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## Sciences

## Study On Emergence Of MDR Pathogens And Its Microbiological Study.

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#### ABSTRACT

Antimicrobial resistance is now a major problem confronting the clinicians for treating patients, as it leads to increased morbidity and mortality, increased hospital stay and severe economic loss. Multi drug resistance is defined as non- susceptibility to at least one agent in three or more antimicrobial categories. There is paucity of data regarding Multi Drug Resistance organisms (MDROs) in health care set up. Unless and until, multi drug resistant organisms are detected and their incidence is known, strategies for their control cannot be adopted properly. To estimate antimicrobial susceptibility profile of isolated MDR pathogens. Observational cross-sectional study was conducted for 3 months during the period of Jan 2024 to Mar 2024 in a tertiary care hospital in Ahmednagar, Maharashtra. Bacterial strains were isolated from different clinical samples and identified by conventional methods. Antimicrobial susceptibility test of bacterial strain was done by Kirby-Bauer disc diffusion method as per CLSI guideline. Out of 200 sample processed, 4 samples were culture negative. Our study identified 56% MDR gram negative pathogens and 44% MDR gram positive pathogens. Among MDR gram negative pathogens, predominant MDR gram negative isolate was Klebsiella (73.58%), followed by E. coli (29.54%), Pseudomonas aeruginosa, (36.36%) Acinetobacter (55.55%). Among overall gram-negative isolates maximum resistance was observed for third generation cephalosporin (64.02%) and fluoroquinolones (53.95%). Among MDR gram positive isolates, predominant was S. aureus with MDR 40.42% followed by enterococci with MDR 55.55%. Among overall gram-positive pathogens maximum resistance was observed for erythromycin (84.21%) and ciprofloxacin (28.07%). This study highlights prevalence of MDR pathogens. Preventive measures such as the continuous surveillance of the health care centers and treatment based on antibiogram and a strict implementation of infection control practices are essential in containing the threat of drug resistance in the health-care setting, especially in emerging economies like India.

**Keywords:** Multi drug resistance, Antibiogram, Antimicrobial susceptibility testing, surveillance, Stewardship, Infection control practices, Methicillin resistant *S. aureus*.

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#### INTRODUCTION

Antimicrobial resistance is rapidly becoming a global focus of attention, especially with rising number of microorganisms resistant to available antimicrobials. It includes both gram positive and gram negative bacteria with global prevalence of 60% or more [1]. In 2011, WHO declared "combat drug resistance: No action today, no cure tomorrow." [2].

Multidrug resistant organisms (MDRO) are described as acquired non sensitivity to one or more agents in at least three groups of antimicrobials [3], cause around 7,00,000 deaths worldwide every year and it is estimated that they will cause 10 million deaths by 2050, with a severe loss of economic resources [4]. Presently, antimicrobial resistance (AMR) poses a major threat to patients' treatment as it leads to increased morbidity and mortality, increased hospital stay, and severe economic loss to the patient and nation [5, 6].

The clinical isolates such as *Pseudomonas aeruginosa*, Methicillin resistant *Staphylococcus aureus*, Vancomycin resistant *enterococci and* members of family Enterobacteriaceae, *for example Klebsiella pneumoniae*, *Eschericia. coli*, *Proteus* rapidly develope antimicrobial resistance and spread in the hospital environment [7]. Hence, an evidence-based knowledge regarding the local antimicrobial resistance pattern is fundamental for guiding both antimicrobial treatment and empirical therapy of specific pathogens [8].

#### **Objectives**

To estimate antimicrobial susceptibility profile of isolated MDR pathogens

#### **MATERIAL AND METHODS**

This cross-sectional study was conducted in Department of Microbiology, in tertiary care hospital in Ahmednagar, Maharashtra, India.

#### Study period and clinical samples

Samples such as blood, urine, sputum, pus swabs, stool, other body fluid, ear swabs, throat swabs and nasal swabs were obtained from patients admitted in various clinical department during the study period from 1 Jan 2024 to 31Mar 2024.

The culture and identification were done on the specimen according to the standard operating procedure of the microbiology laboratory. The blood agar, MacConkey agar, chocolate agar were used to isolate organism from clinical specimen. Identification of organism was based colony morphology, biochemical test. Antimicrobial susceptibility testing was carried out according to Kirby Bauer's disc diffusion method and was reported in conformity with CLSI guidelines [9]. After adjustment to 0.5 Mcferland, standard inoculums was swabbed on Muller Hinton agar. Antibiotic discs were placed on media, incubated at 37°c for 24 hrs.

#### Antibiotic discs used for gram positive organisms

Penicillin (10μg), Ciprofloxacin (5μg), Erythromycin (15μg), Tetracycline (30μg), Gentamicin (10μg), Vancomycin (30μg), and Linezolid (30μg).

Antibiotic discs used for gram negative organisms- Amikacin(30μg), Ceftazidime(30μg), Ceftazidime+ clavulanic acid (30/10μg), ciprofloxacin(5μg), Imipenem(10μg), Colistin (10μg).

#### Drug resistance

The classification of drug resistance as below:

Multiple drug resistance- resistance to > 1 agent in three or more antimicrobial category drug [10].



#### RESULTS

This cross-sectional study was conducted in Department of Microbiology, in tertiary care hospital during the study period 1 Jan 2024 to 31 March 2024.

During this study period total 200 samples (pus, urine, sputum, blood, ET swab, Ascitic fluid were processed from various In patient department (Medicine, surgery, OBGY, Medical and surgical ICU, Neonatal ICU, orthopedic and pediatric department) of the hospital.

Out of 200 specimens, 4 specimens showed no growth for bacteriological diagnosis. Only 196 specimens were positive, with 139(71%) for gram negative isolates and 57(29%) for gram positive isolates.

Among 139 isolated gram-negative organisms, 78 (56.11%) were multidrug resistant gram negative isolate and from 57-gram positive isolates, 25(43.85%) were multidrug resistant

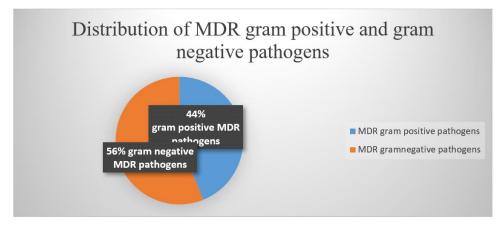
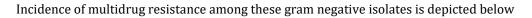
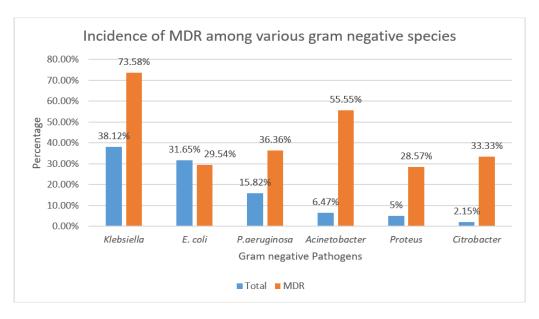
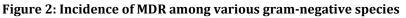


Figure 1: Distribution of MDR gram positive and gram-Negative pathogens

In our study, *Klebsiella spp.* (53) was most commonly isolated gram-negative organism followed by *E. coli* (44), *P. aeruginosa* (22), *Acinetobacter spp.* (9), *Proteus spp.* (7), *Citrobacter* (3) and single isolate of *Enterobacter*.



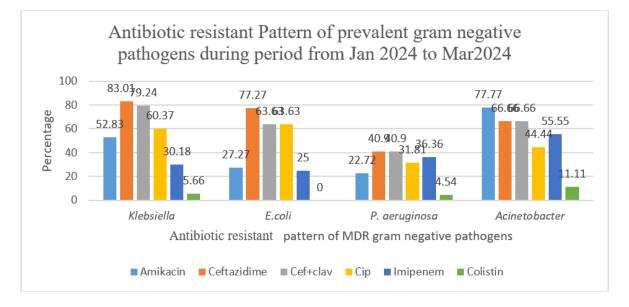




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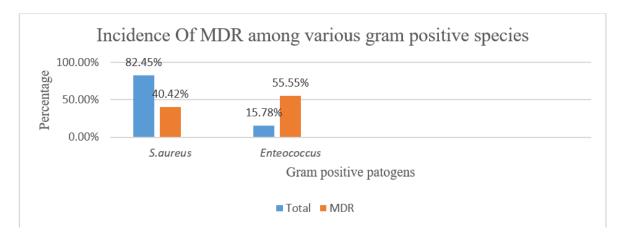


Among the gram-negative isolates, maximum resistance was observed with Ceftazidime (69.78%), Ceftazidime+clavulanic acid (64.02%), ciprofloxacin (53.95%), amikacin (41%). Resistance to Imipenem (29.49%) and Colistin (6.47%) was less.



#### Figure 3: Antibiotic resistant Pattern of prevalent gram-negative pathogens during period from Jan 2024 to Mar2024

Among gram positive isolates, most common isolated organism was *S. aureus* (n=47), with multidrug resistance of 40.42% followed by *enterococcus* with multidrug resistance of 55.55%. Single isolated *streptococcus* was not multidrug resistant. Incidence of multidrug resistance among gram positive isolate is given below

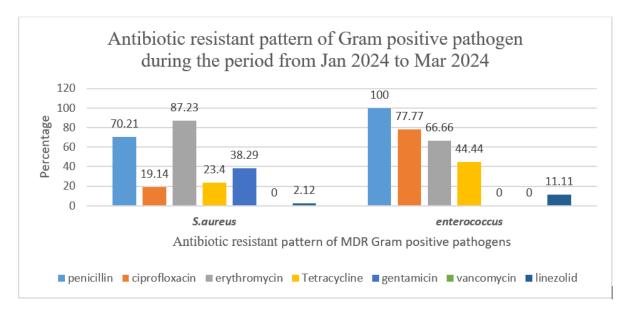


#### Figure 4: Incidence of MDR among various gram-positive species

Among the gram-positive pathogen, maximum resistance was observed with erythromycin (84.21%) and penicillin (75.43%). If we consider species wide distribution of multidrug resistance among gram positive pathogens, *S. aureus* was resistant to erythromycin (87.23%), penicillin (70.21%), gentamicin (38.29%), and tetracycline (23.40%).

Whereas, *enterococcus* was 100% resistant to penicillin and 77.77% resistant to ciprofloxacin and erythromycin 66.66%.





# Figure 5: Antibiotic resistant pattern of prevalent gram-positive pathogens, during the period from Jan 2024 to Mar2024.

### DISCUSSION

Infections are major problem in emerging and developing countries where hand hygiene and sanitation remain not up to international standards. Lack of microbial and antimicrobial data are also a problem in guiding physician in treating the patient with infection before the definitive treatment applied for the best outcome. Wonder drug penicillin started the era of antibiotics in 1928 and since then it has tremendously developed modern medicine. Persistent use of antibiotics, self-medication, and exposure to infections in hospitals has provoked the emergence of multidrug resistant (MDR) bacteria responsible for 15.5% Hospital Acquired Infection (HAIs) in the world [11-14].

In our study, majority of multi drug resistant organisms identified were gram negative isolates (56%) compared to MDR gram positive isolates (44%). These findings are consistent with Anuradha S De et.al [15]. Daniely M. Silva et.al [16]. The global scenario shows that Gram positive infections are more prevalent in the Western world, however, Gram negative bacterial infections dominate in India and Asia-Pacific region [17]. Comparable results were also found by Halim et al [18] where gram-negative bacteria took the upper hand among all nosocomial pathogens (53%) while gram-positive organisms represented 37.9%

Among the gram-negative pathogens, majority of isolates were *Klebsiella pneumoniae* followed by *E. coli*. These results were similar to results reported by other researchers studying bacterial strain in Egypt [18-20] as well as from countries other than Egypt.

In our study, prevalence of various gram-negative organisms was as follows- *Klebsiella* (53/139), *E. coli* (44/139), *P. aeruginosa* (22/139), *Acinetobacter* (9/139), *proteus* (7/139), *citrobacer* (3/139), *and enterbacter* (1/139). Similar findings were also observed by Nazneen et al., study conducted at cancer center, Aurangabad, Marathwada [21]. In our study, the predominant pathogen, *K. pneumoniae sub sp. pneumoniae*, showed 73.58% MDR level. This pathogen is one of the major drivers of infections in health care settings belonging to the family of *enterobacterales*. Literature has documented that currently *enterobacterales* resistance to 3rd generation cephalosporins is now above 10% and 2.7% for carbapenem, citing the rapid spread of ESBL producing strains as the main cause for these findings [22].

In our study, predominant MDR gram positive pathogen was *S. aureus* (40.42%) and *Enterococcus* (55.55%). The methicillin resistance *S. aureus* (MRSA) is associated with poor clinical outcome in numerous infections include prolonged hospital stay [23, 24]. Currently, MRSA isolates are estimated to account for 25% of *S. aureus* isolates, with a prevalence of up to 50% or more in some areas [25]. In India, the incidence of MRSA is increasing, with prevalence rates varying from 23.6% to as high as 59.3% [26-28].

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Our study identified 55.55% MDR *enterococci* isolates. Our study reports 100 % resistance towards penicillin, 77.77% resistance for ciprofloxacin and 66.66% resistance for erythromycin by *enterococci*. Community-acquired infections due to *enterococci* are on the rise due to intensive use of broad-spectrum antibiotics. Moreover, since vancomycin-resistant *enterococci* (VRE) from animal sources such as poultry and human foods of animal origin play an important role in human colonization and infection, a significant level of VRE colonization may be found among persons not associated with the health care setting [29].

#### CONCLUSION

Our study highlights that an antibiogram may help the clinicians to understand local antibiotic-resistant pattern and help them to make an informed decision about the initial empirical antibiotic.

Regular monitoring of pattern of resistance of bacteriological isolates is critical to develop much needed antibiotic policy to combat these infections early. Continuous antibiotic stewardship is required and should be monitored on regular basis to improve outcomes.

Strict infection prevention and control practices, with judicious antibiotics prescription policy, may help in tackling the emerging threat of multiple drug-resistant bugs by obviating the selection pressure.

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#### REFERENCES

- [1] Oduyebo OO, Olayinka AT, Iregbu KC, Versporten A, Goossens H, Nwajiobi-Princewill PI, et al. A point prevalence survey of antimicrobial prescribing in four Nigerian tertiary hospitals. Ann Trop Pathol 2017; 8: 42-6
- [2] A Sharma. Antimicrobial resistance: no action today, no cure tomorrow. Indian Journal of Medical Microbiology 2011;29(2):91–92.
- [3] Simmons BP, Larson EL. Multiple drug resistant organisms in healthcare: the failure of contact precautions. J Infect Prev 2015; 16 (4):178–81.
- [4] Mulani MS, Kamble EE, Kumkar SN, et al. Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. Frontiers in Microbiology 2019;10: Article No. 539.
- [5] LH Rosenberger, T Hranjec, AD Politano et al. Effective cohorting and 'super isolation' in a single intensive care unit in response to an outbreak of diverse multi-drug-resistant organisms. Surgical Infections 2011; 12(5): 345–350.
- [6] E Morales, F Cots, M Sala et al. Hospital costs of nosocomial multi-drug resistant Pseudomonas aeruginosa acquisition. BMC Health Services Research 2012; 12(1):122.
- [7] Arias CA, Murray BE. Antibiotic-resistant bugs in the 21st century—a clinical super-challenge. The New England Journal of Medicine 2009; 360 (5):439–443.
- [8] Tamma PD, Cosgrove SE, Maragakis LL. Combination therapy for treatment of infections with gram-negative bacteria. Clin Microbiol Rev 2012; 25(3):450–470.
- [9] Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. Twenty-second Informational Supplement (M100-S22). Wayne, PA, USA: CLSI; 2016.
- [10] Magiorakos AP, Srinivasan A, Carey R, Carmeli Y, Falagas M, Giske C, et al. Multidrug resistant, extensively drug resistant and pan drug resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology And Infection 2012; 18(3):268-81.
- [11] Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. J Infect Dis 2008; 197:1079–1081.



- [12] Allegranzi B, Nejad SB, Combescure C, Graafmans W, Attar H, Donaldson L, et al. Burden of endemic health-care-associated infection in developing countries: systematic review and metaanalysis. Lancet 2011; 377: 228–241.
- [13] Ibrahim ME, Bilal NE, Hamid M. Increased multi-drug resistant Escherichia coli from hospitals in Khartoum state, Sudan Afr Health Sci 2012; 12: 368–375.
- [14] Pendleton JN, Gorman SP, Gilmore B. Clinical relevance of the ESKAPE pathogens. Expert Rev Anti Infect. Ther 2013; 11:297–308.
- [15] Anuradha S de, Baveja S, D'Souza D and Patwegar S. Antimicrobial resistance among commonly encountered bacteria isolated in 2013- The ESKAPE Menace. Intern Med 2015;5(3).
- [16] Daniely M. Silva, Eulina Maria N. Menezes, Emerson V. Silva; Thaís A. C. Lamounier. Prevalence and antimicrobial susceptibility profile of ESKAPE pathogens from the Federal District, Brazil. J Bras Patol Med Lab 2017; 53(4): 240-245.
- [17] Chaudhry D, Prajapat B. Intensive care unit bugs in India. How do they differ from the Western world? J Assoc Chest Physicians 2017; 5:10.
- [18] Halim MMA, Eyada IK, Tongun RM. Prevalence of multidrug drug-resistant organisms and hand hygiene compliance in surgical NICU in Cairo University Specialized Pediatric Hospital. Egypt Pediatr Assoc Gaz 2018; 66 (4):103–11.
- [19] Sawhney N, Shinu P, Singh VA. Bacteriological profile and antibiotic susceptibility pattern of neonatal septicaemia in a tertiary care hospital. Int J Curr Microbiol App Sci.2015; 4(10):977–84.
- [20] Eshwarappa M, Dosegowda R, Aprameya IV, Khan MW, Kumar PS, Kempegowda P. Clinicomicrobiological profile of urinary tract infection in south India. Indian Journal of Nephrology 2011; 21(1):30-6.
- [21] Nazneen S, Mukta K, et al. Bacteriological trends and antibiotic susceptibility patterns of clinical isolates at Government Cancer Hospital, Marathwada. Indian J Cancer 2016; 53: 583-586.
- [22] Jubeh, B., Breijyeh, Z. and Karaman, R. (2020) Resistance of Gram-Positive Bacteria to Current Antibacterial Agents and Overcoming Approaches. Molecules 2020;25: Article No. 2888.
- [23] Liu F, Wen Z, Wei J, Xue H, Chen Y, Gao W, et al. Epidemiology, microbiology and treatment implications in adult patients hospitalized with pneumonia in different regions of China: a retrospective study. Journal Of Thoracic Disease 2017; 9(10):3875-87.
- [24] Pulido-Cejudo A, Guzman-Gutierrez M,Jalife-Montano A, Ortiz-Covarrubias A, Martinez-Ordaz JL, Noyola-Villalobos HF, et al. Management of acute bacterial skin and skin structure infections with a focus on patients at high risk of treatment failure. Therapeutic Advances In Infectious Disease 2017; 4(5):143-61.
- [25] Sirijan Santajit and Nitaya Indrawattana. 2016. Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. BioMed Research International 2016: Article ID 2475067.
- [26] Tiwari HK, Sen MR. Emergence of vancomycin resistant Staphylococcus aureus (VRSA) from a tertiary care hospital from northern part of India. BMC Infect Dis 2006; 6: 156.
- [27] Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 2009;48: 1-12.
- [28] Lockhart SR, Abramson MA, Beekmann SE, Gallagher G, Riedel S, et al. Antimicrobial resistance among Gram-negative bacilli causing infections in intensive care unit patients in the United States between 1993 and 2004. J Clin Microbiol 2007; 45: 3352-3359.
- [29] Mathur P, Singh S. Multidrug resistance in bacteria: A serious patient safety challenge for India. J Lab Physicians 2013; 5: 5–10.