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PREVALENCE OF ANTI-MICROBIAL RESISTANCE IN GRAM-NEGATIVE ISOLATES AT A TERTIARY CARE HOSPITAL OF SUB HIMALAYAN REGION

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ABSTRACT Antimicrobial resistance (AMR) poses a major threat to global health. An antibiogram is an essential tool to track resistant trends and to assess local susceptibility rates. **Objective:** This study was designed to evaluate the susceptibility pattern of gram negative bacteria isolated from clinical samples. **Materials and Methods:** This is a retrospective study, conducted over a period of two years w.e.f. 1st January 2023 to 31st December 2024.The total number of gram-negative bacteria, isolated from these clinical samples along with their Antimicrobial susceptibility (AST) report was retrieved and analyzed to determine their susceptibility pattern.**Statistical analysis:** The results were analyzed for statistical significance using p-values. **Results:** A total of 10947 samples were received and processed. *Escherichia coli* (n=701) isolates were highest in number followed by *Klebsiella* spp. (n=304). *Escherichia coli* and *Klebsiella spp.* have highest susceptibility to Imipenem (85.1%; 83.72%) followed by Piperacillin-tazobactam (78.72%;80%) and Gentamycin (73.39%;80%). *Pseudomonas spp*. has highest sensitivity to Levofloxacin (80.55%), Piperacillin-tazobactam (80%) followed Imipenem (78.33%) while NF GNB showed lowest sensitivity to most antimicrobials. **Conclusion:** Gram negative bacteria are the majority isolates among clinical samples. Given the high rates of resistance in gram-negative bacteria, it is crucial to use a detailed hospital antibiogram for making informed antibiotic choices. Strict adherence to antibiotic policies, regular update of antibiograms and strengthening antimicrobial stewardship programs is recommended.

KEYWORDS : *Antimicrobial resistance (AMR), antimicrobial susceptibility testing (AST), antimicrobial agents (AMAs), Clinical and Laboratory Standards Institute (CLSI), multi-drug resistance (MDR).*

INTRODUCTION

The burden of infectious diseases with resistant microbes is increasing as per WHO report.[1] Antimicrobial resistance (AMR) is not a new phenomenon but due to high burden of infections, irrational use of antibiotics, over the counter availability of antibiotics, limited availability of antimicrobial susceptibility testing (AST) and improper use of antibiotics in veterinary medicine as well as agriculture sector, AMR has become a global challenge.[2,3]AMR poses a major threat to global health, food safety, and water resources. AMR occurs when microorganisms no longer respond to antimicrobial agents (AMAs) and have adapted as well as evolved the mechanisms of resistance to the lifesaving AMAs.[4,5] There is increased morbidity and mortality, more health care associated infections (HCAIs) as well as longer hospital stay due to AMR. Tertiary care hospitals cater to patients with multi organ failure, complex surgeries, immunosuppressive treatment, and have longer hospitalization. These settings are more susceptible for emergence as well as dissemination of AMR.[6] Both gram positive and gram-negative bacteria contribute to AMR and HCAIs but infections due to gram negative bacteria have become an increasing problem in recent years. [7] Gram negative bacteria which are common cause of urinary, respiratory and gastrointestinal tract infections as well as bacteremia and even nosocomial infections have developed high degree of resistance to antimicrobial agents.[8] The rate of multi drug resistant (MDR) gram negative bacilli (GNB) in the recent studies from India range from 25 % to around 40 % (9) Studies from Eastern Saudi Arabia and China also reported higher prevalence of MDR GNB i.e., 60.4% and 42.5% respectively. [10,11] An antibiogram is an essential tool of an institution, to track resistant trends and to assess local susceptibility rates. It also helps to compare susceptibility rates across institutions as well as to guide empirical antimicrobial therapy. The M39 guidance document for the development and implementation of cumulative antibiograms in healthcare facilities by Clinical and Laboratory Standards Institute(CLSI) helps in development of accurate, reliable and statistically valid antibiogram.[8] Antimicrobial stewardship program (AMSP) has expanded considerably in the last decade and use of antibiogram for implementation of AMSP initiatives is critical to optimize antimicrobial use in order to enhance patient outcome, prevention of AMR and reduce adverse events after antibiotic use.[12,13]

This study was designed to evaluate the susceptibility pattern of gram negative bacteria isolated from clinical samples received during two years. This study will provide information on local resistance trends, enabling healthcare providers to identify emerging resistant pathogens, monitor the effectiveness of current antibiotic treatments, implement targeted antimicrobial stewardship strategies, and contribute to broader regional and national AMR surveillance efforts, ultimately helping to optimize patient care and combat the growing threat of drug-resistant infections.

MATERIALS AND METHODS

This is a retrospective study, conducted in Department of Microbiology, Dr.Yashwant Singh Parmar Government Medical College (Dr. Y.S.P.G.M.C.), Nahan Distt Sirmour, Himachal Pradesh over a period of two-year w.e.f. 1st January 2023 to 31st December 2024 after approval from the institutional ethical committee vide approval number IEC/DYSPGMC/2023/14. Informed consent was waived due to the non-interventional observational nature of the cohort.

All the clinical samples received in Microbiology laboratory, which include urine, pus, sputum, blood, sterile body fluids, stools and other samples (Endocervical swabs, Nasal swabs, Genital ulcer swabs, Skin

scrapping and Tissues) were included in this study. The samples received were processed as per standard operating procedures of Bacteriology laboratory. The gram-negative bacteria so obtained were subjected to AST by Kirby-Bauer disc diffusion method on Muller Hinton agar. The antibiotic discs used for performing AST as well as the interpretation were made in accordance with CLSI M100 document.[14]

We divided the initial antibiotics into eight categories. (appendix 1) Isolates resistant to three or more antibiotic classes were considered multidrug-resistant (MDR).[15]

Inclusion criteria:

1. All clinical samples received during the specific timeframe.
2. Only gram-negative bacterial isolates were taken into consideration along with their AST report.

Exclusion criteria:

1. Samples collected outside the designated study period or external to Dr.Y.S.P.G.M.C. & H. Nahan, Himachal Pradesh.
2. Samples taken for surveillance purpose.
3. Gram positive isolates and bacterial isolates lacking complete or available AST report.
4. Duplicate samples will not be included

RESULTS:

A total of 10947 samples were received and processed w.e.f. 1st January 2023 to 31st December 2024.The distribution of samples received during the study period is shown in table 1.

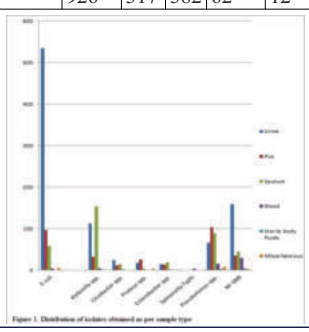
Table1: Distribution of samples received during the study period

S.No:	Sample type	Number of Sample Received	Number of Growth Positive Isolates
1.	Urine	6083	1361
2.	Pus	1463	647
3.	Sputum	1533	370
4.	Blood	1125	172
5.	Sterile body fluids	530	21
6.	Miscellaneous	177	39
Total		10947	2610

Amongst the urine samples (n= 6083), 1361(22.37%) samples showed significant growth of which 926 (68.03%) were gram negative bacteria. In pus, (n=1463) out of 647 (44.22%) growth positive samples, 317 (49%) were gram negative of which 178 belonged to Enterobacterales and 139 isolates were Non Fermenter Gram Negative Bacilli (NF GNB). Similarly, in sputum, blood, sterile body fluids and miscellaneous samples, 317, 62, 12 and 22 samples grew gram negative bacteria respectively. The distribution of Enterobacterales and NF GNB in all the specimens is shown in table 2 and figure 1.

Table2: Distribution of isolates obtained as per sample type

Sr. No	Organism	Urine	Pus	Sputum	Blood	Sterile body fluids	Miscellaneous	Total
1.	E.coli	534	97	58	05	01	06	701
2.	Klebsiella spp.	112	32	153	05	02	--	304
3.	Citrobacter spp.	24	11	14	02	01	--	52
4.	Proteus spp.	17	25	04	--	--	04	50
5.	Enterobacter spp.	14	13	19	02	--	02	50
6.	Salmonella Typhi	--	--	--	04	--	--	04
7.	Pseudomonas spp.	66	104	89	15	03	07	284
8.	NF GNB	159	35	45	29	05	03	276
Total		926	317	382	62	12	22	1721



The *E.coli* (40.73%) isolates were highest in number followed by *Klebsiella spp*. (17.66%) in conglomerated samples. In urine, pus and sputum samples, the highest number of isolates were *E coli*, *Pseudomonas spp*. and *Klebsiella spp* respectively. NF GNB were in maximum number in growth positive blood cultures samples.

Escherichia coli and *Klebsiella spp*. had highest sensitivity to Imipenem (85.1%; 83.72%) followed by Piperacillin-tazobactam (78.72%; 80%) and Gentamicin (73.39%; 80%) and were least sensitive to Amoxycillin-clavulanate (11.15%;12.98%). *Pseudomonas spp.* had highest sensitivity to Levofloxacin (80.55%), Piperacillin-tazobactam (80%) followed by Imipenem (78.33%) while least sensitive to Ceftazidime (15.38%). NF GNB showed highest sensitivity to Gentamicin (70.31%), Piperacillin-tazobactam (52.94%) and Levofloxacin (54.3%) while these isolates are least sensitive to Amoxicillin-clavulanate (14.43%).

The rate of MDR was 34.9%, 47%, 24.5% and 15.2 % among *E. coli,* NF GNB, *Klebsiella* spp. and *Pseudomonas spp,* respectively [Table 3, figure 2]

Table 3: Comparative Analysis of MDR Percentages in Different Organisms

Sr No	Organism	Year 2023		Year 2024		p-value
		No of Isolates	MDR (%)	No of Isolates	MDR (%)	
1	Escherichia coli	338	75(22.2)	361	169 (46.8)	0.0001
2	NF GNB	128	58(45.3)	129	64 (49.61)	0.5330
3	Pseudomonas spp	121	18(14.87)	169	26 (15.38)	0.9999
4	Klebsiella spp	69	13(18.84)	131	36 (27.48)	0.2262

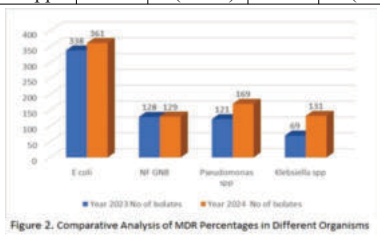


Figure 2. Comparative Analysis of MDR Percentages in Different Organisms

DISCUSSION

AMR is a big concern in healthcare and the bacterial pathogens as well as their antimicrobial sensitivity changes with time and place. This study highlights the AST of gram-negative bacterial isolates at a newly established tertiary care hospital. Our findings provide an insight into the common bacteria causing infections and their sensitivity pattern to various antimicrobial agents which aids in the formulation of hospital antibiograms. Hospital antibiograms are used to guide empirical antimicrobial treatment and are an important tool in monitoring the change in trends of AMR.

Gram negative bacteria are predominant isolates obtained among majority of clinical samples in our study wherein 1721 (65.9%) of all isolates during the study period were gram negative bacilli. These findings align with the study done by Sankarankutty *et al* and Karupiah *et al.* [16,17] The prevailing isolation of Gram-negative bacteria might be due to their simple nutritional requirements, frequent existence in the clinical setting and their ability to resist many antibiotics and detergents in the hospital area. Enterobacterales are the most common and important pathogens which accounts for majority of gram negative bacteria isolated from clinical samples. [13] *Escherichia coli* and *Klebsiella spp.* are predominant bacterial species isolated among Enterobacterales as observed by Kumar *et al and* Dodoo *et al.* [18,19] *Escherichia coli* isolates were the most common organism followed by *Klebsiella spp*. among enterobacterales in our study also. The predominance of *E. coli* and *Klebsiella* spp. in clinical samples could be because these bacteria are normal residents of the gut of individuals that can be displaced from the normal habitat to other sterile sites and openings on the skin and soft tissues, which results in disseminated infections. These observations co-relates with previous study, showing widespread of *E.coli.* [20]

Of the ESKAPE (*Enterococci, S. aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp.*) pathogens known to have antimicrobial resistance, the majority consists of gram-negative bacteria. MDR gram-negative bacteria have been associated with high mortality rates (30–70%). [21]

Our findings reveal that most GNBs show depict high resistance to Cephalosporins, Amoxycillin-clavulanic acid wherein around 11%, 12%, 12% and 14% of *E. coli, Klebsiella spp, Pseudomonas spp* and NF GNB, respectively were sensitive to Amoxycillin-clavulanic acid. The sensitivity rates to cephalosporins (ceftriaxone in enterobacterales and ceftazidime in *Pseudomonas spp*.) varied between 30% to 55% in four commonly isolated gram negative bacteria in the present study. Similar results were found in studies by *Iffat et al* [22] and *Balan et al.* [23]

The factors responsible for high prevalence of resistance to 3rd generation cephalosporins in Enterobacterales may include the over the counter availability of such as oral cephalosporins, and co-amoxiclav, the use of the same or related antibiotics in the poultry and fishing industries and the high use of cephalosporins in healthcare settings without adequate antimicrobial stewardship.

Fluoroquinolones sensitivity in our study is 71.05% for *Klebsiella spp.*, 80.55% for *Pseudomonas spp.* and 54.3% for NF GNB while *E.coli* showed only 19.04% sensitivity. *Hossam et al* in their study observed 60% sensitivity in case of *Klebsiella spp,* 45% in *Pseudomonas spp* and 33% for *E.coli* for fluoroquinolones [24] Enterobacterales and NF GNB showed higher sensitivity to aminoglycosides in our study which align with findings of study by *Krithu et al.*[25] The sensitivity percentage shown by *E.coli* for Cotrimoxazole in our study is similar to the study by *Chen HM. et al.* [26] More than 80% strains of *E.coli* and *Klebsiella spp* were sensitive to Imipenem. These findings are in corroboration with the study conducted by *Kannan et al.* [13]

In our study, 70-80% isolates of *E. coli, Klebsiella spp.* and *Pseudomonas spp* were sensitive to Carbapenems, Piperacillin-tazobactam and aminoglycosides in conjunction with previous studies.[22,26,27] However, NF GNB showed higher resistance to all antimicrobial agent classes as compared to Enterobacterales probably due to intrinsic resistance to common antibiotics. Around 50% of the isolates were sensitive to levofloxacin and piperacillin tazobactam whereas the sensitivity to cephalosporins was only 30%. The high degree of resistance of NF GNB to third-generation cephalosporins could be due to rampant use of this antibiotic in hospital settings. The sensitivity to imipenem was only 43.54% amongst the NF GNB isolates which is startling. Carbapenem resistance may be due to OXA24/40-like carbapenemases, OXA-23-like carbapenemases, metallo-β-lactamases, and serine carbapenemases

Though we did not speciate the NF GNB in our study, the most common NF GNB in most recent studies are *Pseudomonas spp*, *A. baumannii and S. maltophilia.* [28,29] The efficacy of carbapenems in treatment of infections caused by NF GNBs (except Stenotrophomonas maltophila which is intrinsically resistant to carbapanems) is decreasing. Injudicious and excessive use of these drugs should be regulated to preserve their activity against NF GNBs which are resistant to many antibiotics inherently. Presently, treatment options for these infections are limited to more toxic antimicrobial agents such as colistin and aminoglycosides. Our study also revealed that sensitivity of NF GNB to gentamycin was maximum (70.3%). The difficulty in treating infection caused by NF GNB jeopardizes the patient's life and puts the healthcare system at a significant strain. Additionally, the cost of treatment could be devastating for the patient, healthcare system, and society.

The phenomenon of antimicrobial drug resistance is growing at an alarming rate, with the frequency of MDR and XDR bacteria increasing daily. In 2023, there were 338 isolates of *E. coli* of which 22.2%(n=75) were MDR. However in 2024, of the 361 *E. coli* isolates, 46.8% (n= 169) were found to be MDR which is a significant increase ($p<0.005$) Similar findings were noted in *Klebsiella spp.* and NF GNB wherein the %age of MDR isolates increased from 18.84% to 27.48% and 45.3% to 49.61% in *Klebsiella spp.* and NF GNB respectively from 2023 to 2024.($p>0.005$) There was a marginal increase in MDR rate in *Pseudomonas spp.* (14.87% to 15.38% from the year 2023 to 2024). In a study from India, the MDR Gram-negative load was found to be 44.3%. [30] Another study from a tertiary care hospital in Korea in 2023 reported that 18 % of all gram negative isolates belonged to the MDR category. The MDR rate among Enterobacteriaceae was 13.9 % and among *Pseudomonas spp.* was 2.4%. [31] Similarly, another study by *Pydi et al,* found MDR rate among gram negative bacilli to be 20.62%. [32] The rate of MDR gram negative bacilli were significantly higher in ICUs as noted by *Miana et al* and *Joseph and Boloor* [33,34] wherein the rates varied from around 64% to 92% for all gram negative bacilli. Such high rates of MDR could be because ICU-admitted patients have induced immunosuppression, comorbidities and invasive medical interventions that increase their risk for nosocomial infections. Additionally these patients are on prolonged and multiple antibiotics which increase the risk of recurrent infections due to antimicrobial-resistant pathogens.

A high prevalence of MDR NF GNB (47%) and MDR E. coli (34.9%) in the present study raises the concern of rapidly emerging antibiotic resistance in this group of bacteria in our region. Additionally, these bacteria have the capability to survive in hospital environment, and thus improved infection-control measures and antibiotic stewardship are essential to prevent or slow their emergence and spread.

Limitations: The speciation of NF GNB gram negative bacilli could not be done conventionally due to scarcity of resources and manpower. The AST data presented in the study is not stratified by hospital location and by infectious syndromes, which would be of more help in selecting the most suitable drug for empiric therapy for a syndrome.

CONCLUSION

Gram negative bacteria are the majority isolates among all clinical samples. Given the high rates of resistance in gram-negative bacteria, it is crucial to use a detailed hospital antibiogram for making informed antibiotic choices and to implement strong measures against multi-drug resistance. A regularly updated local antibiogram helps to promote rational use of antibiotics and strengthen antimicrobial stewardship programs. It will help track resistance trends over time by highlighting increasing or decreasing resistance which will further help the hospital to prepare empiric treatment guidelines for common infective syndrome like Urinary tract infection, skin and soft tissue infections and pneumonia based on local susceptibility.

Additionally, the steps such as banning over-the-counter sales of antibiotics and implementing the national AMR plan should be fully supported. Most classes of antibiotics are available for use in humans and animals. AMR can be reduced when antimicrobials are used only as a treatment, rarely for prophylaxis, and never as growth promoters. Therefore, stringent and efficient control of the types and amounts of antimicrobials used in veterinary and agriculture practice will aid in controlling the spread of resistant bacteria to the environment.

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