Antimicrobial resistance profiles of bacterial isolates from clinical specimens of patients referred to private laboratory during 2023.

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 private laboratory during 2023.

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

Result:
In our study, 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 urine sample. Predominant organism isolated in urine was *E.coli* (91/100 isolates) and in blood samples 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. pneumonaie*. Cumulative MDR isolates rate in this study was 64.29% which is alarming.
Discussion: The most prevalent bacteria in this study, *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.

Keywords: Antimicrobial resistance profile, Clinical samples, Bacterial isolates

1. 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 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 private laboratory during 2023.

2. Material and method:

2.1 Study Design: It was a retrospective study. Dr. Mulla's Laboratory is one of the oldest and NABL accredited private laboratory dealing with different pathological and Microbiological tests. Average sample load of 100-250 exclusively for culture and sensitivity test from different private and corporate hospital of Surat city per month.

2.2 Data collection and inclusion and exclusion criteria:

All consecutive samples were included in the study. Samples received from different hospital of Surat city during the year 2023 for culture and sensitivity test will be analysed to know the burden of AMR at local level. Repeat samples from same patient received within 3 days were excluded. Mismatched or leakage samples which were rejected by lab were excluded.

2.3 Culture and antimicrobial sensitivity test:

Different types of samples were received during 2023 for culture and antimicrobial sensitivity test like blood, pus, stool, body fluids, urine etc. which were processed for aerobic culture on different inhouse prepared culture media like Blood agar, Chocolate agar, Mac Conkey agar, TCBS agar etc. as per standard laboratory protocol and incubated at 37°C Incubator. Samples showing growth of colony were processed for Microscopy and phenotypic identification of microorganisms.

From the isolates antimicrobial sensitivity test were done using manual Kerbey Bauer disk diffusion method on Muller Hinton agar. The plates were incubated overnight. After incubation was completed, the zone inhibition diameter was measured in millimeters (mm). The zones were interpreted as susceptible, intermediate, or resistant according to 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 were used to detect the resistance patterns of each isolate.

2.4 Data analysis: All data were entered in WHONET software. Analysis of data was done to know frequency, distribution of different types of samples percentage for AMR in different samples.

3. Result:

In our study, as per Table no.1 and Table no.2 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 urine sample. Predominant organism isolated in urine was *E.coli*(91/100 isolates) and in blood samples 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. pneumonaie.*

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

Table 3: Analysis of E.coli isolates and its drug sensitivity pattern 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%

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 fluids. In blood samples, all 13 isolates (100%) 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%), Gentamycin (60%), Meropenem (50%) and Ertapenem (50%).

91 *E.coli* isolates from urine samples show good sensitivity to Amikacin (75%), Ertapenem (74%), Imipenem (83%), meropenem (83%) and to 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 its drug sensitivity pattern 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%

As per Table no.4, *K. pneumoniae* was isolated from 16 samples, out of which one was from blood, 6 were from pus, 6 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, cefotaxime; 50% resistance was found in Ertapenem and Gentamycin 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, cefuroxime; 67% found in amoxiclav, cefepime, cefotaxime, 50% found in Imipenem and 17% found in amikacin, Ertapenem, meropenem and piperacillin-tazobactam. In the blood isolate, resistance was found in Ampicillin, Amoxiclav, cefepime, cefotaxime, ceftriaxone, cefuroxime, and for piperacillin-tazobactam.

Table 5: Analysis of S.Typhi isolates and its 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*, resistant to Ciprofloxacin was 45%. Ampicillin (77%), Ceftriaxone (90%), cefixime(74%), Azithromycin(65%), Chloramphenicol(94%) were overall sensitive as per Table no.5.

Table 6 : Analysis of proteus vulgaris isolates and its 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%

P.vulgaris 2 isolates were found in urine with overall 50% sensitivity to almost all drugs were found as per table no.6.

Table 7 : Analysis of A.baumannii isolates and its drug sensitivity pattern in different samples

	Pus sample		
***A.baumannii*-2 isolates**	**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%

As per table no.7, 2 isolates of *A.baumannii* were found from pus samples out of which one was resistant to Cephalosporine groups and carbapenem group and another was sensitive to both the groups.

Table 8: Analysis of Enterococci isolates and its drug sensitivity pattern in different samples

	Pus-1 isolate			**Urine-1 isolate**		
***Enterococci*-2 isolates**	**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 its drug sensitivity pattern 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%

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 from were overall showing sensitivity for Amikacin (57%), Gentamycin (57%), Imipenem (57%), Meropenem (71% isolates), Netilmicin (57%) and to piperacillin-tazobactam (71% isolates). Resistance was seen in Ceftazidime (71%). In urine samples sensitivity was found 100% for Amikacin, ciprofloxacin and gentamicin, 67% for Imipenem, meropenem, Netilmicin and 33% for Aztreonam and Piperacillin-tazobactam. Resistant was found 67% for ceftazidime.

Table 10: Analysis of S.aureus isolates and its 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%

14 isolates of *S.aureus* were found out of which one was from blood, 11 were from pus, 1 from swab and one was from sputum. In pus isolates, resistance was found 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.

4. 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 Centre. .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 Fahim1[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 lowest resistance [11]. Furthermore, Fahim
also reported in Egypt that gram-negative isolates exhibited high resistance to almost all the classes of antibiotic in use with the least frequency recorded against nitrofurantoin, amikacin, followed by imipenem and meropenem [12]. Factors that may have contributed to the emergence and prevalence of resistance, includes 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 immune-suppressed 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, *Acinetobacte*r 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.

5. Conclusions

The most prevalent bacteria in this study, *Escherichia coli*. Overall, the multidrug resistance 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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