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RESEARCH ARTICLE

STUDY ON EMERGENCE OF MDR PATHOGEN AND ITS MICROBIOLOGICAL STUDY

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Abstract

Antimicrobial resistance is now a major challenge to clinicians for treating patients.Hence, this study was undertaken to detect the incidence of multidrug-resistant (MDR),extensively drug-resistant (XDR) and pandrug-resistant (PDR) bacterial isolates in a tertiary care hospital.

Aims and Objectives:
This study was undertaken to detect the incidence of MDR, XDR and PDR bacterial isolates in a tertiary care hospital and to help in antibiotic policy of a hospital to guide the clinicians for empirical usage of antibiotics and prevent drug resistance.

Methodology: This research study was conducted to isolate multi-drug resistant pathogen.All the clinical samples like pus, swab, urine, body fluids, medical devices, blood culture, sputum, drain etc., received for culture and sensitivity in Microbiology laboratory during Jan 2022 to December 2022 were included. They were inoculated and then incubated, and gram staining was performed. Antibiotic resistance and susceptibility were measured by the disk diffusion method according to the Clinical and Laboratory standards institute (CSLI) guidelines.

Result: Out of 5501patients 2008 (37%) patients were showing culture positive during one year. The predominant gram negative isolate in our study was E.coli (19%) followed by klebsillaspp (18%), staphylococcus aureus (16%) and pseudomonas (13%) respectively. It shows that colistin was found to be more sensitive for gram negative organisms followed by aminoglycosides and carbapenem group, while resistance was observed towards fluoroquinolone, 1st and 2nd generation cephalosporine groups. &Vancomycin, Teicoplanin and Linezolide were highly active drugs against Gram positive organisms.

Conclusion: The vast majority of isolated organisms in this study were gram-negative bacteria, and most were showing high antimicrobial resistance. The antibiograms should be developed and regularly updated at every ward and hospital. There is a need to bring more awareness about the proper use of antimicrobials among healthcare workers, and antimicrobial stewardship programs can help in this matter.

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Introduction:-

In 2011, WHO declared "combat drug resistance: no action today, no cure tomorrow."[1] In recent years, strains of multidrug resistant organisms have become quadrupled worldwide.[2] 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.[3,4]

Antimicrobial resistance (AMR) has become a challenge in modern-day medical practice. The pace at which microbes are becoming resistant to antibiotics is greater than the discovery of novel antimicrobial agents. This problem is substantially greater in developing countries like India with limited resources and public awareness [5]. Hence, there is a need to study these patterns. The way to do so is to develop antibiograms at the institutional or even ward level. Antibiograms function to guide the use of antibiotics for prophylactic and empirical purposes. They display the current trends of microbes isolated from different patients and the antibiotic resistance patterns of these isolates [6].

It is essential to develop and analyze these antibiograms so that physicians, surgeon & pediatrician, are better aware of the current trends of antimicrobial resistance in their respective wards and institutions. It also guides the development of antimicrobial stewardship programs, which may help tackle the problem of antimicrobial resistance in an organized manner [7].

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), and pandrug-resistant (PDR) bacteria have been well defined [8]. Multidrug resistant (MDR) was defined as acquired nonsusceptibility 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). Pandrug resistant (PDR) was defined as nonsusceptibility to all agents in all antimicrobial categories. Hence, this short term study was undertaken to detect the incidence of MDR, XDR, and PDR bacterial isolates in a tertiary care hospital of Central India.

Aim and Objevctive:-

1. This study was undertaken to detect the incidence of MDR, XDR, and PDR bacterial isolates in a tertiary care hospital.
2. To Help in antibiotic policy of a hospital to guide the clinicians for empirical usage of antibiotics and prevent drug resistance

Material and Method:-

Study design:-

This is a retrospective study which was initiated in the Department of Microbiology of the NAMO MEDICAL EDUCATION AND RESEARCH INSTITUTE & SVBCH, Silvassa, DNH after obtaining approval from the ethical committee.

Study period:-

1 year, from January 2022 – December 2022.

Sample size:-

All the samples that are received in bacteriology section of Microbiology laboratory for bacterial culture and sensitivity are included.

Inclusion criteria:-

All the samples received from the OPD, wards and Critical areas like Medical Intensive care unit, Surgical Intensive care unit, Neonatal Intensive care unit, Obstetric Intensive care unit were included.

Exclusion criteria:-

Samples that are received from the same patient within a span of 3 days are considered as repeat isolates and are not included in the analysis of antibiogram.

Methodology:-

Sample Collection:-

All the clinical samples like pus, swab, urine, body fluids, medical devices, blood culture, sputum, drain etc., received for culture and sensitivity in bacteriology section at Microbiology laboratory were given a Laboratory Identification number.

Sample Processing: - (1ST DAY FOLLOWUP)

All samples were cultured on Blood agar, Mac conkey agar, Chocolate agar and Nutrient agar as per the protocol of sample processing & incubated at 37°C for 24 hours in aerobic environment. Also smears were prepared from all the samples and they were subjected to gram stain, in order to see whether the organism is a gram positive or gram negative.

Further processing of sample: (2ND DAY FOLLOWUP)

Culture plates were observed for growth after 24 hours of incubation. If any visible growth was observed over the plates, then Colony smear was prepared and gram staining was carried out. Depending on the colony morphology and result of gram stain, a provisional identification of the organism was done. Different biochemical test is performed according to morphology andast is performed.

Further processing of sample: (3rd DAY FOLLOWUP)

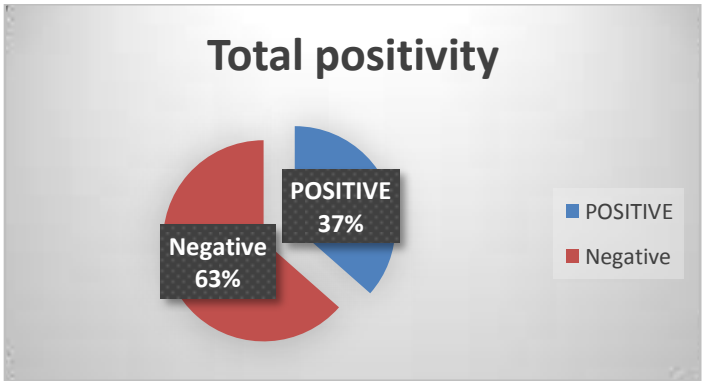
Identification of organism was done and antibiotic susceptibility was measured by the disk diffusion method according to the Clinical and Laboratory standards institute (CLSI) guidelines.

Quality Control:

Quality Control:
Staphylococcus aureus ATCC 25923- Oxacillin susceptible
Staphylococcus aureus ATCC 43300- Oxacillin resistant
Staphylococcus aureus ATCC BAA966- D-Zone test Negative
Staphylococcus aureus ATCC BAA966- D-Zone test Positive
Klebsiella pneumonia ATCC 700603- ESBL positive
Escherichia coli ATCC J53RI (TEM ESBL) - ESBL positive
Escherichia coli ATCC 25922- ESBL Negative.

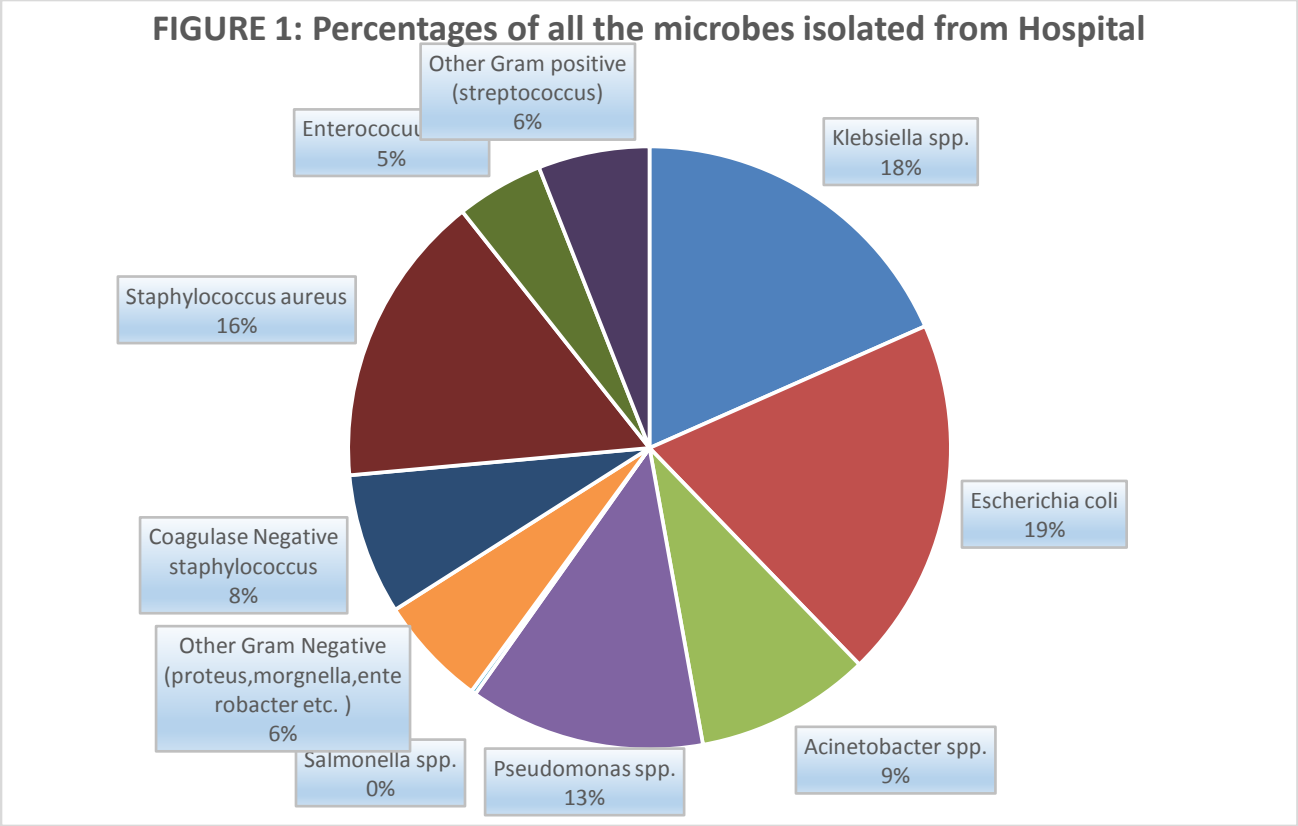
Result:-

1. Present study was conducted in Microbiology department of tertiary care hospital. Out of 5501patients 2008 (37%) patients were showing culture positive during one year (January 2022 to December 2022).

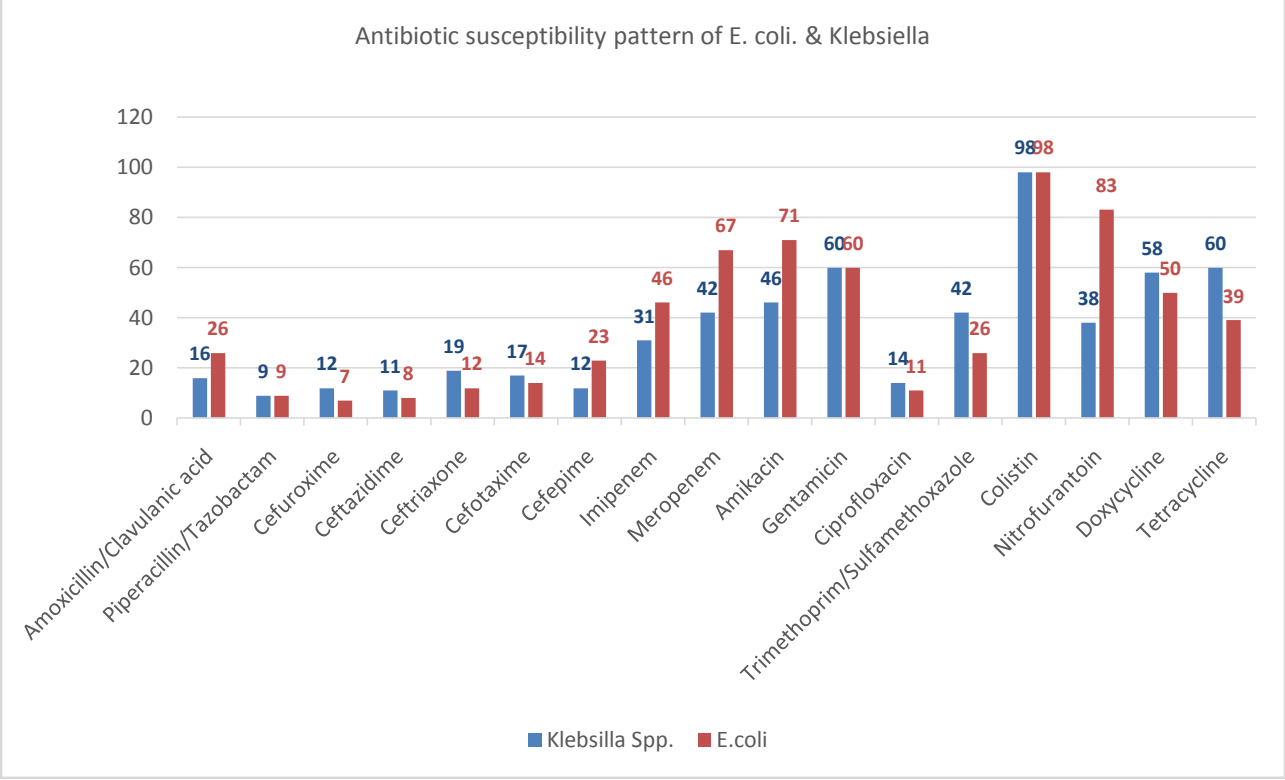


2. **Microorganisms isolated from culture:**

ORGANISMS	TOTAL NO. OF ORGANISMS
Gram positive cocci	683
Gram negative bacilli	1325
Total	2008

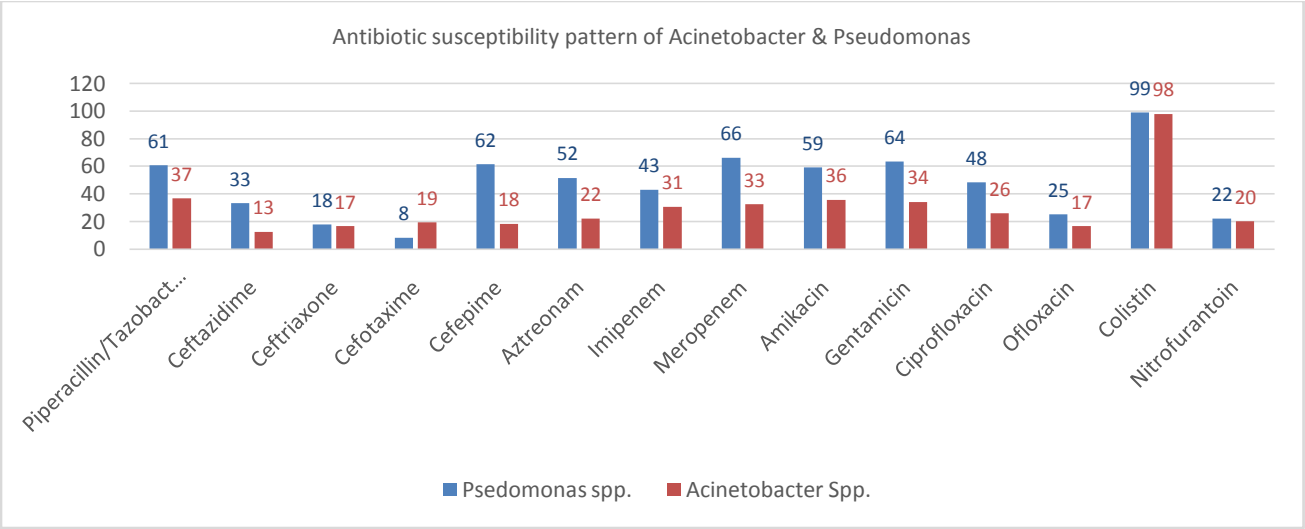


3. Antibiotic susceptibility pattern of E. coli.& Klebsiella



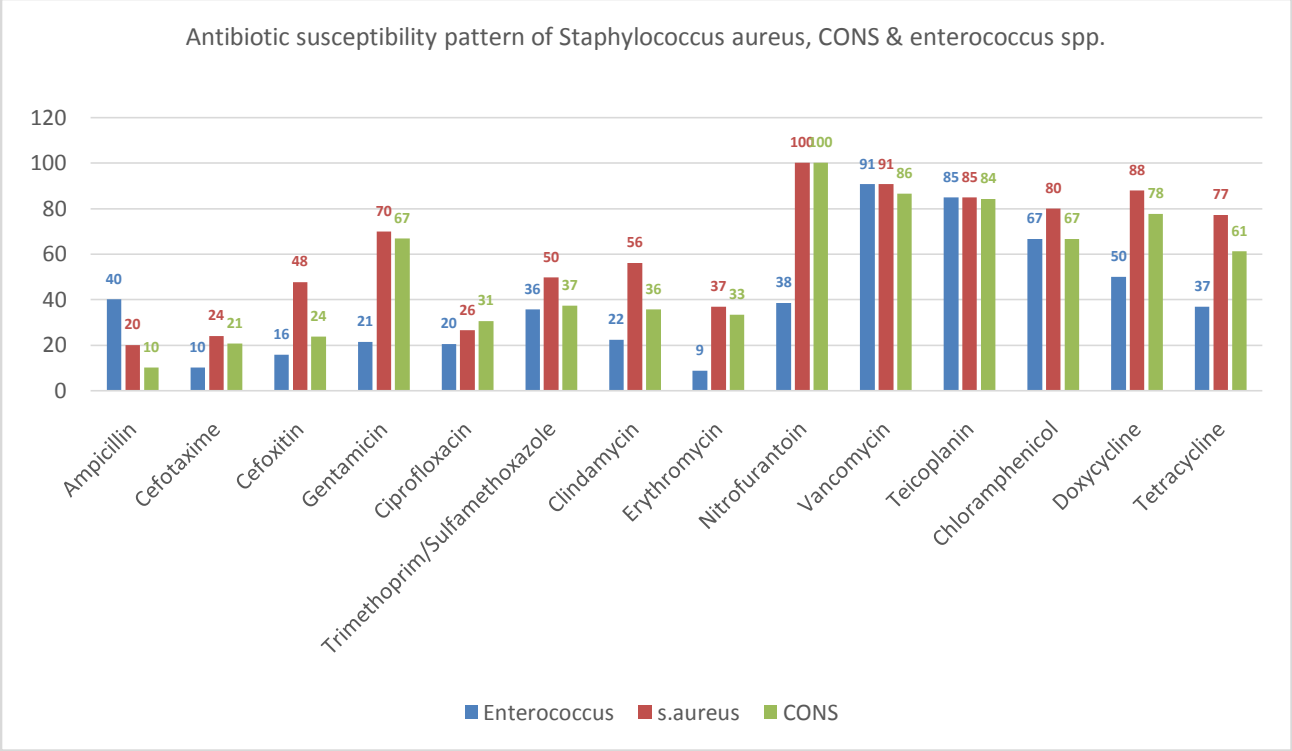
It shows that colistin was found to be more sensitive for both of the organisms followed by aminoglycosides and carbapenem group, while resistance was observed towards fluoroquinolone, 1st and 2nd generation cephalosporine & piperacillin tazobactam.

4. Antibiotic susceptibility pattern of Acinetobacter&Pseudomonas



It shows that colistin was found to be more sensitive for both of the organisms followed by aminoglycosides and carbapenem group, while resistance was observed towards fluoroquinolone , 1st and 2nd generation cephalosporine.

7.Antibiotic susceptibility pattern of Staphylococcus aureus, CONS & enterococcus spp.



Vancomycin, Teicoplanin and Linezolide were highly active drugs against Gram positive organisms.

Discussion:-

The choice of empirical antimicrobial therapy is made on the basis of local guidelines and antibiogram patterns that demonstrate the trends of antimicrobial resistance and organisms commonly isolated from a particular hospital or ward. Hence, it is very important for the physician to familiarize himself with this information. With time, newer trends of antimicrobial resistance are being discovered, so the patterns of antibiotic resistance are dynamic in nature [9]. That's the reason antibiograms are regularly updated based upon hospital protocols.

The clinical and financial burden to patients and health care providers for MDROs is really challenging. Barbara Soule, Joint Commission Resources Practice Leader, Infection Prevention and Control Services, has told, "Patients who are infected with MDROs often have an increased risk of prolonged illness and mortality. The cost of care for these patients can be more than double as compared to those without MDRO infection". Since the year 2000, only 4 new classes of antibiotics have been approved by Food and Drug Administration (FDA), US, for example, linezolid, streptogramins, daptomycin, and tigecycline. The first 3 drugs are effective against MRSA and VRE. Tigecycline has also effect on Gram negative bacilli. The problem is that the bacteria are developing resistance at a much faster pace than the new drug development [11]. Regarding public health attention, MDROs are described as superbugs having very limited treatment options. For some MDROs, only 1 or 2 antibiotics can be effective with toxic side effects. In 2009, Boucher et al. have reported ESKAPE organisms as "Bad Bugs," where E stands for Enterococcus faecium, S for Staphylococcus aureus, K for Klebsiella pneumoniae, A for Acinetobacter baumannii, P for Pseudomonas aeruginosa, and E for Enterobacter species [12]. In the year 2009 only, Peterson has reported the ESCAPE group of organism, which was the same as the above list but K was replaced by C, that is, Clostridium difficile, and the last E stands for Enterobacteriaceae [13]

Gram-negative bacterial infections have been recently reported to be significantly increased worldwide. Our results confirmed that the most common pathogens isolated were Gram-negative bacteria (65.98%), which may be due to their wide prevalence in the hospital environment. Additionally; their frequent resistance to antibiotics may play a role in their persistent and spread. The predominant gram negative isolate in our study was E.COLI followed by klebsilla spp , which was similar to other studies by Zainab et al 2012 (14) and Morfin Otero et al 2012 (15) have reported E.coli as predominant organism &klebsilla is predominant in rajan et all . A similar study conducted by Ziab et al 2013 (16) reported Pseudomonas aeruginosa as predominant gram negative bacilli isolated from ICU and also according to AMR Annual Report (2021) E.coli is predominate organism followed by klebsilla among 95728 isolates.(29)

For **E.coli**1st most common microorganism in our study (n=389) Our study showed high resistance to third-generation cephalosporins (88% ceftriaxone) and 77 % for cefepime (4th generation) Qadeer et al.'s study showed high resistance to third-generation cephalosporins (93% ceftazidime and 90% ceftriaxone) [17]; similarly, more than 90% E. coli were found to be resistant to third-generation cephalosporin by Al mohammady et al. [18]. Carbapenem resistance was 52% for imipenem and 47.3% for meropenem. Carbapenem resistance was as low as 10% in Qadeer et al. [17]. Almost similar results reported by Bayram et al. [19] showed 13.1% E. coli resistance to imipenem. Gunjal et al. [22] reported that 28.10% of E. coli isolates were resistant to amikacin and 48.20% resistance to gentamicin, whereas resistance amikacin and gentamicin were 29% and 40%, respectively, in our study. Colistin showed only resistance by 1% to E. coli strains in the present study. our resistance pattern is similar to AMR annual report in which high resistance toward cephalosporins (cefotaxime (84%)and ceftazidime(82 %) followed by carbapemans(36%) . whereas least resistance drug was aminoglycoside (22%) group and colistin(1%). (29)

Klebsiella, 2nd most common microorganism in our study (n=369), showed high carbapenem resistance (69% meropenem and 58% imipenem), while in Qadeer et al.'s study, similar resistance was observed (56% meropenem and 55% imipenem) [17], whereas Sheth et al. showed 100% sensitivity to carbapenems [20] and Rajan et al. documented 28.13% carbapenem resistance [21]. In the present study, a high pattern of resistance was seen with third-generation cephalosporins (81% ceftriaxone) and 88% for cefepime (4th generation) and aminoglycosides (40% gentamicin, 54% amikacin). Also, in Qadeer et al.'s study, a high pattern of resistance was seen with third-generation cephalosporins (94% ceftazidime, 82% ceftriaxone, and 70% cefoperazone/sulbactam) and aminoglycosides (61% gentamicin, 48% amikacin) [17]. Gunjal et al. has reported 60% resistance to amikacin and 80% resistance to gentamicin [22]. The most effective drug was colistin, which showed 1% resistance in our study. Whereas, tigecycline was found to be the effective antibiotic against multidrug-resistant Klebsiella in Qadeer et al.'s study [17]. our resistance pattern is similar to AMR annual report in which high resistance toward cephalosporins

(cefotaxime (80%)and ceftazidime(81%) followed by carbapemans(58%) resistance to carbapenem was observed more in klebsilla compare to E.coli . whereas least resistance drug was colistin(4%). (29)

Among the non-fermenting gram negative bacteria, in our study pseudomonas (13%) followed by Acinetobacter spp.(9%) Whereas as in AMR REPORT Acinetobacter baumannii (49.5%) was more common followed by Pseudomonas aeruginosa (46.4%),A. baumannii and P. aeruginosa causes serious healthcare associated infections such as pneumonia, bloodstream infections andpostoperativewoundinfections.[29]

In our study, Pseudomonas (n=254) showed resistance to carbapenems (57% imipenem, 34% meropenem). In Qadeer et al.'s study [17], Pseudomonas showed less resistance to carbapenems (59% imipenem/meropenem), whereas a study published by Rakhee et al. [23] showed 20.8% resistance to imipenem and the study published by Rajan et al. [21] showed 12.9% carbapenem resistance to Pseudomonas. Pseudomonas also showed high resistance to third generation cephalosporins (83% ceftriaxone) and 58% to cefepime (4th generation), while aminoglycosides showed (36% gentamicin and 41% amikacin). In Qadeer et al.'s study [17], Pseudomonas aeruginosa resistance to third- generation cephalosporins was (53% cefoperazone/sulbactam and 39% to ceftazidime) and for aminoglycosides was (48% gentamicin and 41% amikacin). Radji et al. showed 60.9% resistance to ceftriaxone and found that amikacin was the most effective antibiotic against Pseudomonas with 15.6% resistance [24]. We found colistin to be the most effective antibiotic against Pseudomonas with 1.01% resistance.

Our study shows carbapenem resistance among Acinetobacter (n=189), 69 % for imipenem and 68% for meropenem. Qadeer et al.'s study showed 100% resistance to carbapenems [17]. Another study conducted by Khan has reported 79% resistance to imipenem [25], while Rajan et al. showed 52% carbapenem resistance among Acinetobacter [21]. In our study, Acinetobacter was highly resistant to third-generation cephalosporins (83% ceftriaxone), aminoglycosides (66% gentamicin and 64% amikacin), and quinolones (74% ciprofloxacin). In Qadeer et al.'s study, Acinetobacter was also highly resistant to third generation cephalosporins (100% ceftazidime), aminoglycosides (97% gentamicin and 95% amikacin), and fluoroquinolones (100% ciprofloxacin and moxifloxacin) [17]. The most effective drug was colistin which showed 1% resistance, in Qadeer et al.'s study, the most effective drug was colistin which showed 3% resistance. Similar results of colistin effectiveness against Acinetobacter were seen in the study by Rajan et al. [21].

Resistance to carbapenems in Acinetobacter baumanniiwas recorded as 87.5% in the year 2021, limiting the availability of available treatment options.In A.baumannii, there is no significant change in the susceptibility trends to all the tested antibiotics compared to last year. Susceptibility to minocycline was close to 50 %(45% to 65.6%) making it most susceptibile antibiotic after colistin for Acinetobacter baumannii. In Pseudomonas aeruginosa, more than 60% susceptibility was observed for various aminoglycosides and fluoroquinolones in 2021. There is a consistent increase insusceptibility to all the major antipseudomonal drugs in the last few years.(29)

The most common gram-positive organism in this study was Staphylococcus aureus, which was the 3rd most common microorganism in our study. A vast majority of this microbe was isolated from the wound tissue specimen. It showed 91% sensitivity toward linezolid and vancomycin. It was also highly sensitive towards aminoglycosides like gentamycin (77%). Out of a total of 50 %were designated as methicillin-resistant Staphylococcus aureus (MRSA). MRSA is a nuisance in any hospital and has the ability to prolong hospital stay and the cost of treatment for the patient. Hence, its prevention and treatment should be of utmost priority for any healthcare facility [28].similar antibiotic patteren were observed for enterococcus and CONS.

Staph. aureus (coagulase-positive staphylococci):MRSA was 53% of them where sensitivity to vancomycin and linezolid were 91% and 100%, respectively. The resistance of MRSA to vancomycin may be because of its prolonged and frequent abuse in empirical use. Savanur et al. [26], in their study, dedicated that among the Gram-positive organisms; coagulase negative staphylococcus (CoNS) (15.6%) was most commonly isolated followed by Streptococcus (2.32%), while Chidambaram et al. [27] reported that among the Gram-positive isolates, Enterococcus (4.79%) was the common isolate obtained followed by Staphylococcus aureus (3.72%).

In AMR annual report, The overall proportion of MRSA was 42.6 % (Cefoxitin) and 33.8%(Oxacillin) respectively.Penicillin susceptibility was extremely low as expected (9.5% in MSSA and 6.9% among CoNS).Susceptibility to erythromycin,clindamycin,ciprofloxacin,co-trimoxazole and high level mupirocin was more evident in MSSA when compared to MRSA. The anti MRSA antibiotics such as vancomycin and tigecycline

showed excellent in vitro activity (100% against MRSA isolates) .Vancomycin resistance in enterococci (E.faecalisandE.faecium) was 14.9%, however, the rate was 6 times higher in E. faecium compared to E. faecalis (25.4% vs 3.8%). 37.5% of Enterococcus faecium causing blood stream infections (BSIs) werevancomycinresistant.(29) in our study MRSA and VRE was observed 53 % and 9% respectively.

The slightly increased incidence of drug resistant strains observed in our study may be because our hospital is a tertiary care center in a rural setup and patients from adjoining districts and even villages are admitted for treatment. Before attending the hospital, most of the patients get different antibiotics from general practitioners or due to over-the counter sells of antibiotics often in improper dose.

Our study was limited by the absence of clinical data to distinguish between hospital-acquired and community-acquired infections and the data required to differentiate between true infection and colonization. Another limitation is our dependence on the first-isolate approach in order to reduce the bias that may be present in an all-isolate approach. Although the first-isolate approach is recommended by CLSI, it might underestimate the resistance rate of nosocomial infections.

Early detection and close monitoring of MDR, XDR, or even PDR bacterial strains must be started by all clinical microbiology laboratories to reduce the menace of antimicrobial resistance which is now a global problem.

Conclusion & Summary:-

AMR is a growing problem in low to middle-income countries. It is important to understand the local prevalence of most common organisms isolated from different specimens and their antibiotic susceptibilities. For this purpose, it is important to develop antibiograms at the levels of hospitals and wards. It can help the physicians to choose empirical antimicrobials according to the local trends of AMR. & also, there is a need to develop antimicrobial stewardship programs in hospitals to tackle the misuse of antimicrobials.

This local prevalence study will aid in establishing an effective antimicrobial stewardship to preserve the potentials of the current antimicrobial agents. Resistance to important antibiotic groups, including quinolones, piperacillin-tazobactam, and carbapenems, has increased substantially over the past few years. We can solve these issues by various techniques for example; high resistance of gram negative to carbapenem emphasize the implementation of carbapenem sparing techniques. In order to adequately implement antimicrobial stewardship as a tool to combat antimicrobial resistance in hospital, further prospective multicenter epidemiological studies are needed.

There is paucity of data regarding MDROs in health care setup not only in India but also worldwide. Unless and until multidrug resistant organisms are detected and their incidence is known, the strategies for their control cannot be adopted properly in healthcare setup. Hence, detection, prevention of transmission of MDROs by following infection control practices, antimicrobial surveillance, and stewardship are need of the hour. Misuse and overuse of antibiotics, over-the-counter selling of antibiotics without prescription to common people, must be stopped by strict implementations of rules and regulations.

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