RESEARCH ARTICLE

ANTIMICROBIAL RESISTANCE PROFILES OF BACTERIAL ISOLATES FROM CLINICAL SPECIMENS OF PATIENTS REFERRED TO A PRIVATE LABORATORY DURING 2023

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Manuscript Info

Abstract

Introduction: AMR is a global health and development threat that emerged as one of the major public health problems of the 21st century and warns against the effective prevention and treatment of an ever-increasing range of infections. Identifying the most common bacterial pathogens and their respective AMR profiles would be valuable to optimize treatment and reduce morbidity and mortality associated with infectious disease. Thus, up-to-date information on microbial resistance is needed at local and national levels to guide the rational use of the existing antimicrobials. Therefore, this study aimed to determine the antimicrobial resistance patterns of the bacterial isolate from different clinical specimens referred to a private laboratory during 2023.

Material and methods: Samples received from different hospitals in Surat City during the year 2023 for culture and sensitivity tests was analysed to know the burden of AMR at the local level. Different types of samples were received during 2023 for culture and antimicrobial sensitivity tests, like blood, pus, stool, body fluids, urine, etc., which were processed for aerobic culture on different in-house prepared culture media. From the isolates, antimicrobial sensitivity tests were done using the manual Kirby-Bauer disk diffusion method on Muller Hinton agar.

Result: In our study, the predominant samples were urine (272/646 total samples) and blood (271/646 total samples), followed by pus (51/646 samples). Predominant culture positivity was found in the urine sample. The predominant organism isolated in urine was E. coli (91/100 isolates), and in blood samples the predominant isolates were S. Typhi (31/46 isolates), followed by E. coli (13/46 isolates). In pus samples, S. aureus followed by E. coli were isolated predominantly, followed by P. aeruginosa and K.pneumoniae. The cumulative MDR isolate rate in this study was 64.29%, which is alarming.

Discussion: The most prevalent bacteria in this study is Escherichia coli. Overall, the multidrug resistance rates found in this study were alarming: 64.29%. Therefore, strengthening antimicrobial resistance surveillance at the national level and antimicrobial sensitivity testing at local diagnostic centres are very important in reducing the challenges of antimicrobial resistance.

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Introduction:-

AMR is a global health and development threat that emerged as one of the major public health problems of the 21st century and warns against the effective prevention and treatment of an ever-increasing range of infections. [1] The

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World Health Organization (WHO) has declared that AMR is one of humanity's top 10 global public health threats. [2] The problem of antimicrobial resistance is not only the cause of the development of the resistance but also the transmission of the resistant strains from one person to another, especially in a health facility setting. The problem worsens in countries where poor sanitation makes transmitting the bacteria easy. [3]

The emergence of antimicrobial resistance is multifactorial, and tackling its development is challenging. Consequently, infections caused by resistant bacteria are unresponsive to conventional drugs, resulting in prolonged and severe illnesses, higher mortality rates, and considerable healthcare costs. Therefore, understanding the antimicrobial resistance profiles of bacterial pathogens is essential to optimize treatments and reduce the risks associated with infections. Understanding and acting on the local or national AMR situation is critical to gaining consensus on implementing appropriate interventions. [4] Furthermore, identifying the most common bacterial pathogens and their respective AMR profiles would be valuable to optimize treatment and reduce morbidity and mortality associated with infectious disease. [5,6] Thus, up-to-date information on microbial resistance is needed at local and national levels to guide the rational use of the existing antimicrobials. Therefore, this study aimed to determine the antimicrobial resistance patterns of the bacterial isolate from different clinical specimens referred to a private laboratory during 2023.

Material and Method:

Study Design: It was a retrospective study.
Dr. Mulla's Laboratoryis one of the oldest and NABL-accredited private laboratories dealing with different pathological and microbiological tests. Average sample load of 100-250 exclusively for culture and sensitivity tests from different private and corporate hospitals in Surat City per month.

Data collection and inclusion and exclusion criteria:
All consecutive samples were included in the study. Samples received from different hospitals in Surat City during the year 2023 for culture and sensitivity tests will be analyzed to know the burden of AMR at the local level. Repeat samples from the same patient received within 3 days were excluded. Mismatched or leakage samples that were rejected by the lab were excluded.

Culture and antimicrobial sensitivity test:
Different types of samples were received during 2023 for culture and antimicrobial sensitivity tests, like blood, pus, stool, body fluids, urine, etc., which were processed for aerobic culture on different in-house prepared culture media, like blood agar, chocolate agar, MacConkey agar, TCBS agar, etc., as per standard laboratory protocol and incubated in a 37°C incubator. Samples showing growth of colony were processed for microscopy and phenotypic identification of microorganisms
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From the isolates, antimicrobial sensitivity tests were done using the manual Kirby-Bauer disk diffusion method on Muller Hinton agar. The plates were incubated overnight. After incubation was completed, the zone of inhibition diameter was measured in millimeters (mm). The zones were interpreted as susceptible, intermediate, or resistant according to the CLSI 2023 guideline [CLSI 2023]. The definition of CDC was used in this study for multidrug resistance (MDR): resistance of bacterial isolates to at least one antibiotic in three or more drug classes was used to detect the resistance patterns of each isolate.

Data Analysis:-

All data were entered in WHONET software. Analysis of data was done to know the frequency and distribution of different types of sample percentages for AMR in different samples.

Result:-

In our study, as per Table no.1 and Table no.2, the predominant samples were urine (272/646 total samples) and blood (271/646 total samples), followed by pus (51/646 samples). Predominant culture positivity was found in the urine sample. The predominant organism isolated in urine was E. coli (91/100 isolates), and in blood samples the predominant isolate was S. Typhi (31/46 isolates), followed by E. coli (13/46 isolates). In pus samples, S. aureus followed by E. coli were isolated predominantly, followed by P. aeruginosa and K. pneumoniae.
Table 1: Different samples and their culture positivity rate

Sample type	Culture positive No(percentage)	Culture negative No(percentage)	Total No of samples
Blood	46(17%)	225(83%)	271
Urine	100(37%)	172(63%)	272
Body Fluid	1(25%)	3(75%)	4
Swab	4(80%)	1(20%)	5
Sputum	7(20%)	28(80%)	35
Pus	37(73%)	14(27%)	51
Tissue	0(0%)	4(100%)	4
Stool	0(0%)	4(100%)	4
Total	195 (30%)	451(70%)	646

Table 2: Analysis of different isolates in different samples

	No of isolates in different samples					
	Blood	urine	Body fluid	Pus	Sputum	Swab
E.coli	13	91	1	10	0	3
S.Typhi	31	0	0	0	0	0
S.aureus	1	0*	0	11	1	1
K. pneumoniae	1	3	0	6	6	0
A.baumannii	0	0	0	2	0	0
Enterococci	0	1	0	1	0	0
Pseudomonas aeruginosa	0	3	0	7	0	0
Proteus vulgaris	0	2	0	0	0	0

*: No isolates found

Table 3: Analysis of E. coli isolates and their drug sensitivity patterns in different samples

E,coli-13 isolates	blood-3			Body Fluid	1 isolate		Pus	10 isolates		swab-3			Urine -93		
	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Amikacin	0%	33%	67%	0%	0%	100%	20%	10%	70%	0%	0%	100%	15%	3%	75%
Ampicillin	100%	0%	0%	100%	0%	0%	100%	0%	0%	100%	0%	0%	96%	1%	0%
Amoxiclav	100%	0%	0%	100%	0%	0%	100%	0%	0%	100%	0%	0%	95%	0%	3%
Cefepime	67%	0%	33%	0%	0%	100%	90%	10%	0%	67%	33%	0%	82%	5%	13%
Cefotaxime	67%	0%	33%	0%	0%	100%	100%	0%	0%	67%	0%	33%	82%	1%	17%
Ceftriaxone	67%	0%	33%	0%	0%	100%	100%	0%	0%	67%	0%	33%	82%	2%	16%
Cefuroxime	100%	0%	0%	0%	0%	100%	90%	0%	10%	100%	0%	0%	86%	0%	14%
Ertapenem	33%	0%	67%	100%	0%	0%	50%	0%	50%	0%	0%	100%	20%	5%	74%
Gentamicin	33%	0%	67%	0%	0%	100%	30%	10%	60%	33%	0%	67%	29%	4%	67%
Imipenem	33%	0%	67%	0%	0%	100%	70%	10%	20%	0%	0%	100%	15%	2%	83%
Meropenem	33%	0%	67%	0%	0%	100%	50%	0%	50%	0%	0%	100%	18%	4%	77%
Piperacillin-Tazobactam	0%	0%	100%	0%	0%	100%	70%	0%	30%	0%	0%	100%	24%	8%	69%
Cefuroxime	100%	0%	0%	100%	0%	0%	90%	0%	10%	100%	0%	0%	86%	1%	13%

*: 0 means no resistance/sensitivity or intermediate was found for that antibiotic.

As per Table no. 3, E. coli was isolated in 13 blood samples, 10 pus samples, 3 swab samples, 91 urine samples, and one body fluid. In blood samples, all 13 isolates (100%) were resistant to Ampicillin, Amoxiclav, and Cefuroxime. And 67% resistant to Cefotaxime, Ceftriaxone, and Cefepime. All blood isolates were 100% sensitive to Piperacillin-tazobactam, while 67% sensitivity was found for Amikacin, Ertapenem, Meropenem, Imipenem, and Gentamycin.

All 10 E. coli isolates from pus were resistant to Ampicillin, Amoxiclav, Cefotaxime, and Ceftriaxone. While sensitive to Amikacin (70%), Gentamicin (60%), M
eropenem (50%), and Ertapenem (50%).

91 E. coli isolates from urine samples show good sensitivity to Amikacin (75%), Ertapenem (74%), imipenem (83%), meropenem (83%), and Piperacillin-tazobactam (69%). While predominant resistance was found in Amoxiclav (96%), Cefuroxime (86%), Cefepime (82%), Ceftriaxone (82%), and Cefotaxime (82%).

Table 4: Analysis of K. pneumoniae isolates and their drug sensitivity patterns in different samples

K.pneumoniae-16 isolates	Blood-1 isolate			pus-6 isolates			Sputum-6 isolates			Urine-3 isolates		
	R	I	S	R	I	S	R	I	S	R	I	S
Amikacin	0%	0%	100%	33%	0%	67%	17%	33%	50%	33%	0%	67%
Ampicillin	100%	0%	0%	100%	0%	0%	100%	0%	0%	100%	0%	0%
Amoxiclav	100%	0%	0%	67%	17%	17%	67%	33%	0%	100%	0%	0%
Cefepime	100%	0%	0%	67%	0%	33%	67%	17%	17%	100%	0%	0%
Cefotaxime	100%	0%	0%	67%	0%	33%	67%	17%	17%	100%	0%	0%
Ceftriaxone	100%	0%	0%	100%	0%	0%	100%	0%	0%	100%	0%	0%
Cefuroxime	100%	0%	0%	33%	0%	67%	100%	0%	0%	100%	0%	0%
Ertapenem	0%	0%	100%	50%	0%	50%	17%	0%	83%	33%	0%	67%
Gentamicin	0%	0%	100%	50%	0%	50%	33%	0%	67%	67%	0%	33%
Imipenem	0%	0%	100%	33%	0%	67%	50%	17%	33%	33%	0%	67%
Meropenem	0%	0%	100%	33%	0%	67%	17%	17%	67%	33%	0%	67%
Piperacillin-Tazobactam	100%	0%	0%	33%	17%	50%	17%	17%	67%	33%	0%	67%
Cefuroxime	100%	0%	0%	100%	0%	0%	100%	0%	0%	100%	0%	0%

*: 0 means no resistance/sensitivity or intermediate was found for that antibiotic.

As per Table no. 4, K. pneumoniae was isolated from 16 samples, out of which one was from blood, 6 were from pus, 6 were from sputum, and 3 were from urine samples. From isolates of pus, 100% resistance was noted in Amikacin, Ceftriaxone, and Cefuroxime; 67% resistance was noted in Amoxiclav, Cefepime, and Cefotaxime; 50% resistance was found in Ertapenem and gentamicin, and 33% was found in Amikacin, Cefuroxime, Imipenem, Meropenem, and Piperacillin-tazobactam. In sputum isolates of K. pneumoniae, 100% resistance was found in Ampicillin, Ceftriaxone, and Cefuroxime; 67% was found in Amoxiclav, Cefepime, and Cefotaxime; 50% was found in Imipenem; and 17% was found in Amikacin, Ertapenem, Meropenem, and Piperacillin-tazobactam. In the blood isolate, resistance was found in Ampicillin, Amoxiclav, Cefepime, Cefotaxime, Ceftriaxone, Cefuroxime, and Piperacillin-tazobactam.

Table 5: Analysis of S. Typhi isolates and their drug sensitivity pattern in different samples

S.Typhi-31 Isolates			
	R	I	S
Ampicillin	23%	0%	77%
Ceftriaxone	10%	0%	90%
Cefixime	13%	13%	74%
Ciprofloxacin	45%	42%	13%
Azithromycin	19%	16%	65%
Chloramphenicol	3%	3%	94%

In 31 isolates of S. Typhi, resistance to ciprofloxacin was 45%. Ampicillin (77%), Ceftriaxone (90%), Cefixime (74%), Azithromycin (65%), and Chloramphenicol (94%) were overall sensitive as per Table No. 5.

Table 6: Analysis of Proteus vulgaris isolates and their drug sensitivity pattern in different samples

Proteus vulgaris-2 isolates	Urine		
	R	I	S
Amikacin	0%	50%	50%
Ampicillin	100%	0%	0%
Amoxiclav	50%	0%	50%
Cefepime	50%	0%	50%
Cefotaxime	50%	0%	50%
Ceftriaxone	50%	0%	50%
Cefuroxime	100%	0%	0%
Ertapenem	50%	0%	50%
Gentamicin	0%	0%	100%
Imipenem	50%	0%	50%
Meropenem	50%	0%	50%
Piperacillin-tazobactam	50%	50%	0%
Cefuroxime	100%	0%	0%

*: 0 means no resistance/sensitivity or intermediate was found for that antibiotic.
P. vulgaris 2 isolates were found in urine with an overall 50% sensitivity to almost all drugs,as per table no. 6.

Table 7: Analysis of A. baumannii isolates and their drug sensitivity patterns in different samples

A.baumannii-2 isolates	Pus sample		
	R	I	S
Amikacin	0%	50%	50%
Ampicillin	100%	0%	0%
Amoxiclav	50%	0%	50%
Cefepime	50%	0%	50%
Cefotaxime	50%	0%	50%
Ceftriaxone	50%	0%	50%
Cefuroxime	50%	0%	50%
Ertapenem	50%	0%	50%
Gentamicin	0%	0%	100%
Imipenem	50%	0%	50%
Meropenem	50%	0%	50%
Piperacillin-tazobactam	50%	50%	0%
Cefuroxime	100%	0%	0%

*: 0 means no resistance/sensitivity or intermediate was found for that antibiotic.
As per table no. 7, 2 isolates of A. baumannii were found from pus samples, out of which one was resistant to Cephalosporin and Carbapenem groups, and another was sensitive to both groups.
Table 8: Analysis of Enterococci isolates and their drug sensitivity pattern in different samples

Enterococci-2 isolates	Pus-1 isolate			Urine-1 isolate		
	R	I	S	R	I	S
Amikacin	0%	0%	100%	0%	0%	100%
Ciprofloxacin	100%	0%	0%	100%	0%	100%
Doxycycline	100%	0%	0%	100%	0%	100%

Erythromycin	100%	0%	0%	0%	0%	100%
Gentamicin	100%	0%	0%	0%	0%	100%
Linezolid	0%	0%	100%	0%	0%	100%
Teicoplanin	0%	0%	100%	0%	0%	100%
Tetracycline	100%	0%	0%	100%	0%	100%
Vancomycin	0%	0%	100%	0%	0%	100%

As per table no. 8, 2 Enterococci isolates were found, one in pus and one in urine. Both were susceptible to almost all drugs except Ciprofloxacin, Tetracycline, and Doxycycline.

Table 9: Analysis of P. aeruginosa isolates and their drug sensitivity patterns in different samples

P. aeruginosa-10 isolates	Pus-7 isolates			Urine-3 isolates		
	R	I	S	R	I	S
Amikacin	43%	0%	57%	0%	0%	100%
Aztreonam	43%	29%	29%	33%	33%	33%
Ceftazidime	71%	14%	0%	67%	0%	0%
Ciprofloxacin	14%	0%	29%	0%	0%	100%
Gentamicin	43%	0%	57%	0%	0%	100%
Imipenem	43%	0%	57%	33%	0%	67%
Meropenem	29%	0%	71%	33%	0%	67%
Netilmicin	0%	0%	57%	33%	0%	67%
Piperacillin-tazobactam	29%	0%	71%	0%	67%	33%

*: 0 means no resistance/sensitivity or intermediate was found for that antibiotic.
As per Table no. 9, 10 isolates of Pseudomonas aeruginosa were found, out of which 7 were from pus and 3 were from urine samples. All isolates were overall showing sensitivity for Amikacin (57%), Gentamicin (57%), Imipenem (57%), Meropenem (71% of isolates), Netilmicin (57%), and Piperacillin-tazobactam (71% of isolates). Resistance was seen in Ceftazidime (71%). In urine samples sensitivity was found to be 100% for Amikacin, Ciprofloxacin, and Gentamicin; 67% for Imipenem, Meropenem, and Netilmicin; and 33% for Aztreonam and Piperacillin-tazobactam. Resistance was found to be 67% for Ceftazidime.

Table 10: Analysis of S. aureus isolates and their drug sensitivity pattern in different samples

S.aureus-14 isolates	Blood-1 isolate			Pus-11 isolates		
	R	I	S	R	I	S
Amoxiclav	100%	0%	0%	91%	0%	9%
Cefoxitin	100%	0%	0%	27%	0%	73%
Ciprofloxacin	100%	0%	0%	64%	9%	27%
Clindamycin	100%	0%	0%	82%	0%	18%
Erythromycin	100%	0%	0%	82%	0%	18%
Gentamicin	100%	0%	0%	0%	9%	91%
Linezolid	0%	0%	100%	18%	0%	82%
Teicoplanin	0%	0%	100%	0%	0%	100%
Tetracycline	0%	0%	100%	18%	9%	73%
Vancomycin	NT	NT	NT	NT	NT	NT
Penicillin G	100%	0%	0%	91%	0%	9%

NT: Not tested

14 isolates of S. aureus were found, out of which one was from blood, 11 were from pus, 1 was from a swab, and one was from sputum. In pus isolates, resistance was found to be 91% for Amoxiclav and Penicillin G, 82% for Clindamycin and Erythromycin, 64% for Ciprofloxacin, 27% for Cefoxitin, and 18% for Linezolid and Tetracycline, as per table no. 10.

Discussion:-

Understanding the distribution of microbial pathogens and their associated infections is required to control infectious diseases and monitor antimicrobial resistance. The current study aimed at establishing the prevalence of common pathogenic microorganisms, including their antimicrobial susceptibility patterns and distribution according to specimens in a private diagnostic center. The excessive use of antibiotics, among other factors, has led to extensive antimicrobial resistance. If this trend continues unabated, then all other antibiotic options will be exhausted, making the treatment of associated infections extremely difficult. Hence, the WHO identified it as an international health problem of prime concern [7]. To control this rising predicament, all-inclusive antibiotic and other relevant stewardship, especially in poor countries, are essential.

However, enough data concerning antimicrobial resistance are inaccessible to exactly measure the degree of the problem. The few available studies regarding results on microbiological samples suggest that there are hotbeds of emerging high-level resistance [8]. In this study, gram-negative bacteria were more prevalent than gram-positive isolates, similar to reports by Newman and colleagues and Fahim [9]. The high prevalence of microbial isolates reported in this study highlights the need for effective monitoring and surveillance of microbial infections in resource-limited health care facilities [10].

obtained from adults corresponds with the high number of adult clients recorded. We report a high prevalence of microorganisms with variable susceptibility patterns to key antimicrobials. All microorganisms isolated showed resistance to more than one antimicrobial agent. Cotrimoxazole, erythromycin, vancomycin, Chloramphenicol, and Cefuroxime were among the top five antimicrobials with a high prevalence of resistance. However, Amikacin, gentamicin, and nitrofurantoin were the three most effective antibiotics. This is similar to an earlier report where Amikacin was among the group with the lowest resistance [11]. Furthermore, Fahim

It was also reported in Egypt that gram-negative isolates exhibited high resistance to almost all the classes of antibiotics in use, with the least frequency recorded against nitrofurantoin and Amikacin, followed by imipenem and meropenem [12]. Factors that may have contributed to the emergence and prevalence of resistance include uncontrolled use of these drugs, non-compliance with treatment, and geographical location/unsanitary environment. Another significant factor for increased resistance to antibiotics is the use of substandard and counterfeit drugs and the unauthorized sale of antibiotics without prescription. [13, 14, 15] Interesting. Antibiotic-resistant bacterial infections are among the most challenging public health concerns, especially in developing countries. The absence of effective antibiotic treatment will challenge clinicians to manage infectious diseases and their complications, particularly in immunosuppressed patients.

This study showed that gram-negative bacteria were predominantly isolated from most clinical samples; E. coli was the most commonly isolated bacterial pathogen, followed by K. pneumoniae. From all 840 isolates, E. coli accounts for 51.43%, regardless of specimen type. This finding agrees with other studies done in India (53.3%). [15] The most concerning part of this study was that a significant number of bacterial isolates showed drug resistance against the majority of antibiotics used for sensitivity testing. E.coli, Klebsiella spp., Acinetobacter spp., and Citrobacter spp. were highly resistant to commonly prescribed drugs like sulfamethoxazole-trimethoprim (cotrimoxazole), Ceftriaxone, Ampicillin, Ciprofloxacin, Cefotaxime, Cefepime, and Ceftazidime. However, these bacteria are highly susceptible to amoxicillin/clavulanic acid, Amikacin, Doripenem, Meropenem, and Imipenem. The overall observed high rate of MDR could be linked to irrational use and/or self-medication of antibiotics, possibly contributing to the resistance rates in the study area. This study has some limitations. Since our study was retrospective, it could not indicate the current antimicrobial resistance patterns of the isolates. This study also couldn't determine whether the identified resistance was due to hospital-acquired or community-acquired causes.

Conclusions:-

The most prevalent bacteria in this study is Escherichia coli. Overall, the multidrug-resistant isolates found in this study were alarming, 64.29%. Therefore,strengthening antimicrobial resistance surveillance at the national level and antimicrobial sensitivity testing at local diagnostic centres are very important in reducing the challenges of antimicrobial resistance.

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