

Prevalence of Multi Drug Resistant and Extensively Drug Resistant in Gram Negative Bacterial Isolates From Different Clinical Samples

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ABSTRACT

Antibiotic resistance is a worldwide problem that can cross international boundaries and spread between continents very easily and speedily. World health readers have described antibiotic resistant organisms as “nightmare bacteria” that pose a “catastrophic threat” to people in every country in the world. The use of antibiotic is the single most important factor leading to antibiotic resistance around the world. Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria. Gram-positive and Gram-negative bacteria are both affected by the emergence and rise of antimicrobial resistance. As this problem continues to grow, harmonized definitions with which to describe and classify bacteria that are resistant to multiple antimicrobial agents are needed, so that epidemiological surveillance data can be reliably collected and compared across healthcare settings and countries. Present study will help to know the prevalent drug-resistant bacterial strain and their resistance pattern and also to detect the incidence of Multi drug resistant (MDR), Extensive drug resistant (XDR) in Gram negative bacterial isolates in tertiary care hospital. This Prospective study will be conducted in central laboratory, Department of Microbiology, F.H medical college & hospital, Tundla Agra. Total 371 gram negative bacterial isolates were obtained in two and half year study. Male patients 53% and female patient's 47%. most of the bacteria isolated from Urine specimen 157 followed by pus sample 73. MDR was reported in GNB about 61.18% and XDR 35.3%, *Klebsiella spp* 120 was the most reported bacteria in our study followed by *E. coli*, 112.

Keywords: Multi Drug resistant, Extensive Drug Resistance, Gram negative bacteria.

1. INTRODUCTION

Antibiotic resistance is a worldwide problem that can cross international boundaries and spread between continents very easily and speedily. World health readers have described antibiotic resistant organisms as “nightmare bacteria” that pose a “catastrophic threat” to people in every country in the world. The use of antibiotic is the single most important factor leading to antibiotic resistance around the world [1].

Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria. Gram-positive and Gram-negative bacteria are both affected by the emergence and rise of antimicrobial resistance. As this problem continues to grow, harmonized definitions with which to describe and classify bacteria that are resistant to multiple antimicrobial agents are needed, so that epidemiological surveillance data can be reliably collected and compared across healthcare settings and countries. In the strictest sense, multidrug-resistant organisms (MDROs) are labeled as such because of their in vitro resistance to more than one antimicrobial agent. Infections with MDROs can lead to inadequate or delayed antimicrobial therapy, and are associated with poorer patient outcomes [2].

In 2011, WHO declared “combat drug resistance: no action today, no cure tomorrow.”. In recent years, strains of multidrug resistant organisms have become quadrupled worldwide. Presently, antimicrobial resistance (AMR) poses a major threat to

patient's treatment as it leads to increased morbidity and mortality, increased hospital stays, and severe economic loss to the patient and nation. The clinical isolates such as *Pseudomonas aeruginosa*, Methicillin Resistant *Staphylococcus aureus* (MRSA), Enterococci especially Vancomycin Resistant Enterococci (VRE), and members of Family Enterobacteriaceae, for example, *Klebsiella pneumoniae*, *E. coli*, and *Proteus* sp., rapidly develop antibiotic resistance and spread in the hospital environment [3]. Actually, the health care planners have declared "Health for All by the year 2000." In the last two decades, there were so much increase of infectious diseases that the standard of public health in many parts of the world is equivalent to preantibiotic era [4]. As per standardized international terminology created by European Centre for Disease Control (ECDC) and Centre for Disease Control & Prevention (CDC), Atlanta, the multidrug-resistant (MDR), extensively drug-resistant (XDR), bacteria have been well defined. Multidrug resistant (MDR) was defined as acquired nonsusceptibility to at least one agent in three or more antimicrobial categories [5]. Extensively drug resistant (XDR) was 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 antimicrobial categories [6].

2. MATERIAL & METHODS

This study was carried out in the Department of Microbiology, F.H Medical College & Hospital, Tundla Agra. A total 371-Gram negative bacterial isolates were obtained from July 2022 to December 2024.

Aim of the study: To detect the prevalence of multi drug resistant, extensive drug resistant and pan-drug resistant bacterial isolates in tertiary care hospital.

Statistical analysis: Chi – Square analysis of study as per following formula

$$\chi^2 = \sum (O_i - E_i)^2 / E_i$$

Where

- O_i = observed value (actual value)
- E_i = expected value.

The Chi-Square test gives a P-value to help you know the correlation if any!

A hypothesis is in consideration, that a given condition or statement might be true, which we can test later. For example

- A very small Chi-Square test statistic indicates that the collected data matches the expected data extremely well.
- A very large Chi-Square test statistic indicates that the data does not match very well. If the chi-square value is large, the null hypothesis is rejected.

Objectives of the study:

1. Present study will help to know the prevalent drug resistant bacterial strain and their resistance pattern.
2. Close monitoring of drug resistant strain that will help in the formulation of antibiotic policy and infection control measure to reduce the incidence of antimicrobial drug resistance.

Study Design: Laboratory based prospective study.

Selection Criteria of Patients: All clinical isolates (Urine, Pus, blood, fluids, sputum etc.) received in microbiology laboratory from both IPD and OPD patients.

Methodology: Clinical samples received in microbiology lab are included in the study using a preformed structured format. Details such as name, age, sex, address, IP no., date of admission, and clinical data like presenting complaints, personal history, past medical history, high risk factors, immunocompromised status, physical examination findings and details of clinical diagnosis will be collected.

Technique:

- Smears are prepared from pus, wound swab, sediments of body fluids and respiratory samples, stained by Gram staining and are examined for presence of inflammatory cells, epithelial cells and the type of microbial flora.
- As soon the samples received in the laboratory, Streak culture method was employed for sample inoculation on culture media plates like Blood agar, MacConkey agar, CLED agar for uropathogens.
- Culture plates were incubated at 37°C aerobically for 24-48 hours
- Antimicrobial sensitivity testing done on Muller Hinton agar by Kirby-Bauer disc diffusion method as per CLSI 2022 guidelines [7].

3. RESULTS

A total number of 271 Gram Negative bacteria isolated from different clinical specimens like urine, pus, sputum, body fluids etc. Male patients (53%) are more reported then female patients (47%) in our study.

Chart 1: Male and Female Percentage

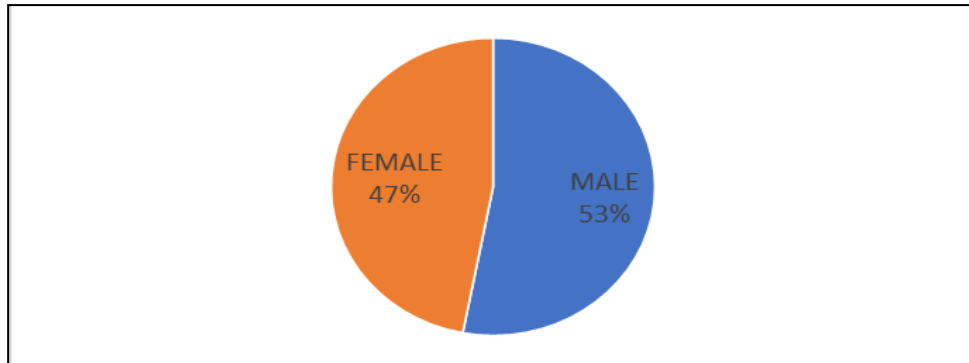


Table 2: AGEWISE DISTRIBUTION

AGE	No. Of Samples	%
Neonates	12	4
1-20 years	22	8
21-40 years	112	41
41-60	60	22
60	65	23
Total	271	100%

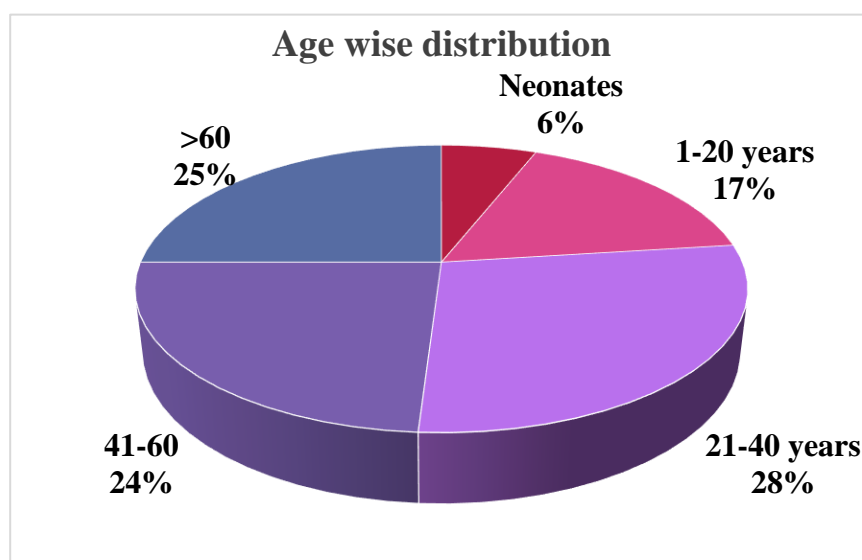
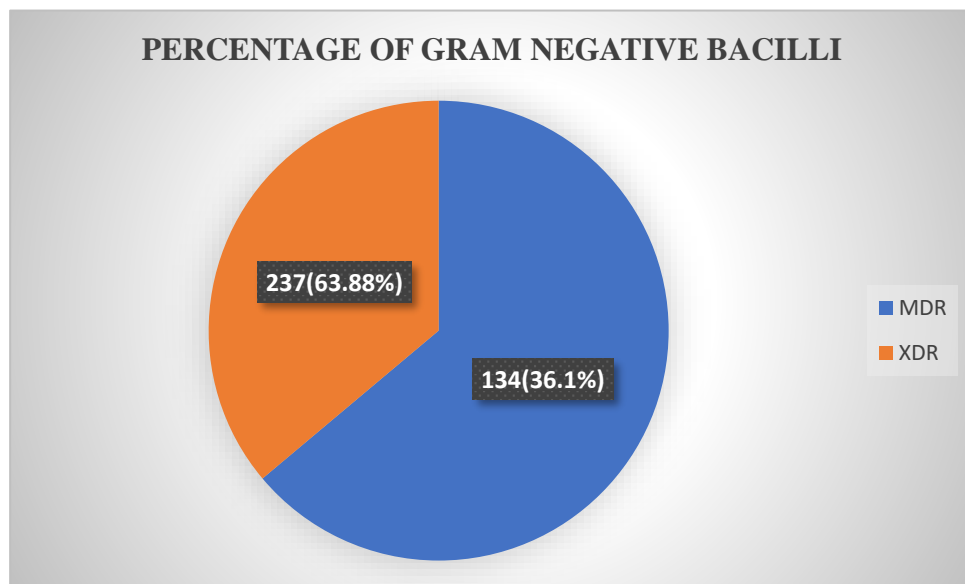


Table 3: DISTRIBUTION OF CLINICAL SAMPLES

AREA		PERCENTAGE
CRITICAL AREA	ICUs	53%
NON-CRITICAL AREA	WADS	44%
	OPD	3%

Table 4: PERCENTAGE OF GRAM NEGATIVE BACILLI (TOTAL - 371)

MDR	XDR
237(63.88%)	134(36.13%)

**Table 5: Antibiotic drug resistance in Gram negative bacilli**

ORGANISM	MDR	XDR	TOTAL
Escherichia coli	77	33	112
Klebsiella species	62	57	120
Pseudomonas	45	17	63
Citrobacter	15	4	19
Acinetobacter	22	14	36
Proteus	14	7	21

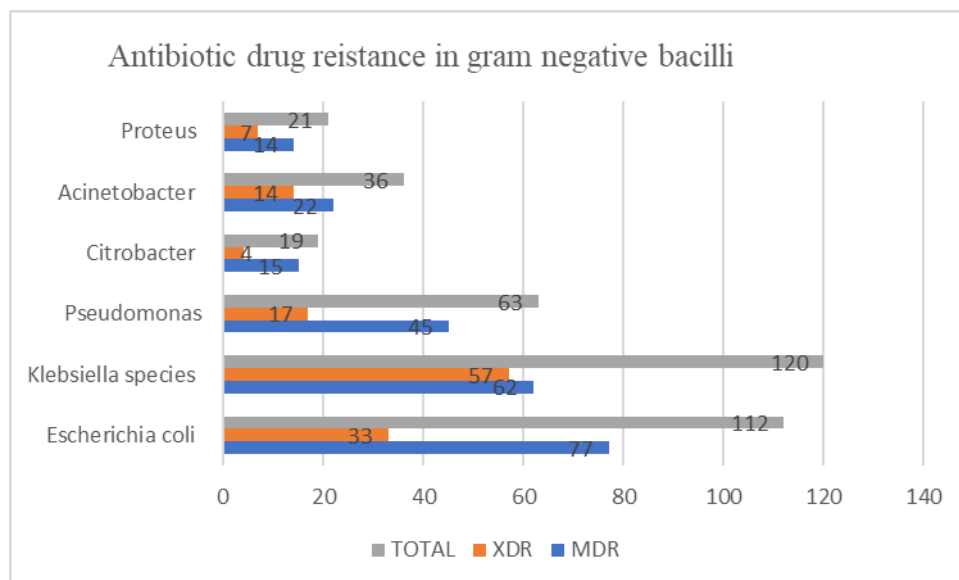
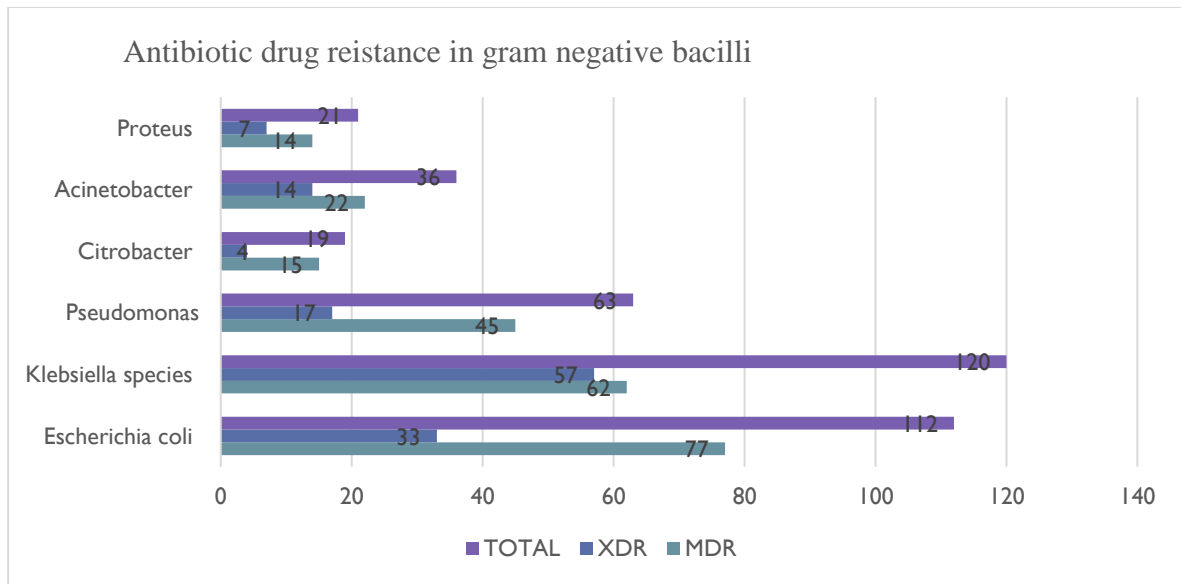
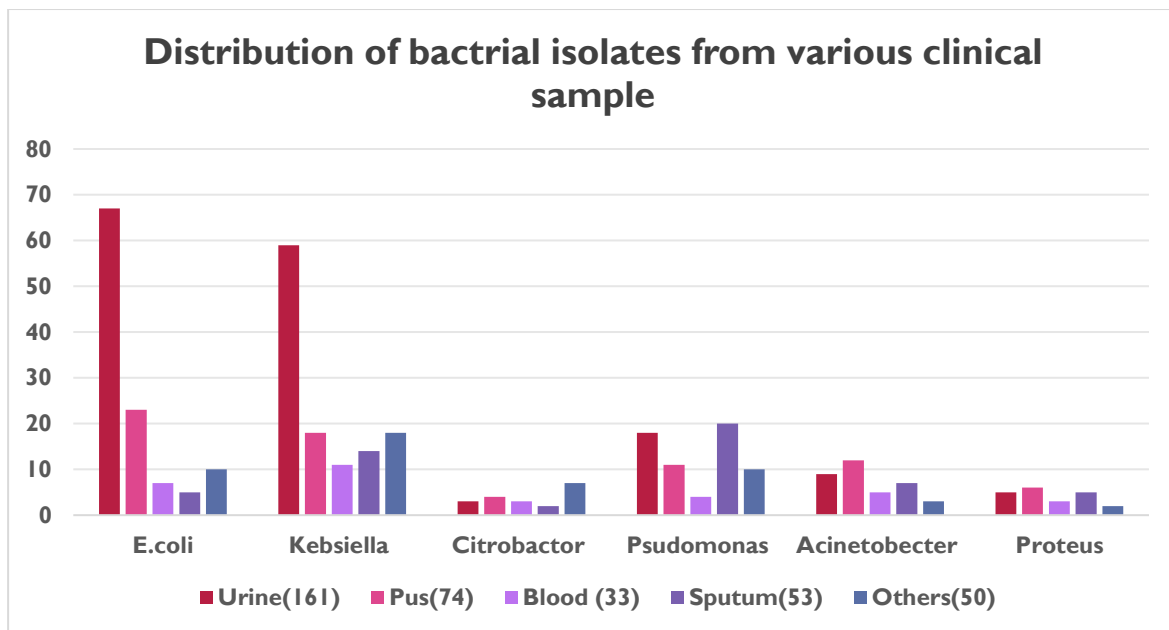


Table 6: Distribution of bacterial isolates (Gram negative bacteria) from various clinical samples

Sample	E.coli	Klebsiella	Citrobacter	Pseudomonas	Acinetobacter	Proteus
Urine(161)	67	59	3	18	9	5
Pus(74)	23	18	4	11	12	6
Blood (33)	7	11	3	4	5	3
Sputum(53)	5	14	2	20	7	5
Others(50)	10	18	7	10	3	2



4. DISCUSSION

In 2011, WHO declared “combat drug resistance: no action today, no cure tomorrow.” In recent years, strains of multidrug resistant organisms have become quadrupled worldwide. Presently, antimicrobial resistance (AMR) poses a major threat to patient’s treatment as it leads to increased morbidity and mortality, increased hospital stay, and severe economic loss to the patient and nation. The clinical isolates such as *Pseudomonas aeruginosa*, and the members of Family *Enterobacteriaceae*, for example, *Klebsiella pneumoniae*, *E. coli*, and *Proteus* sp., rapidly develop antibiotic resistance and spread in the hospital environment [8]. Actually, the health care planners have declared “Health for All by the year 2000.” As per standardized international terminology created by European Centre for Disease Control (ECDC) and Centre for Disease Control & Prevention (CDC), Atlanta, the multidrug-resistant (MDR), extensively drug-resistant (XDR), bacteria have been well defined. Multidrug resistant (MDR) was defined as acquired non susceptibility to at least one agent in three or more antimicrobial categories. Extensively drug resistant (XDR) was defined as nonsusceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two antimicrobial categories [9].

This Prospective study will be conducted in central laboratory, department of microbiology, F.H medical college & hospital, Tundla Agra. Total 371, gram negative bacterial isolates were obtained in two and half year study. Male patients 53% and female patient’s 47%. most of the bacteria isolated from Urine specimen 157 followed by pus sample 73. MDR was reported in GNB about 61.18% and XDR 35.3%. *Klebsiella spp* 120 was the most reported bacteria in our study followed by *E. coli* 112.

Table 7: Comparison of MDR & XDR in Gram Negative Bacterial isolates with Different Studies

S. Basalk et al. in 2012 ¹⁰	Reported MDR & XDR in GNB 13.8% and 37.1%
Partab SK, Raj K et al. In 2020 ¹¹	Reported MDR about 14% and XDR 35% in Gram negative isolates.
A Mathew, T Arbino et al in 2011 ¹²	find that the XDR in GNB is about 16% and MDR is about 40%.
T Bajpai, Ganesh S et al. In 2014 ¹³	observed that MDR 18% and XDR 7.2% reported in urine specimen.
Ibrahim ME, Bilal NE et al 2015 ¹⁴	observed that MDR is about 17% and XDR 5.6% in gram negative isolates.
J Lakshmi, V Puranet et al. in 2021 ¹⁵	Find that the MDR in GNB isolates about 16.5% and XDR about 9%.

In another study of Silpi Basak, Priyanka Singh et al were observed The antibiotic susceptibility profile of 1060 bacterial strains was studied. 393 (37.1%) bacterial strains were MDR, 146 (13.8%) strains were XDR, and no PDR was isolated. All (100%) Gram negative bacterial strains were sensitive to colistin whereas all (100%). Ibrahim ME, Bilal NE et al observed Of the 232 *E. coli* isolates, the majority were from urine (65.1%). MDR *E. coli* were present in 214 (92.2%). A study of Trupti Bajpai, Ganesh S et al. observed Out of the 314 urine samples tested, 120 isolates were detected among which 91 isolates were Gram negative bacilli. Among the 91 Gram negative isolates, 69 (75.8%) were MDR, 11 (12%) were XDR whereas 2 (2.1%) [16]. In a study of Degefu Beyene, I., Adane Bitew et al Out of 238 fermentative Gram-negative bacilli isolates, 94.5% were MDR of which 8.8% and 0.8% were XDR and PDR, respectively. Among 144 strains of *E. coli*, 99.3% were MDR of which 18.1% were XDR. Similarly out of 72 isolates of *K. pneumoniae*, 90.3% were MDR of which 11.2% and 2.8% were XDR and PDR respectively [17]. As this problem continues to grow, harmonized definitions with which to describe and classify bacteria that are resistant to multiple antimicrobial agents are needed, so that epidemiological surveillance data can be reliably collected and compared across healthcare settings and countries. Present study will help to know the prevalent drug resistant bacterial strain and their resistance pattern and also to detect the incidence of Multi drug resistant, Extensive drug resistant bacterial isolates in tertiary care hospital.

5. CONCLUSION

Applying these definitions for MDR and XDR worldwide would allow comparability of data and promote better comprehension of the problem of high antimicrobial resistant bacteria. This has been possible until now, not only due to varied definitions that are being used but also because of difference antimicrobial agents that are used for routine antimicrobial susceptibility testing. The proposed definitions of XDR and MDR present an opportunity for clinical microbiology laboratories to review and if necessary expand the number of antimicrobial agents routinely tested against various organisms and organism groups. The addition testing may be carried out in clinical microbiology laboratory by using a supplemented panel or by submitting the isolate to reference laboratory to allow definitive classification of these bacteria.

Moreover, it must be emphasized that although the XDR and MDR important characterization of drug resistance, in this era of extreme resistance and despite differences in the interpretation of MDR and XDR that can depend on geographical area and endemicity, countries should place high importance on monitoring resistant bacteria that are XDR and MDR because of their public health impact.

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