**ORIGINAL RESEARCH**

# A study on correlates of pure and mixed growth of multidrug resistant gram negative bacilli

[1]Dr.Bhavana S Nath, [2]Dr.Archana Rao K, [3]Dr. Shamsunder BV

[1]Research Scientist, VRDL, Department of Microbiology, VIMS, Bellary, Karnataka, India
[2]Assistant Professor, Department of Microbiology, Rajarajeshwari Medical College and Hospital, Bangalore, Karnataka, India
[3]Assistant Professor, Department of Microbiology, MMCRI, Mysore, Karnataka, India

**Corresponding Author**
Dr. Bhavana S Nath
Research Scientist, VRDL, Department of Microbiology, VIMS, Bellary, Karnataka, India

Received: 12March, 2023 Accepted: 18April, 2023

**ABSTRACT**
WHO's recent global report on AMR surveillance has revealed the gaps in information on pathogens of major public health importance. The report also points out the significant disparities in surveillance quality, varying methods of data collection and lack of common platform for data-sharing, as well as the lack of common standards on such issues. The study included multidrug resistant Gram negative bacilli isolated from various clinical samples from patients from all the hospitals. Sample size was calculated as 133, rounded off to 150, assuming 1% alpha error, and 15% relative precision, 69% to 95% sensitivity of Colistin among MDR gram negative bacteria.Diabetic foot (20%) and post-operative state (20%) showed higher chances of mixed infection with MDR organisms followed by sepsis (16.7%), ulcer (16.7%), surgical site infections (10.5%) and peritonitis (8.3%).Burns, compound fracture, lower respiratory tract infections, road traffic accidents, tracheostomy site infections and urinary tract infections yielded only pure growth.

**Key words:**MDR gram negative bacteria, pure and mixed growth, correlates



## Introduction

Since Alexander Fleming discovered Penicillin in 1928, the treatment of bacterial infections has come a long way. Today we have many classes of antibiotics, totaling to more than 100 active antibiotics in our armamentarium, yet we are not close to a near win against bacterial infections. (1)

Many of the antibiotics used in the past have become redundant over the period of time because of bacterial resistance acquired through various means.(1) While appearance of resistance is a continuous phenomenon in microorganisms, its amplification and spread is through an array of practices conducted by human beings. Diseases due to resistant organisms take longer to cure, require expensive and at times toxic drugs for longer periods, often making the disease untreatable. The resistant organisms can also move across countries through travel and trade and are a threat to global health security.

In January 2013, the World Economic Forum warned that antimicrobial resistance (AMR) is one of the major global health security risks that the world needs to address and called attention to GDP losses from AMR ranging from 0.4% to 1.6%.(2) As per the WHO report on global surveillance of antimicrobial resistance, there were more than 23,000 deaths and 2 million illnesses in the United States of America due to antibacterial resistance. A similar report from the European Union from 2007 showed greater than 25,000 deaths and greater than 2.5 million extra hospital days due to antibacterial resistance in 2007. The overall societal costs were 1.5 billion Euros per year. (3)

WHO's recent global report on AMR surveillance has revealed the gaps in information on pathogens of major public health importance. The report also points out the significant disparities in surveillance quality, varying methods of data collection and lack of common platform for data-sharing, as well as the lack of common standards on such issues. Although there is inadequate information on the magnitude of the problem in the South-East Asia Region, it is reported that there is a significant burden of drug resistance in the region. (2)

Similarly, emergence of Multidrug resistant (MDR) bacteria represents an enormous challenge to modern health care systems.Multidrug resistance (MDR) is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, Extensive drug resistance (XDR) is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) and Pan drug resistance (PDR) is defined as non-susceptibility to all agents in all antimicrobial categories. [(4)]

MDR microorganisms were named as the 'ESKAPE' pathogens (*Enterococcus faecium*,*Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacterbaumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.) to emphasize that they 'escape' the effects of antibacterial agents. [(5)]

The World Health Organization in its IDSA report, 2010, has identified antibiotic resistance as one of the 3 greatest threats to human health. [(6)]The situation is especially worrisome with MDR Gram-negative bacteria, namely, *Pseudomonas aeruginosa*, *Acinetobacterbaumannii*and *Klebsiella pneumoniae*, against which no new antibiotics will be available for many years to come.IDSA has placed these 3 very problematic pathogens on a hit-list of top-priority dangerous pathogens.

**Methodology**

The present study was carried out in the Department of Microbiology, The study included multidrug resistant Gram negative bacilli isolated from various clinical samples from patients from all the hospitals. Sample size was calculated as 133, rounded off to 150, assuming 1% alpha error, and 15% relative precision, 69% to 95% sensitivity of Colistin among MDR gram negative bacteria.

**Inclusion criteria**

Multidrug resistant Gram negative clinical isolates

**Exclusion criteria**

1. Non multidrug resistant Gram negative organisms
2. All Gram positive organisms
3. All Gram negative cocci
4. All organisms showing inherent resistance to Colistin such as *Proteus species*, *Vibrio species*, *Burkholderia species.*

**Processing of specimens**

Gram negative organisms were identified as per standard protocol by Gram stain, catalase, oxidase, motility, Oxidation-Fermentation test, nitrate reduction, indole, Methyl Red, Voges–Proskauer, citrate, urease, Triple Sugar Iron agar, sugar fermentation and amino acid decarboxylation tests.

Antibiotic susceptibility testing was done on Mueller Hinton agar using Kirby-Bauer disk diffusion method as per CLSI. Gram negative isolates were tested against 9 groups of antibiotics.

**Results**

**Table 1: Pure and mixed growth of the samples**

| Pure growth | Mixed growth | TOTAL SAMPLES |
|---|---|---|
| 129 (92.8%) | 10 (7.2%) | **139 (100%)** |

Of the 139 samples, 129 (92.8 %) yielded pure growth-single isolate and 10 (7.2%) yielded mixed growth - ≥ 2 isolates.

9 of the mixed growth samples yielded 2 isolates and 1 sample yielded 3 isolates.

Therefore, the total MDR isolates were 150.

**Table 2: Sex co-relation to pure and mixed growth**

| | Mixed | | Pure | | Total |
|---|---|---|---|---|---|
| | Number | Percentage | Number | Percentage | |
| Female | 5 | 9.1 % | 50 | 90.9 % | 55 |
| Male | 5 | 6 % | 79 | 94% | 84 |

Of the 55 females included in the study, samples from 5 (9.1%) females were mixed growth and 50 (90.9%) were pure growth.

Of the 84 males, samples from 5 (6%) were mixed growth and 79 (94%) were pure growth.

**Table 3: Age co-relation to pure and mixed growth samples**

| | Mixed | | Pure | |
|---|---|---|---|---|
| | Number | Percentage | Number | Percentage |
| < 1 year | 1 | 5.3% | 18 | 94.7% |
| 1-5 years | 0 | .0 | 6 | 100.0% |
| 6 – 20 years | 1 | 7.7% | 12 | 92.3% |
| 21 – 40 years | 0 | .0 | 35 | 100.0% |
| 41- 60 years | 3 | 7.9% | 35 | 92.1% |

| | | | | |
|---|---|---|---|---|
| >60 years | 5 | 17.9% | 23 | 82.1% |

Chi-square test, p=0.2

From the above table, majority (17.9%) of mixed growth samples were from patients aged above 60 years.

Samples from patients aged < 1 year, 6- 20 years and 41-60 years predominantly yielded pure growth. Samples from patients aged 1-5 years and 21- 40 years yielded only pure growth.

**Table 4: Co-relation of clinical conditions with pure and mixed growth**

| | **Mixed** | | **Pure** | |
|---|---|---|---|---|
| | **Number** | **Percentage** | **Number** | **Percentage** |
| Burns | 0 | .0 | 2 | 100% |
| Compound fracture | 0 | .0 | 3 | 100% |
| COPD | 0 | .0 | 3 | 100% |
| CSOM | 0 | .0 | 3 | 100% |
| Diabetic foot | 3 | 20% | 12 | 80% |
| LRTI | 0 | .0 | 21 | 100%% |
| Post op | 1 | 20% | 4 | 80% |
| Peritonitis | 1 | 8.3% | 11 | 91.7% |
| RTA | 0 | .0 | 1 | 100% |
| Sepsis | 2 | 16.7% | 10 | 83.3% |
| SSI | 2 | 10.5% | 17 | 89.5% |
| TSI | 0 | .0 | 1 | 100% |
| Ulcer | 1 | 16.7% | 5 | 83.3% |
| UTI | 0 | .0 | 36 | 100% |

(COPD- chronic obstructive pulmonary disease, CSOM- chronic suppurative otitis media, LRTI- lower respiratory tract infection, RTA- road traffic accident, SSI- surgical site infection, UTI – urinary tract infection)

Diabetic foot (20%) and post-operative state (20%) showed higher chances of mixed infection with MDR organisms followed by sepsis (16.7%), ulcer (16.7%), surgical site infections (10.5%) and peritonitis (8.3%). Burns, compound fracture, lower respiratory tract infections, road traffic accidents, tracheostomy site infections and urinary tract infections yielded only pure growth.

**Table 5: Sample wise distribution of pure and mixed growth**

| | **Mixed growth** | | **Pure growth** | |
|---|---|---|---|---|
| | **Number** | **Percentage** | **Number** | **Percentage** |
| Blood | 2 | 8.7% | 21 | 91.3% |
| Exudate | 7 | 14.3% | 42 | 85.7% |
| Sputum | 1 | 2.9% | 33 | 97.1% |
| Urine | 0 | .0 | 33 | 100.0% |

Majority of the exudate samples yielded mixed growth (14.3%), followed by blood (8.7%) and sputum (2.9%)

All the urine samples (100%) yielded pure growth.

Outer circle denotes pure growth and inner circle mixed growth

**Table 6: Organisms isolated from pure and mixed growth**

| | **Mixed growth** | | **Pure growth** | |
|---|---|---|---|---|
| | **Number** | **Percentage** | **Number** | **Percentage** |
| *Acinetobacter*spp. | 4 | 19.0% | 19 | 14.7% |
| *Citrobacter*spp. | 1 | 4.8% | 9 | 7.0% |
| *E.coli* | 7 | 33.3% | 47 | 36.4% |
| *Enterobacter* spp. | 1 | 4.8% | 5 | 3.9% |
| *Klebsiella* spp. | 5 | 23.8% | 34 | 26.4% |
| *Pseudomonas* spp. | 3 | 14.3% | 15 | 11.6% |

(p>0.05, chi-square test)

*E.coli* was predominantly isolated from pure (36.4%) and mixed growth (33.3%), *Klebsiella* spp. 26.4 % and 23.8% from pure and mixed growth respectively, *Acinetobacter* spp. 14.7% and 19% from pure and mixed growth respectively, *Pseudomonas* spp. 11.6% and 14.3% from pure and mixed growth respectively, *Citrobacter* spp. 7% and 4.8% from pure and mixed growth respectively.

**Discussion**

**Table 7: Isolation of MDRO from clinical samples in various studies**

| Study | Year | Place | Samples | | | |
|---|---|---|---|---|---|---|
| | | | Blood | Sputum | Exudate | Urine |
| Behera*et al.* [7] | 2007 | New Delhi | 26% | 44% | 21% | 8% |
| Somily*et al*. [8] | 2010 | Riyadh | 1.8% | - | 9.8% | 7.3% |
| Rajput & Naik [9] | 2013 | Surat | 3.1% | 9.4% | 48.5% | 38% |
| Saman*et al.* [10] | 2013 | Navi Mumbai | 5.4% | 3.9% | 21% | 21% |
| Present study | 2014 | MMCRI, Mysore | 16.7% | 23.3% | 38% | 22% |

(MDRO- multi drug resistant organisms)

In the present study, higher number of MDR organisms were isolated from exudate- 38% compared to other studies. Patients with open wounds are at an increased risk of getting colonized/ infected with hospital environmental strains and have higher chances of showing drug resistance.

Present study showed higher number of isolates from sputum compared to others. Number of urinary isolates co related with Saman*et al*.

Blood though a sterile compartment yielded 16.7% of the isolates. A similar trend towards an increased incidence of MDR Gram-negative organisms causing bloodstream infections has been reported by Wisplinghoff*et al.*and Munoz *et al*.

**Table 8: Age group predominance in various studies**

| Study | Year | Place | Mean Age(years) |
|---|---|---|---|
| J. Huang *et al.* [11] | 2006 | China | 54 |
| Somily*et al*. [8] | 2010 | Riyadh | >50 |
| Porwal*et al.* [12] | 2011 | Chennai | 52.3 |
| Present study | 2014 | MMCRI, Mysore | >60 |

In the present study most of the patients were above 60 years of age. This was comparable with the other studies.

According to Dantas RCC *et al*.,advancing age is a risk factor for colonization and infection with MDR pathogens.

**Table 9: Male- female ratio in various studies**

| Study | Year | Place | Male: Female ratio |
|---|---|---|---|
| J. Huang *et al.*[11] | 2006 | China | 1.1:1 |
| Karnik*et al.* [12] | 2009 | Mumbai | 1.1:1 |
| Somily*et al*. [8] | 2010 | Riyadh | 1.7:1 |
| Present study | 2014 | MMCRI, Mysore | 1.5:1 |

In the present study, the male: female ratio was 1.5: 1which was comparable with Somily*et al*.and Porwal*et al.*

**Table 10: MDR Gram negative bacterial isolates in various studies**

| Study | Year | Place | Predominant isolates | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *E.coli* | Kleb | Acineto | Pseud | Citro | Entero |
| J. Huang *et al*. [11] | 2006 | China | - | 13.3% | 68.4% | 21.1% | - | - |
| Karnik*et al*. [13] | 2009 | Mumbai | - | 4.7% | 57% | 33.3% | - | - |
| Porwal*et al.*[12] | 2011 | Chennai | 26% | 44% | 20% | 10% | - | - |
| Mezzatesta*et al*. [14] | 2013 | Italy | - | 32.4% | 67.5% | - | - | - |
| Rajput & Naik [15] | 2013 | Surat | 33.3% | 19% | 20.6% | 23.8% | - | - |
| Rajenderan*et al.* [16] | 2014 | Vellore | 22.8% | 22.3% | 38.2% | 16.5% | - | - |
| Present study | 2014 | MMCRIMysore | 36% | 26% | 15.3% | 12% | 6.6% | 4% |

(Kleb –*Klebsiella* spp., Acineto- *Acinetobacter* spp., Pseud- *Pseudomonas* spp., Citro- *Citrobacter* spp., Entero – *Enterobacter* spp.)

In the present study, *E.coli* was the predominant isolate (36%) which co-related with the study of Rajput & Naik (33.3%).
Most other studies showed *Acinetobacter* spp. to be predominant, whereas here it was 15.3%.

**Conclusion**

- Of the 139 samples, 129 (92.8 %) yielded pure growth-single isolate and 10 (7.2%) yielded mixed growth - ≥ 2 isolates. 9 of the mixed growth samples yielded 2 isolates and 1 sample yielded 3 isolates, the total MDR isolates were 150.
- Majority of the exudate samples yielded mixed growth (14.3%), followed by blood (8.7%) and sputum (2.9%)
- All the urine samples (100%) yielded pure growth.
- *E.coli* was predominantly isolated from pure (36.4%) and mixed growth (33.3%)

**References**


1. Coates A., Halls G., & Hu Y. Novel classes of antibiotics or more of the same? British Journal of Pharmacology. 2011; 163:184-94.
2. Baltz, R., Miao V., & Wrigley, S. Natural products to drugs: daptomycin and related lipopeptide antibiotics. Nat Prod Rep. 2005; 22:717-41.
3. Livermore, D. Tigecycline: what is it, and where should it be used? Journal of Antimicrobials and Chemotherapy. 2005; 56:611-4.
4. Shinabarger D., Marotti K., Murray R., Lin A., Melchior E., Swaney S., Dunyak D., Demyan W. &Buysse J. Mechanism of action of oxazolidinones: effects of linezolid and eperezolid on translation reactions. Antimicrobial Agents and Chemotherapy. 1997; 41:2132-6.
5. Mullane K., Miller M., Weiss K., Lentnek A., Golan Y., Sears P., Shue Y., Louie T. &Gorbach S. Efficacy of fidaxomicin versus vancomycin as therapy for Clostridium difficile infection in individuals taking concomitant antibiotics for other concurrent infections. Clin Infect Dis. 2011; 53:440-7.
6. Brunton LL, Chabner BA et al. Goodman And Gillman's The Pharmaceutical Basis Of Therapeutics. 12th ed.Chemotherapy of microbial diseases.
7. Behera B, Mathur P, Das A, Kapil A, Gupta B, Bhoi S, Farooque K, Sharma V, Misra MC. Evaluation of susceptibility testing methods for polymyxin. International Journal of Infectious Diseases. 2010: e596–e601.
8. Ali M Somily. Comparison of Etest and Disk Diffusion methods for the in vitro evalution of antimicrobial activity of Colistin in multi-drug resistant gram-negative bacilli. Saudi Med J. 2010; 31(5): 507-11.
9. Gohel K, Jojera A, Soni S, Gang S, Sabnis R, Desai M. Bacteriological Profile and Drug Resistance Patterns of Blood Culture Isolates in a Tertiary Care Nephrourology Teaching Institute. Biomed Res Int. 2014; 2014:5.
10. Samant SA, Marathe N, Vaishampain A and Shouche Y. Detection of NDM-1 in Multi Drug Resistant Gram Negative Clinical Isolates from a Tertiary Care Hospital in Navi Mumbai, IndiaInt.J.Curr.Microbiol.App.Sci.2015; 4(3): 20-9.
11. Huang J, Tang YQ, Sun JY. Intravenous Colistin sulfate: a rarely used form of polymyxin E for the treatment of severe multidrug-resistant Gram-negative bacterial infections. Scandinavian Journal of Infectious Diseases, 2010; 42: 260–5.
12. Porwal R, Gopalakrishnan R, Rajesh NJ, Ramasubramanian V. Carbapenem resistant Gram-negative bacteremia in an Indian intensive care unit: A review of the clinical profile and treatment outcome of 50 patients. Indian J Crit Care Med. 2014; 18(11):750-3
13. Karnik ND, Sridharan K, Jadhav SP, Kadam PP, Naidu RK, Namjoshi RD, Gupta V, Gore MS, Surase PV, Mehta PR, Gogtay JA, Thatte UM, Gogtay NJ. Pharmacokinetics of Colistin in critically ill patients with multidrug-resistant Gram-negative bacilli infection. Eur J ClinPharmacol. 2013; 69(7):1429-36.
14. Mezzatesta ML, Caio C, Gona F, Cormaci R, Salerno I, Zingali T, Denaro C, Gennaro M, Quattrone C, Stefani S. Carbapenem and multidrug resistance in Gram-negative bacteria in a single centre in Italy: considerations on in vitro assay of active drugs. Int J Antimicrob Agents. 2014; 44(2):112-6.
15. Rajput & Naik. Detection of Metallo Beta Lactamase Production in Gram Negative Clinical Isolates. Int. J. of Pharm. Life Sci. 2015; 6(2):4272-9.
16. Rajenderan S, Balaji V, Anandan S, Sahni RD, Tansarli GS, Falagas ME. Determination of MIC distribution of arbekacin, cefminox, fosfomycin, biapenem and other antibiotics against gram-negative clinical isolates in South India: a prospective study. PLoS One. 2014; Jul 28; 9(7).