and activation of LPS modifying operons by mutations in two component systems [24,25]. Qureshi et al. [26] concluded that colistin-resistant *Acinetobacter baumannii* occurred almost exclusively among patients who had received colistin methansulfonate for treatment of carbapenem-resistant, colistin-susceptible *Acinetobacter baumannii* infection and Lipid A modification by the addition of phosphoethanolamine accounted for their colistin resistance. Therefore colistin usage appears to be single most important driving force for the development of colistin resistance. Nearly 20% of our colistin resistant isolates were resistant only to colistin and susceptible to all other antibiotics tested. This may have been driven by excess use of colistin by clinicians in the hospital setting and is a worrisome trend.

MDR non fermenters are a lurking threat in almost every hospital setting now, compounded by the ever increasing colistin resistance. Non fermenters can spread from one patient to another and are capable of causing outbreaks of serious infections. Resource limited countries like India struggle to keep up with ideal hospital infection control protocols, so it is even more dangerous in such settings. Thus the need to reduce colistin resistance cannot be emphasized more. The cornerstones of such an approach are judicious use of colistin and strict antibiotic stewardship. De-escalation after obtaining culture results is of paramount importance. Recent CLSI guidelines recommend that for the treatment of *Pseudomonas* and *Acinetobacter baumannii* complex, Colistin should be administered with a loading dose and at the maximum recommended doses, in combination with other agents [27]. Therefore, continuous efforts towards curtailing colistin resistance must be in place lest we are thrown back to the pre-antibiotic era.

*Limitations of the study:* Molecular tests to elucidate mechanisms of resistance in the colistin resistant isolates especially those only resistant to colistin, were unavailable at our institute. Therefore the mechanism of colistin resistance could not be commented upon.

*Future directions*: Larger longitudinal studies to monitor the epidemiology and mechanisms of colistin resistance amongst non fermenters may be taken up. It will be very useful if the trend of colistin resistance is monitored with respect to actual colistin usage in the hospital setting.

## Conclusion

Increasing role of non fermenters as pathogens in the hospital settings is worrying. Judicious use of antibiotics is needed to curb the high antibiotic resistance amongst non-fermenters.

## Acknowledgements

We acknowledge Ms. Dharmishta Rajput and Ms. Rachnaben Rathod for their technical support.

## References

1. Rubin SJ, Granato PA, Wasilauskas BL. Glucose nonfermenting Gram negative bacteria. In: Lennette EH, Balows A, Hausler WJ Jr, et al., eds. Manual of Clinical Microbiology. 4th ed. Washington, D.C: American Society for Microbiology, 1985:330–49.
2. Nonfermenting Gram negative bacilli. In: Winn W Jr, Allen S, Janda W, Koneman E, et al., eds. In: Koneman's Color Atlas and textbook of Diagnostic Microbiology. 6th ed. USA: Lippincott Williams and Wilkins Company, 2006:305–91.
3. Steinberg JP, Rio DC. Gram negative and Gram variable bacilli. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious diseases. 6th ed. Vol. 2. Philadelphia, USA: Elsevier Publication, 2005:2751–68.
4. Gales AC, Jones RN, Forward KR, et al. Emerging importance of multidrug-resistant *Acinetobacter* species and *Stenotrophomonas maltophilia* as pathogens in seriously ill patients: Geographic patterns, Epidemiological features, and trends in the SENTRY antimicrobial surveillance program (1997-1999) Clin Infect Dis. 2001;32:104–13.
5. Malini A, Deepa E, Gokul B, et al. Nonfermenting Gram-Negative Bacilli Infections in a Tertiary Care Hospital in Kolar, Karnataka. J Lab Physicians. 2009;1(2):62-6.
6. Wattal C, Goel N, Oberoi JK. Surveillance of Multidrug Resistant Organisms in a Tertiary Care

Hospital in Delhi, India. J Assoc Physicians India 2010;58:S32-S36.

7. Li J, Nation RL, Turnidge JD, et al. Colistin: the re-emerging antibiotic for multidrug-resistant gram-negative bacterial infections. Lancet Infect Dis. 2006;6:589–601.
8. Dhariwal AK, Tullu MS. Colistin: Re-emergence of the 'forgotten' antimicrobial agent. J Postgrad Med 2013;59:208-15.
9. Performance Standards for Antimicrobial Susceptibility Testing, 26th Edition. Wayne: CLSI; 2016. Clinical and Laboratory Standards Institute.
10. Rit K, Nag F, Raj HJ, et al. Prevalence and susceptibility profiles of nonfermentative gram-negative bacilli infection in a tertiary care hospital of Eastern India. Indian J Clin Pract 2013;24:451-55.
11. Grewal US, Bakshi R, Walia G, et al. Antibiotic susceptibility profiles of non-fermenting gram-negative Bacilli at a Tertiary Care Hospital in Patiala, India. Niger Postgrad Med J. 2017;24(2):121-5.
12. Agarwal S, Kakati B, Khanduri S, et al. Emergence of Carbapenem Resistant Non-Fermenting Gram-Negative Bacilli Isolated in an ICU of a Tertiary Care Hospital.J Clin Diagn Res. 2017;11(1):DC04–DC07.
13. Cai XF, Sun JM, Bao LS,et al. Risk factors and antibiotic resistance of pneumonia caused by multidrug resistant Acinetobacter baumannii in pediatric Intensive Care Unit. World J Emerg Med 2012;3:202-7.
14. Gupta M, Lakhina K, Kamath A, et al. Colistin-resistant *Acinetobacter baumannii* ventilator-associated pneumonia in a tertiary care hospital: an evolving threat. J of Hosp Inf 2016;9(1):72 - 73.
15. Rossi F, Girardello R, Cury AP, et al. Emergence of colistin resistance in the largest university hospital complex of São Paulo, Brazil, over five years. Braz J Infect dis 2017;21(1):98–101.
16. Cai Y, Chai D, Wang R, et al. Colistin resistance of Acinetobacter baumannii: clinical reports, mechanisms and antimicrobial strategies. J Antimicrob Chemother 2012;67:1607–15.
17. Taneja N, Singh G, Singh M, et al. Emergence of tigecycline & colistin resistant Acinetobacter baumanii in patients with complicated urinary tract infections in north India. Indian J Med Res 2011;133:681-84.
18. Behera IC, Swain SK, Sahu MC. Incidence of colistin-resistant Acinetobacter baumannii in an Indian tertiary care teaching hospital. Int J of Appl Res 2017;3(12):283-86.
19. Kombade S, Agrawal GN. Study of multidrug resistant nonfermenting gram-negative bacilli in intensive care unit, Nagpur. Indian J Microbiol Res 2015;2(2):120-25.
20. Aswani J, Pattanayak M, Sao S, et al. Incidence of carbapenem resistant *Pseudomonas aeruginosa* isolated from patients with lower respiratory tract infection in intesive care units. International Journal of Scientific Research And Management. 2015;3(7):289-92.
21. Ramesh N, Prasanth M, Ramkumar S, et al. Colistin susceptibility of gram-negative clinical isolates from Tamil Nadu, India. Asian Biomedicine 2016;10(1):35-39.
22. Moffatt JH, Harper M, Harrison P, *et al.* Colistin resistance in *Acinetobacter baumannii* is mediated by complete loss of lipopolysaccharide production. Antimicrob Agents Chemother 2010;54:4971-7.
23. Pelletier MR, Casella LG, Jones JW, *et al.* Unique structural modifications are present in the lipopolysaccharide from Colistin-resistant strains of *Acinetobacter baumannii*. Antimicrob Agents Chemother 2013;57:4831-40.
24. Macfarlane EL, Kwasnicka A, Ochs MM, et al. PhoP-phoQ homologues in *Pseudomonas aeruginosa* regulate expression of the outer-membrane protein OprH and polymyxin B resistance. Mol Microbiol 1999;34:305- 16.
25. Das P, Sengupta K, Goel G, et al. Colistin: Pharmacology, drug resistance and clinical applications. J Acad Clin Microbiol 2017;19(2):77-85.
26. Qureshi ZA, Hittle LE, O'Hara JA, et al. Colistin-Resistant Acinetobacter baumannii: Beyond Carbapenem Resistance. Clin Infect Dis. 2015;60(9):1295-30.
27. Performance Standards for Antimicrobial Susceptibility Testing, 27th Edition. Wayne: CLSI; 2017. Clinical and Laboratory Standards Institute.

# Journal of Microbiology and Related Research

**Library Recommendation Form**

If you would like to recommend this journal to your library, simply complete the form below and return it to us. Please type or print the information clearly. We will forward a sample copy to your library, along with this recommendation card.

**Please send a sample copy to:**

Name of Librarian

Name of Library

Address of Library

**Recommended by:**

Your Name/ Title

Department

Address

**Dear Librarian,**

I would like to recommend that your library subscribe to the Journal of Microbiology and Related Research. I believe the major future uses of the journal for your library would provide:

1. useful information for members of my specialty.
2. an excellent research aid.
3. an invaluable student resource.

**I have a personal subscription and understand and appreciate the value an institutional subscription would mean to our staff.**

Should the journal you're reading right now be a part of your University or institution's library? To have a free sample sent to your librarian, simply fill out and mail this today!

Stock Manager
Red Flower Publication Pvt. Ltd.
48/41-42, DSIDC, Pocket-II
Mayur Vihar Phase-I
Delhi - 110 091(India)
Phone: Phone: 91-11-45796900, 22754205, 22756995, Cell: +91-9821671871
E-mail: sales@rfppl.co.in

Journal of Microbiology and Related Research
Volume 4 Number 2, July - December 2018
DOI: http://dx.doi.org/10.21088/jmrr.2395.6623.4218.5

# Occurrence of ESBL, AmpC and Carbapenemase Producers among Enterobacteriaeceae in Rural Tertiary Care Hospital

**Sharath Chandru Megha[1], Dasegowda Venkatesha[2], Doddaiah Vijaya[3]**

**Author Affiliation**
[1]Assistant Professor [2]Professor and Head [3]Professor and Head (former), Dept of Microbiology, Adichunchanagiri Institute of Medical Sciences, B.G. Nagara, Karnataka 571448, India.

**Corresponding Author**
**Sharath Chandru Megha,**
Assistant Professor, Dept of Microbiology, Adichunchanagiri Institute of Medical Sciences, B.G. Nagara, Karnataka 571448, India.

**E-mail:** meghaptl88@gmail.com

**Received on** 19.07.2018,
**Accepted on** 09.08.2018

**Abstract**

*Background:* Aim of the study was to know the occurrence of ESBL, AmpC and carbapenemases producers among Enterobacteriaeceae by phenotypic disc diffusion tests. *Materials and methods:* A total of 209 isolates belonging to the family Enterobacteriaceae obtained from different clinical samples received in the Department of Microbiology, AIMS. B.G. Ngara were included in the study. ESBL screening was done, followed by phenotypic confirmatory test by CLSI recommended combination disc method. AmpC screening and confirmation was done by phenylboronic acid test and AmpC disc test. Carbapenemase producers were screened and confirmed by CLSI recommended MHT and Remodified Hodge test for KPC detection and double disc synergy test, EDTA disc potentiation test for MBL detection. *Results:* The most common organism isolated was Escherichia coli 101 (48.31%) followed by Klebsiella species 52 (27.27%). Of the 209 isolates of Enterobacteriaceae 24.88%, 1.91% and 7.17% were pure ESBL, AmpC and carbapenemase producers respectively. 5.7% were ESBL and AmpC co-producers, 11.96% were ESBL and carbapenemase co-producers, 2.39% were AmpC and carbapenemase co-producers and 6.22% were combined ESBL, AmpC and carbapenemase co-producers. *Conclusion:* Cefotaxime/clavulanate disc potentiation test detected maximum number of ESBL producers compared to Ceftazidime/ Clavulunate. Cefoxitin–boronic acid detected maximum number of AmpC producers compared to AmpC disc test. Remodified Hodge test is better than MHT in detecting KPC producers. DDST detected more number of MBL producers compared to EDTA disc potentiation test and is a satisfactory and inexpensive method for characterizing the type of carbapenemase producers, when genotypic methods are not available.

**Keywords:** ESBL; AmpC; Carbapenemases; Phenotypic Methods.

## Introduction

Antimicrobial resistance is emerging in the isolates of *Enterobacteriaceae* and is the major threat to the successful treatment of infections in hospitals [1]. The most common mechanism of resistance is the enzymatic inactivation of thebetalactams by a beta-lactamase [2]. Betalactamases are the enzymes produced by microorganisms which can hydrolyze the betalactam ring of beta lactam antibiotics [3].

Organisms producing ESBLs hydrolyze penicillins, cephalosporins and monobactams [3], and are inhibited by clavulanic acid, tazobactam and sulbactam. They are plasmid coded and are easily transmissible from one organism to the other. They are generally derived from TEM and SHV type [4].

AmpC betalactamase confer resistance to a wide variety of beta lactam drugs including beta lactamase inhibitors like clavulanic acid, sulbactam and tazobactam [5]. They are sensitive to cefepime, cefpirome and carbapenems.

It can be plasmid mediated which are typically associated with multi-drug resistance [6] or

chromosomal mediated AmpC, induced in the presence antibiotics such as cefoxitin and imepenem, but poorly induced by 3rd and 4th generation cephalosporins [7].

Both ESBL and AmpC beta lactamases may be produced together by an organism, the effect of plasmid mediated AmpC betalactamases masks the effects of ESBLs which may then be wrongly reported as ESBL negative. This is due to the intrinsic capability of AmpC betalactamases to resist inhibition by clavulanic acid [8]. Boronic acid enhances the detection of ESBL in AmpC producers [8]. Resistance to cefoxitin in *Enterobacteriaceae* indicates AmpC activity [9]. It is important to distinguish between AmpC and ESBL producers, as AmpC producers are resistant to cephamycins and susceptible to fourth generation cephalosporins whereas ESBLs are resistant to fourth generation cephalosporins and susceptible to cephamycin [10].

Carbapenems (ex., imipenem, meropenem, ertapenem and doripenem) are often the antimicrobials of last choice to treat infections due to ESBL and plasmid mediated AmpC producing organisms [11]. KPC confer resistance to all betalactams including penicillins, cephalosporins, monobactams and carbapenems and are inhibited better by tazobactam than clavulanic acid [12].

Class B Metallo beta-lactamases are mostly of VIM- and IMP- types but recently emerged NDM- type is becoming the most threatening carbepenemase [13]. MBL are zinc containing enzymes, they are inhibited by chelating agent such as EDTA and not inhibited by beta lactamase inhibitors [12].

The need for the present study is to compare and to know the advantage of different phenotypic methods in identifying ESBL, AmpC and carbapenemase producing organisms among *Enterobacteriaceae*.

## Materials and methods

The present study was conducted in the department of Microbiology, AIMS, B.G. Nagar. Ethical committee clearance was taken from the institution. A total of 209 isolates of *Enterobacteriaceae* from 200 different clinical samples like urine, pus, blood, sputum, high vaginal swab. Organisms were isolated and identified as per standard procedures. All the isolates were further tested for the production of ESBL, AmpC and Carbapenemases.

Methods for detection of ESBL, AmpC, carbapenemases.

*Screening test [14]*

All isolates were subjected to screening tests to detect ESBL, AmpC and carbapenemase producers.

After adjusting the bacterial suspension to 0.5 MacFarland's units, lawn culture was done on MHA. Amoxicillin-clavulanic acid disc (20µg+10µg) was placed in the center of the petridish and Cefpodoxime (10 µg), Ceftazidime (30µg) disc were placed on either side of Amoxicillin-clavulanic acid disc at a distance of 20mm. Cefoxitin (30µg) disc was placed at a distance of 20mm from Cefpodoxime and Ceftazidime disc. Meropenem (10µg) disc placed at a distance of >25mm from other discs (Fig. 1). Plate was incubated at $35^0$C for 16-18hrs.

*Interpretation*

1. Extension of zone of inhibition of cefpodoxime or ceftazidime towards Amoxyclav disc was taken as ESBL screening positive (Fig. 1).
2. Blunting of zone of inhibition of Ceftazidime towards cefoxitin was taken as AmpC screening positive.
3. Blunting of zone of inhibition of Ceftazidime towards amoxyclav was taken as inducible AmpC positive.
4. Zone of inhibition around Meropenem disc < 21mm was taken as carbapenemase screening positive.

*Confirmatory tests*

All isolates were subjected to ESBL confirmation test. Those which were screening positive for AmpC and Carbapenemase were also subjected to respective confirmatory tests.

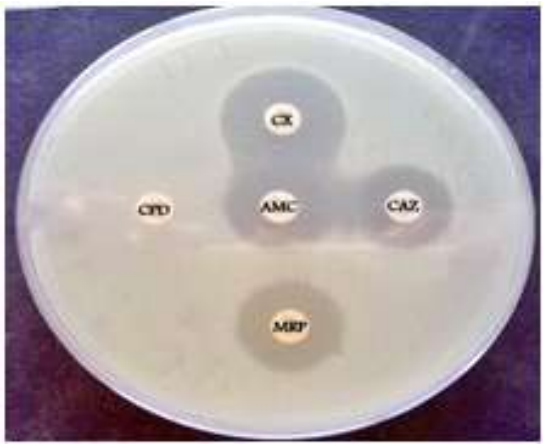


**Fig. 1:** Screening test for ESBL, AmpC and carbapenemase producers.

*1. Confirmatory test for ESBL*

*Combination disc method [14]*

Lawn culture of bacterial suspension was done on MHA. Ceftazidime (30µg), ceftazidime+clavulanic acid(30µg+10µg) and cefotaxime (30µg), cefotaxime+clavulanic acid (30µg+10µg) were placed >30mm apart (Fig. 2). Plates were incubated at $37^0$C for 16-18 hours.

*Interpretation*

≥5mm increase in the zone of inhibition of Ceftazidime+clavulanic acid and/or cefotaxime+clavulanic acid discs as compared to Ceftazidime and/or cefotaximediscs alone was taken as ESBL positive. (Fig. 2).

*2. Confirmatory tests forAmpC*

*Combination disc method [14]*

Lawn culture of bacterial suspension was done on MHA. Cefoxitin (30 µg) and cefoxitin+ Aminophenylboronic acid disc placed > 30mm apart. The plates were incubated at 37˚C for 16-18 hours.

*Interpretation*

≥5mm increase in the zone of inhibition of cefoxitin + aminophenylboronic acid when compared to cefoxitin disc alone was taken as AmpC positive.

*AmpC disc test:*

Lawn culture of *Escherichia coli* ATCC 25922 was done on MHA. Cefoxitin (30µg) disc was placed on it. Test bacterial colony was placed on sterile plain disc of Whatmann filter paper (6mm diameter) adjacent to cefoxitindisc (Fig. 3). The plate was incubated at $37^0$C for 16-18hours.

*Interpretation*

Flattening or indentation of the cefoxitin zone of inhibition in the vicinity of test organism disc was taken as positive AmpC disc test. (Fig. 3).

*3. Confirmatory tests for Carbapenemase [14]*

*KPC type carbapenemase [14,15]*

*Modified and Remodified Hodge test*

Lawn culture of *Escherichia coli* ATCC 25922 was done on MHA. Imipenem (10µg) disc and Imipenem (10µg) +zinc (140µg) disc were placed on the inoculated plate. Test strains were streaked like a line with the inoculation loop at right angles to each other from Imipenem and Imipenem+zinc disc, not touching the disc (Fig. 4). The plates were incubated at $37^0$C for 16-18hours.

*Interpretation*

Enhancement of growth of indicator strains *E.coli* ATCC 25922 around Imipenem+zinc disc as compared to only imipenem disc was considered as Remodified Hodge test positive (Fig. 4).

**Fig. 3:** AmpC disc test

**Fig. 2:** Combination disc method to detect ESBL producer.

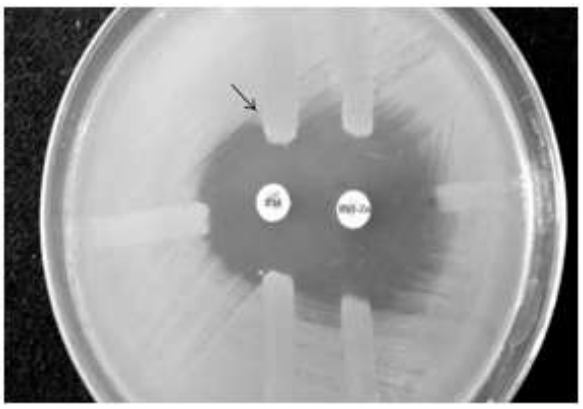

**Fig. 3:** MHT and Remodified Hodge test.

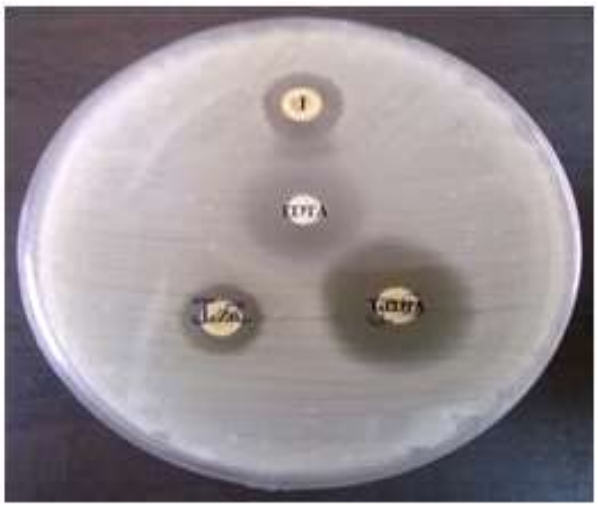

**Fig. 5:** EDTA disc potentiation and Double disc synergy tests

*MBL type carbapenemase [15]*

*1. Double disc synergy test (DDST)*

*2. Combination disc method (EDTA disc potentiation test)*

Lawn culture was done on MHA. EDTA plain disc (750µg) was placed in the center of the plate. Imipenem (10µg) disc, Imipenem+zinc disc (10 µg+ 140 µg) and Imipenem+EDTA (750 µg) were placed at a distance of 20mm each from EDTA plain disc (Fig. 5). The plates were incubated at $37^0$C for 16-18hours.

*Interpretation:* (Fig. 5)

1. Synergy betweenImipenem disc and plain EDTA disc and Imipenem+zinc disc and plain EDTA disc was taken as double disc synergy test positive.

2. ≥ 3mm decrease in the zone of inhibition of Imipenem+zinc disc as compared to Imipenem disc is taken as combination disc test positive.

3. ≥ 7mm increase in the zone of inhibition around Imipenem+EDTA disc as compared to Imipenem alone was considered as EDTA disc potentiation test positive.

## Results

Total of 209 clinical isolates of *Enterobacteriaceae* from different clinical samples like urine, pus, blood, sputum, high vaginal swab collected from out-patients and in-patients admitted in the hospital. Out of 200 clinical samples, majority of the isolates were from urine (55.98%), followed by pus (19.13%), sputum (11.48%), high vaginal swab (6.22%), blood (3.82%), stool (2.87%) and fluid (0.47%).

All isolates were screened for ESBL production, which detected 84 isolates to be ESBL screening positive. Irrespective of screening test results, all the isolates were subjected to phenotypic confirmatory test, which detected 102 isolates to be ESBL positive. 51 (24.4%) were screening positive for AmpC. Combination disc method and AmpC disc tests detected 34 and 31 isolates are AmpC positive respectively.

Of 209 isolates, 115 (55%) were Meropenem resistant and are indicative of carbapenemase production. Out of 115 screening positive isolates, 28 (24.3%) and 30 (26%) were positive for KPC and MBL producers respectively.

Out of 209 isolates, ESBL producers are 102 (48.8%), AmpC 34 (16.3%) and carbapenemase 58 (27.8%) respectively. 52 isolates were ESBL producers, AmpC 4, carbapenemase 15 (8 KPC producers, 7 MBL). 12 isolates were ESBL + AmpC co- producers, 25 ESBL + carbapenemase, 5 AmpC + carbapenemase, 13 ESBL+AmpC+carbapenemase producers. Comparision of various methods in detecting these enzymes are shown in Table.I.

## Discussion

Cephalosporins are the first line drugs used in the treatment of infections caused by gram negative organisms. The extensive use of third-generation cephalosporins has resulted in the increased prevalence of ESBL and plasmid- mediated AmpC among these organisms [16].

Carbapenems form an integral part of treatment regimen for serious and multi drug resistant Gram negative bacterial infections. However, there are reports on increasing prevalence of carbapenem resistance in clinical isolates of *Enterobacteriaceae*mainly due to the production of metallo-β-lactamases (MBL) and *Klebsiellapneumoniae* type carbapenemases (KPC) [17].

The present study was conducted to detect the occurrence of β- lactamases among *Enterobacteriaceae* in rural tertiary care hospital. *E.coli* was the most common (48.3%) organism isolated followed by *Klebsiella* species (27.2%), *Citrobacterspp* (7.17%), *Enterobacterspp* (6.2%), *Proteus spp* (4.78%), *Providenciaspp* (4.3%) and *Morganellamorganii* (1.91%) respectively.

**Table 1:** Comparison of various methods in detection of ESBL, AmpC and carbapenemaseproducers among Enterobacteriaceae.

| Methods | Organisms [N=209] | | | | | | |
|---|---|---|---|---|---|---|---|
| | **E.coli 101(48.3%)** | **Klebsiellaspp 52(27.2%)** | **Enterobacterspp 13(6.2%)** | **Citrobacterspp 15(7.17%)** | **Proteus spp 10(4.78%)** | **Providenciaspp 9(4.3%)** | **Morganellaspp 4(1.91%)** |
| *ESBL detection 102(48.8%)* | | | | | | | |
| Screening test 84(40.1%) | 48(47.5%) | 12(23%) | 5(38.4%) | 4(26.66%) | 7(70%) | 6(66.6%) | 3(75%) |
| Combination disc method 102(48.8%) | 55(54.4%) | 19(36.5%) | 8(61.5%) | 6(40%) | 6(60%) | 5(55.5%) | 3(75%) |
| *AmpC detection 34(16.3%)* | | | | | | | |
| Screening test 51(24.4%) | 21(20.7%) | 15(28.8%) | 7(53.8%) | 4(26.6%) | 1(10%) | 2(22.2%) | 1(25%) |
| Combination disc method 34(16.3%) | 12(11.8%) | 9(17.3%) | 5(38.4%) | 3(20%) | 1(10%) | 2(22.2%) | 1(25%) |
| *Carbapenemase detection 58(27.8%)* | | | | | | | |
| Carbapenemase screening test 115 (55%) | 56(55.4%) | 24(46.15%) | 8(61.5%) | 7(46.6%) | 9(90%) | 7(77.7%) | 3(75%) |
| *1) KPC detection 28 (24.3%)* | | | | | | | |
| MHT 20(17.4%) | 8(0.79%) | 7(13.46%) | 4(30.76%) | 1(6%) | 5(50%) | 3(33.3%) | 1(25%) |
| Re- MHT 28(24.3%) | 8(0.79%) | 6(11.5%) | 4(30.76%) | 1(6%) | 6(60%) | 3(33.3%) | 1(25%) |
| *2) MBL detection 30 (26%)* | | | | | | | |
| EDTA disc potentiation test 23(20%) | 15(14.85%) | 5(9.6%) | 2(15.38%) | 0 | 1(10%) | 3(33.3%) | 1(25%) |
| Double disc synergy test 30 (26%) | 12(11.88%) | 5(9.6%) | 2(15.28%) | 2(13.3%) | 1(10%) | 2(22.2%) | 0 |

In the present study, a total of 209 isolates were screened for the production of ESBL, which showed 84 (40.1%) isolates ESBL screening test positive. Irrespective of the screening test, all the isolates were put for ESBL confirmatory test. Confirmatory test was put using combination disc method, which detected 102 (48.8%) isolates ESBL positive, which is comparable with the study of Dalela G, which detected 135 (61.6%) [18].

In the present study, among the β lactam-inhibitor combination used, CTX and CEC combination detected majority of the ESBL isolates compared to CAZ and CAC. The confirmation of the ESBL production by clavulanic acid inhibition can be difficult in some strains, not only because the activity of the β-lactamase varies with different substrates, but also because the organism may contain additional resistance mechanisms that can mask the presence of the ESBL activity [19]. In the study of Shoorashetty RM et al. [20] and Sturenberg et al. [21] cefepime/clavulanate method could detect maximum ESBL.

Cephamycins are the better screening agents for AmpC production [22]. Confirmatory tests for AmpC was done using combination disc method (Cefoxitin/boronic acid) and AmpC disc test, which detected 34 (35.7%) and 31 (32.6%) respectively. In the study of DL Maraskohle et al., Disc potentiation test using boronic acid detected 59 (35.76%) AmpC producers [23] and study of Vandana KE et al., the PBA (phenyl boronic acid) method detected 24 (58.5%) and 9 (82%) in *E.coli* and *K.pneumoniae* as AmpC positive respectively [24].

In the present study, 20 isolates which were cefoxitin resistant were not AmpC producers. It was observed, though different methods for detection of AmpC β lactamases were used for comparison, that not all cefoxitin resistant isolates were producers of AmpC β lactamases and this range of non producers varied from 20-50% in different studies [7,25]. The resistance in these organisms was considered to be due to the lack of permeation of porins [25]. The limitation of our study was that these isolates needed further evaluation with Modified three dimensional test (M3DT) for AmpC detection. M3DT has an advantage, in this test by using the extract of the organism the effect due to porin mechanism is ruled out and the total effect is due to AmpC β lactamases [23].

Apart from above observation, one Cefoxitin sensitive isolate was a pure AmpC producer. Which is also been reported in the studies of Maraskohle D.L [23] and Hemalatha V [7], where 16.67% and 71.4% of non ESBL Cefoxitin sensitive isolates were AmpC producers. This is because of novel type of AmpC β lactamase with a low level of activity against Cefoxitin which is designated as ACC-1.

In the present study, 115 (55%) isolates were indicative of carbapenemase production. Various studies across the world have reported varying resistance to Imepenem and Meropenem (4-60%) [15].

Out of 115 carbapenemase screening test positive isolates, the MHT detected 20 (17.4%) isolates as carbapenemase producers, while Re-MHT was positive for 28 (24.3%) isolates. Of the above result, Re-MHT detected more KPC than MHT. This is in comparison with the study of AttalRO et al which showed 16 (11.4) isolates to be positive by MHT [34]. Study of SM Amudhan and S Rai detected 113(97.4%), 92 (90.2%) carbapenemase producers respectively [15,27].

Out of remaining 87 screening test positive, which was negative by MHT and Re-modified Hodge test. 23 (20%) were positive by EDTA disc potentiation test and 30 (26%) were positive by double disc synergy test. This can be compared with the study of Srai et al in which EDTA disc diffusion synergy test and combined disc test detected 8 and 9 isolates respectively [15]. Another study by Attal Ro et al showed both methods detected equal numbers of isolates to be carbapenemase positive i.e, 16 (11.4%) among *Pseudomonas aeruginosa* isolates [26].

CLSI recommended combination disc method using clavulanic acid- detected all ESBL but failed to detect ESBL's in the presence of AmpC in 8 isolates. Boronic acid disk potentiation detected all AmpC, and combined enzyme producers correctly compared to AmpC disc test.

In the present study out of 209 isolates, 15(7%) were only carbapenemase (8 KPC and 7 MBL), 25 (11.9%) were combined ESBL and carbapenemase, 5 (2.3%) were combined AmpC and carbapenemase and 13 (6.2%) were ESBL, AmpC, carbapenemase co-producers.

In the study of Oberoi et al coexistence of ESBL and MBL, AmpC and MBL, AmpC & ESBL was seen in 8.79%, 3.67% and 6.59% respectively [28]. A study of Arora et al reported AmpC and MBL coproduction in 46.6% isolates and ESBL and AmpC co production in 3.3% isolates [5].

The present study shows the occurrence of ESBL, AmpC and carbapenemase in 48.8%, 16.26% and 27.7% respectively among *Enterobacteriaceae*. Early detection will help in the management, thus preventing the development and dissemination of drug resistant organisms.

## Conclusion

Increased rates of antimicrobial resistance among members of family *Enterobacteriaeceae,* which are known to cause clinically significant infections, suggest monitoring mechanisms of antimicrobial. Phenotypic methods for detection of these resistant mechanisms are faster, cost effective, easier to perform and less labour intensive though it's not confirmatory.

Combination disc method using cefotaxime/ clavulanic acid combination, detected maximum number of ESBL, but it should be performed with other test using boronic acid for the detection of ESBL in the presence of co-production of AmpC.

Cefoxitin-boronic acid method is simple, highly sensitive and easier in detecting AmpC compared to AmpC disc test. Re-MHT using zinc sulphate, detected higher numbers of KPC compared to MHT. Double disc synergy test using zinc sulphate detected maximum number of MBL compared to combination disc method using EDTA.

In order to prevent the spread of these multidrug resistant organisms, it is necessary to identify and detect them routinely in the laboratories using simple phenotypic methods as it helps the clinician to provide appropriate antimicrobial therapy. It is advisable for all the health care settings to have hospital infection control committee with hospital antibiotic policy, with regular updates.

## References


1. Khanna A, Singh N, Aggarwal A, Khanna M. The antibiotic resistance pattern in *Citrobacter species*: An emerging nosomial pathogen in a tertiary care hospital. J ClinDia Res. 2012;6(4):642-44.
2. Laghawe A, Jaitly N. Thombare V. The simultaneous detection of ESBL and the AmpC β-lacatamases in Gram negative bacilli. J ClinDiagReas. 2012; 6(4):660-63.
3. Thomson KS. Controversies about Extended spectrum betalactamases. Emerginf dis. 2001;7(2):333-36.
4. Rupp ME, Fey PD. Extended Spectrum β lactamase (ESBL) –Producing *Enterobacteriaceae*- Consideration for Diagnosis, Prevention and Drug Treatment. 2003;63(4):353-65.
5. Arora S, Bal M. The AmpC β-lacaamase producing bacterial isolates at a Kolkata hospital. Ind J Med Res. 2005;122:224-33.
6. Ratna AK, Menon I, Kapur I, Kulkarni R. Occurrence and detection of AmpC β lactamases at a referral hospital in Karnataka. Indian J Med Res. 2003;118:29-32.
7. Hemalatha V, Padma M, Umasekar, Vinodh TM, Arunkumar AS. Detection of AmpC beta lactamases production in *Escherichia coli* and *Klebsiella* by an inhibitor based method. Indian J Med Res. 2007;126:220-23.
8. Courdon PE, Moland ES, Kenneth ST. Occurrence and detection of AmpC beta-lactamases among *Escherichia coli, Klebsiellapneumoniae* and *Proteus mirabilis* isolates at a Veterans medical center. J ClinMicrobiol. 2000;38(5):1791-96.
9. Rosco diagnostic. Screening and detection of plasmid mediated AmpC beta lactamses.
10. Song W, Kwon B, You-Nae L, Chae-Hoon L, Sang H, Jeong SH. Detection of Extended-spectrum β lactamase Inhibitor in Clinical Isolates of *Klebsiella spp*. and Escherichia coli. J ClinMicrobiol. 2007;45(4): 1180-84.
11. Hara G L, Gould I, Endimiani A, Pardo PR et al. Detection, treatment and prevention of carbapenemase producing *Enterobacteriaeceae*: Recommendation from and international working group. J Chemother. 2013 Jun;25(3):129-40
12. Bush Karen, Jacoby GA. Updated Functional Classification of β lactamases. Antimicrobial Agent Chemother. 2010;54(3):969-76.
13. Overturf, G. Carbapenemases: a brief review for pediatric infectious disease specialists. Pediatr Infect Dis J. 2010;29:68–70.
14. Clinical and Laboratory standard institute. Performance standards for antimicrobial susceptibility testing; 23rd informational supplement. 2013;31(1):44-61.
15. Rai S, Manchanda V, Singh NP, Kaur IR. Zinc-dependent carbepenemases in clinical isolates of family *Enterobacteriaceae*. Indian J Med Microbiol 2011;29(3):275-9.
16. Rudresh SM, Nagarathnamma T. Extended spectrum β-lactamase producing *Enterobacteriaeceae* and antibiotic co-resistance. Ind J Med Res. 2011;133(1):116-18.
17. Triple-disk assay for phenotypic detection of predominant Carbapenemases. Indian J Med Res 2013 Dec;138;1025-26.
18. Dalela G. Prevalence of Extended spectrum β lactamase (ESBL) producers among Gram negative bacilli from various clinical isolates in a tertiary care hospital at Jhalawar, Rajasthan, India. J Clin Diag Res. 2012;6(2):182-87.
19. Steward CD, Rasheed JK, Hubert SK et al. Characterization of the clinical isolates of *Klebsiellapneumoniae* from 19 laboratories by using the National Committee for Clinical Laboratory Standards extended- spectrum β-lactamase detection methods. J.ClinMicrobiol 2001;39:2864-72.

20. Shoorashetty RM, Nagarathnamma T, Prathibha J. Comparison of the boronic acid disc potention test and cefepime-clavulunic acid method for the detection of ESBL among among AmpC producing *Enterobacteriaceae*. Indian J Med Microbiol 2011; 29(3):297-301.
21. Sturenberg E, Mack D. Extended Spectrum β lactamases: Implications for the clinical microbiology laboratory, therapy and infections control. Journal of Infection. 2003;47(4):273-95.
22. Ananthan S, Subha A. Cefoxitin resistance mediated by loss of a porin in clinical strains of *Klebsiellapneumoniae* and *Escherichia coli*. Ind J Med Microbiol. 2005;23:20-3.
23. Maraskolhe D.L, Deotale VS, Mendiratta. D.K, Narang P. Comparision of three laboratory tests for detection of AmpC β- lactamases in *Klebsiellaspp* and *E.coli*. J ClinDiag Res. 2014;8(6):DC05-08.
24. Vandana KE, Honnavar P. AmpC beta lactamases among ESBL producing *Escherichia coli* and *Klebsiellapneumoniae*- If you don't look, Youwont find. Journal of Clinical and Diagnostic Research. 2009;3(4):1653-56.
25. Manchanda V, Singh NP, Goyal R, Kumar A, Thukral SS. Phenotypic Characteristics of clinical isolates of *Klebsiella pneumoniae* and evaluation of available phenotypic techniques for the detection of extended spectrum beta-lactamases. Ind J Med Res. 2005;122:330-37.
26. Attal RO, Basak S, Mallick S K, Bose S. Metallo beta lactamases producing *Pseudomonas aeruginosa*: An emerging threat to clinician. J Clin and Diag Res 2010 August;4:2691-6.
27. S M Amudhan, U Sekar, Arunagiri K, B Sekar. OXA beta-lactamase-mediated carbepenem resistance in *Acinetobacter baumanii*. Indian J Med Microbiol 2011;29(3):269-74.
28. Oberoi L, Singh N, Sharma P, Agarwal A. ESBL, MBL and AmpC β lactamases Producing Superbugs-Havoc in the Intensive Care Units of Punjab India. J ClinDiag Res. 2013;7(1):70-73.

# Subject Index

## Author Index

# Guidelines for Authors

Manuscripts must be prepared in accordance with "Uniform requirements for Manuscripts submitted to Biomedical Journal" developed by international committee of medical Journal Editors

**Types of Manuscripts and Limits**

Original articles: Up to 3000 words excluding references and abstract and up to 10 references.

Review articles: Up to 2500 words excluding references and abstract and up to 10 references.

Case reports: Up to 1000 words excluding references and abstract and up to 10 references.

**Online Submission of the Manuscripts**

Articles can also be submitted online from http://rfppl.co.in/customer_index.php.

I) First Page File: Prepare the title page, covering letter, acknowledgement, etc. using a word processor program. All information which can reveal your identity should be here. use text/rtf/doc/PDF files. Do not zip the files.

2) Article file: The main text of the article, beginning from Abstract till References (including tables) should be in this file. Do not include any information (such as acknowledgement, your name in page headers, etc.) in this file. Use text/rtf/doc/PDF files. Do not zip the files. Limit the file size to 400 Kb. Do not incorporate images in the file. If file size is large, graphs can be submitted as images separately without incorporating them in the article file to reduce the size of the file.

3) Images: Submit good quality color images. Each image should be less than 100 Kb in size. Size of the image can be reduced by decreasing the actual height and width of the images (keep up to 400 pixels or 3 inches). All image formats (jpeg, tiff, gif, bmp, png, eps etc.) are acceptable; jpeg is most suitable.

Legends: Legends for the figures/images should be included at the end of the article file.

If the manuscript is submitted online, the contributors' form and copyright transfer form has to be submitted in original with the signatures of all the contributors within two weeks from submission. Hard copies of the images (3 sets), for articles submitted online, should be sent to the journal office at the time of submission of a revised manuscript. Editorial office: Red Flower Publication Pvt. Ltd., 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi – 110 091, India, Phone: 91-11-22754205, 45796900, 22756995. E-mail: author@rfppl.co.in. Submission page: http://rfppl.co.in/article_submission_system.php?mid=5.

**Preparation of the Manuscript**

The text of observational and experimental articles should be divided into sections with the headings: Introduction, Methods, Results, Discussion, References, Tables, Figures, Figure legends, and Acknowledgment. Do not make subheadings in these sections.

**Title Page**

The title page should carry

1) Type of manuscript (e.g. Original article, Review article, Case Report)

2) The title of the article, should be concise and informative;

3) Running title or short title not more than 50 characters;

4) The name by which each contributor is known (Last name, First name and initials of middle name), with his or her highest academic degree(s) and institutional affiliation;

5) The name of the department(s) and institution(s) to which the work should be attributed;

6) The name, address, phone numbers, facsimile numbers and e-mail address of the contributor responsible for correspondence about the manuscript; should be mentoined.

7) The total number of pages, total number of photographs and word counts separately for abstract and for the text (excluding the references and abstract);

8) Source(s) of support in the form of grants, equipment, drugs, or all of these;

9) Acknowledgement, if any; and

l0) If the manuscript was presented as part at a meeting, the organization, place, and exact date on which it was read.

**Abstract Page**

The second page should carry the full title of the manuscript and an abstract (of no more than 150 words for case reports, brief reports and 250 words for original articles). The abstract should be structured and state the Context (Background), Aims, Settings and Design, Methods and Materials, Statistical analysis used, Results and Conclusions. Below the abstract should provide 3 to 10 keywords.

**Introduction**

State the background of the study and purpose of the study and summarize the rationale for the study or observation.

**Methods**

The methods section should include only information that was available at the time the plan or protocol for the study was written such as study approach, design, type of sample, sample size, sampling technique, setting of the study, description of data collection tools and methods; all information obtained during the conduct of the study belongs in the Results section.

Reports of randomized clinical trials should be based on the CONSORT Statement (http:// www. consort-statement. org). When reporting experiments on human subjects, indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000 (available at http://www.wma. net/e/policy/l 7-c_e.html).

**Results**

Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first. Do not repeat in the text all the data in the tables or illustrations; emphasize or summarize only important observations. Extra or supplementary materials and technical details can be placed in an appendix where it will be accessible but will not interrupt the flow of the text; alternatively, it can be published only in the electronic version of the journal.

**Discussion**

Include summary of key findings (primary outcome measures, secondary outcome measures, results as they relate to a prior hypothesis); Strengths and limitations of the study (study question, study design, data collection, analysis and interpretation); Interpretation and implications in the context of the totality of evidence (is there a systematic review to refer to, if not, could one be reasonably done here and now?, What this study adds to the available evidence, effects on patient care and health policy, possible mechanisms)? Controversies raised by this study; and Future research directions (for this particular research collaboration, underlying mechanisms, clinical research). Do not repeat in detail data or other material given in the Introduction or the Results section.

**References**

List references in alphabetical order. Each listed reference should be cited in text (not in alphabetic order), and each text citation should be listed in the References section. Identify references in text, tables, and legends by Arabic numerals in square bracket (e.g. [10]). Please refer to ICMJE Guidelines (http://www.nlm.nih.gov/bsd/uniform_ requirements.html) for more examples.

**Standard journal article**

[1] Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35: 540-7.

[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand 2003; 61: 347-55.

**Article in supplement or special issue**

[3] Fleischer W, Reimer K. Povidone iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3-9.

**Corporate (collective) author**

[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792-801.

**Unpublished article**

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

**Personal author(s)**

[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

**Chapter in book**

[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O,

Kidd EAM, editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. p. 7-27.

**No author given**

[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

**Reference from electronic media**

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www.statistics.gov.uk/downloads/theme_health/HSQ20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

More information about other reference types is available at www.nlm.nih.gov/bsd/uniform_requirements.html, but observes some minor deviations (no full stop after journal title, no issue or date after volume, etc).

**Tables**

Tables should be self-explanatory and should not duplicate textual material.

Tables with more than 10 columns and 25 rows are not acceptable.

Table numbers should be in Arabic numerals, consecutively in the order of their first citation in the text and supply a brief title for each.

Explain in footnotes all non-standard abbreviations that are used in each table.

For footnotes use the following symbols, in this sequence: *, ¶, †, ‡‡,

**Illustrations (Figures)**

Graphics files are welcome if supplied as Tiff, EPS, or PowerPoint files of minimum 1200x1600 pixel size. The minimum line weight for line art is 0.5 point for optimal printing.

When possible, please place symbol legends below the figure instead of to the side.

Original color figures can be printed in color at the editor's and publisher's discretion provided the author agrees to pay.

Type or print out legends (maximum 40 words, excluding the credit line) for illustrations using double spacing, with Arabic numerals corresponding to the illustrations.

**Sending a revised manuscript**

While submitting a revised manuscript, contributors are requested to include, along with single copy of the final revised manuscript, a photocopy of the revised manuscript with the changes underlined in red and copy of the comments with the point to point clarification to each comment. The manuscript number should be written on each of these documents. If the manuscript is submitted online, the contributors' form and copyright transfer form has to be submitted in original with the signatures of all the contributors within two weeks of submission. Hard copies of images should be sent to the office of the journal. There is no need to send printed manuscript for articles submitted online.

**Reprints**

Journal provides no free printed reprints, however a author copy is sent to the main author and additional copies are available on payment (ask to the journal office).

**Copyrights**

The whole of the literary matter in the journal is copyright and cannot be reproduced without the written permission.

**Declaration**

A declaration should be submitted stating that the manuscript represents valid work and that neither this manuscript nor one with substantially similar content under the present authorship has been published or is being considered for publication elsewhere and the authorship of this article will not be contested by any one whose name (s) is/are not listed here, and that the order of authorship as placed in the manuscript is final and accepted by the co-authors. Declarations should be signed by all the authors in the order in which they are mentioned in the original manuscript. Matters appearing in the Journal are covered by copyright but no objection will be made to their reproduction provided permission is obtained from the Editor prior to publication and due acknowledgment of the source is made.

**Approval of Ethics Committee**

We need the Ethics committee approval letter from an Institutional ethical committee (IEC) or an institutional review board (IRB) to publish your Research article or author should submit a statement that the study does not require ethics approval along with evidence. The evidence could either be consent from patients is available and there are no ethics issues in the paper or a letter from an IRB stating that the study in question does not require ethics approval.

**Abbreviations**

Standard abbreviations should be used and be spelt out when first used in the text. Abbreviations should not be used in the title or abstract.

**Checklist**

- Manuscript Title
- Covering letter: Signed by all contributors
- Previous publication/ presentations mentioned, Source of funding mentioned
- Conflicts of interest disclosed

**Authors**

- Middle name initials provided.
- Author for correspondence, with e-mail address provided.
- Number of contributors restricted as per the instructions.
- Identity not revealed in paper except title page (e.g.name of the institute in Methods, citing previous study as 'our study')

**Presentation and Format**

- Double spacing
- Margins 2.5 cm from all four sides
- Title page contains all the desired information. Running title provided (not more than 50 characters)
- Abstract page contains the full title of the manuscript
- Abstract provided: Structured abstract provided for an original article.
- Key words provided (three or more)
- Introduction of 75-100 words
- Headings in title case (not ALL CAPITALS).
  References cited in square brackets
- References according to the journal's instructions

**Language and grammar**

- Uniformly American English
- Abbreviations spelt out in full for the first time. Numerals from 1 to l0 spelt out
- Numerals at the beginning of the sentence spelt out

**Tables and figures**

- No repetition of data in tables and graphs and in text.
- Actual numbers from which graphs drawn, provided.
- Figures necessary and of good quality (color)
- Table and figure numbers in Arabic letters (not Roman).
- Labels pasted on back of the photographs (no names written)
- Figure legends provided (not more than 40 words)
- Patients' privacy maintained, (if not permission taken)
- Credit note for borrowed figures/tables provided
- Manuscript provided on a CDROM (with double spacing)

**Submitting the Manuscript**

- Is the journal editor's contact information current?
- Is the cover letter included with the manuscript? Does the letter:

1. Include the author's postal address, e-mail address, telephone number, and fax number for future correspondence?
2. State that the manuscript is original, not previously published, and not under concurrent consideration elsewhere?
3. Inform the journal editor of the existence of any similar published manuscripts written by the author?
4. Mention any supplemental material you are submitting for the online version of your article. Contributors' Form (to be modified as applicable and one signed copy attached with the manuscript)

# Instructions to Authors

Submission to the journal must comply with the Guidelines for Authors.
Non-compliant submission will be returned to the author for correction.

To access the online submission system and for the most up-to-date version of the Guide for Authors please visit:

http://www.rfppl.co.in

Technical problems or general questions on publishing with JMRR are supported by Red Flower Publication Pvt. Ltd's Author Support team (http://rfppl.co.in/article_submission_system.php?mid=5#)

Alternatively, please contact the Journal's Editorial Office for further assistance.

Editorial Manager
Red Flower Publication Pvt. Ltd.
48/41-42, DSIDC, Pocket-II
Mayur Vihar Phase-I
Delhi - 110 091(India)
Mobile: 9821671871, Phone: 91-11-22754205, 45796900, 22756995
E-mail: author@rfppl.co.in