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ANTIMICROBIAL RESISTANCE PATTERN IN A TERTIARY CARE HOSPITAL: RETROSPECTIVE STUDY

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ABSTRACT: Introduction: Antibiotics' efficacy is declining over a period of time because of haphazard use in humans and animals. Empirical use of antibiotics and reduction in the new drug development strategies by pharmaceutical industry can lead to antibiotic resistance crisis in near future. Hence, identifying the common causative organism and their resistance pattern in the geographical region would be helpful in choosing appropriate antibiotics for the diseases. **Methods:** This retrospective study is done at GMERS Medical College and hospital, Junagadh, Gujarat, India. Bacterial isolates and antibiotic susceptibility data from May 2021 to May 2023 were studied from culture and sensitivity register maintained by the microbiology department. Data were analyzed by descriptive statistics. **Results:** Among 43 positive samples 62.8 % were male and 37.2 % were female. Major samples were of sputum 39.53% and urine 32.56%. Organisms identified were *Klebsiella* 37.2%, *E. coli* 30.2%, *Pseudomonas* 16.3%, *Acinetobacter* 4.65%, *S. aureus* 9.3% and *Enterococcus* 2.3%. *E. coli* (69 %) were resistant to ampicillin/sulbactam and co-trimoxazole. *Klebsiella* (75%) were resistant to tetracyclin and ciprofloxacin. *Pseudomonas* (85.71 %) were resistant to tetracycline and chloramphenicol. *Acinetobacter*, *S. aureus* and *enterococcus* showed resistant to many antibiotics. **Discussion:** Different level of antibiotic resistance observed by gram negative and positive bacteria in present study can challenge us in future infection control. It can lead to limited treatment options, increased healthcare costs and patient morbidity & mortality. Thereby prudent use of antibiotic by using antibiotic sensitivity test is requirement for the current antibiotic stewardship programs.

INTRODUCTION: The discovery of antibiotics in the mid-twentieth century was one of the greatest discoveries in the history of medicine related to the reduction of morbidity and mortality of human and live-stock. Since then the fatal and severe bacterial infection outcomes saw a great shift and became easily treatable.

However, eventually bacterial strains started developing resistance to the antibiotics and there is the rise of emergence of antibiotic-resistant (ABR) strains. This results in reduction in the efficacy of antibacterial agents making the treatment difficult, costly or in worst scenario even impossible¹.

It is gestimated that ABR infections may be responsible for about 700,000 deaths per year. If proactive actions are not taken against this, it is predicted that ABR infections may have a higher mortality rate that of cancer and become the common cause of death by the 2050². Empirical use of antibiotics as well as decline in the new drug

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development by pharmaceutical industry due to reduced incentives and challenging regulatory requirements might lead to antibiotic resistance crisis in near future³. World Health Organization (WHO) has been focusing on the emergence of hospital and community acquired resistant infections because of inappropriate and irrational uses of antibiotics in humans and animals for therapeutic and non-therapeutic uses⁴. A study conducted in India also evidenced the same by evaluating the samples of cow dung and drinking water showing the presence of MDR *E. coli*⁵. Geographical variation in antimicrobial sensitivity has been among different bacteria in different region of India. North India studies showed *Vibrio cholerae* being resistant to furazolidone, cotrimoxazole and nalidixic acid but sensitive to tetracycline around Delhi, but the resistance was noted against tetracycline in other parts⁶.

The national scenario of AMR is less known in India because of the unavailability of central monitoring agency. In outlook of this certainty and because of the geographical and time based disparity in antibiotic sensitivity pattern reported by many studies, our study was undertaken. Our study aim is to determine the prevalence pathogens causing community acquired infections medical college hospital in saurashtra region of Gujarat and the antibiotic resistance pattern to provide a database for reference. So, we designed the current study of retrospective analysis of commonly prevalent pathogens and their resistance pattern during previous 3 years in patients in a tertiary care hospital. Increase awareness and reporting for these findings will help in preventing the immersed strains from spreading in the community. Our aim was to identify the microorganisms commonly causing the community acquired infection in the tertiary care hospital and to find their antibiotic resistance pattern and to provide the effective antibiotic database for the reference to use in such patients in future.

MATERIAL AND METHODS: Present study was done at GMERS Medical College and hospital, Junagadh, Gujarat, India after due approval from the Institutional Ethics Committee (IEC), of our institution (Approval number:- IEC/12/2023, date: 21/07/2023). We included in-patient as well as out-patient data of urine, pus, sputum, CSF as well as

blood culture and sensitivity from May 2021 to May 2023 through register maintained by microbiology department. Culture and sensitivity testing (HiMedia / Pathoteq 'Bio-Disc-12') was carried out as per the Clinical and Laboratory Standards Institute (CLSI) guidelines by microbiology department.

Demographic profile, specimen type, bacterial isolates and antibiotic susceptibility pattern were collected using a data collection sheet. Only positive cultures which showed significant bacterial growth were included in this study. The incomplete data were excluded from study such as non-reporting of the isolated organism, intermediate antibiotics susceptibility and incomplete demographic details.

The antibiotic sensitivity and resistance pattern against gram positive and gram negative antimicrobials including ampicillin+sulbactam, cotrimoxazole, piperacillin, tetracycline, amikacin, cloxacillin, linezolid, roxythromycin, chloramphenicol, cefotaxime *etc.* of standard strengths were scrutinized. The data were analyzed according to age, gender, organisms isolated and antibiotics sensitivity / resistance pattern with the help of Microsoft office software 2010.

RESULTS: As per inclusion criteria, total forty-three positive samples were identified during study period. Most of the positive samples were of sputum (39.53%), urine (32.56%) and pus (25.58%) from indoor and outdoor patient departments **Fig. 1**.

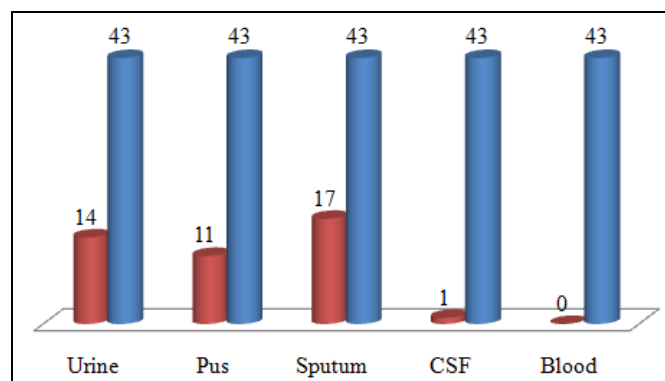


FIG. 1: TYPES OF POSITIVE SAMPLES (n=43)

Out of 43 samples, 27 (62.8 %) were male and 16 (37.2 %) were female patients. Male predominance was seen among the sputum samples **Fig. 2**.

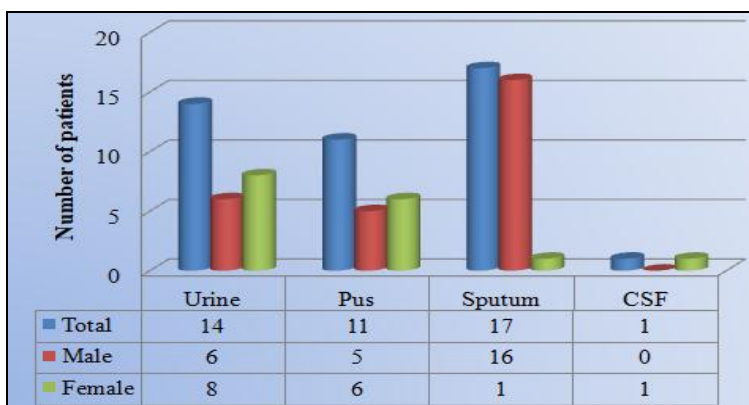


FIG. 2: DEMOGRAPHIC CHARACTERISTICS OF THE PATIENTS (n=43)

According to the age group, 11 in 0-25 years, 17 in 26-50 years, 14 in 51-75 years and 1 in >76 years of age were culture positive. Urine and pus positive cultures were nearby same among all the age

groups up to 75 years. Sputum positive cultures were relatively high amongst the age of 26-75 years
Fig. 3.

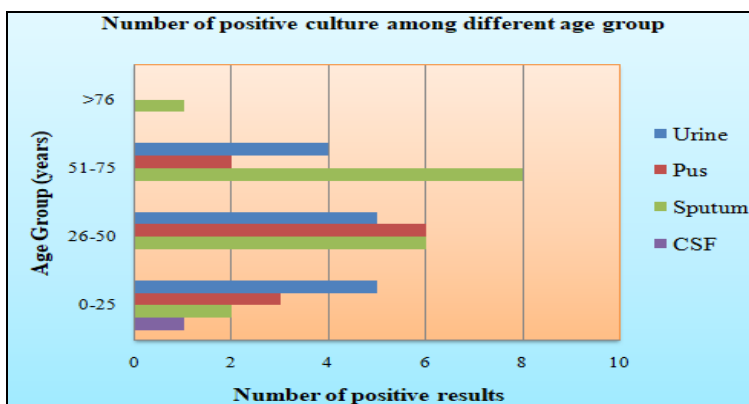


FIG. 3: AGE GROUP (0-25 YEARS, 26-50 YEARS, 51-75 YEARS, >76 YEARS) WISE POSITIVE CULTURE

In majority of cases we found gram negative isolates like *Klebsiella* (37.2%), *E. coli* (30.2%) and *Pseudomonas* (16.3%). Among other gram negative isolates we found *Acinetobacter* (4.65%) only in 2 urine culture samples. While gram

positive isolates like *S. aureus* (9.3%) and *Enterococcus* (2.3 %) were only seen in pus cultures. Majority of sputum samples (n=13) were having *Klebsiella* growth while urine samples (n=9) were having *E. coli* growth **Fig. 4.**

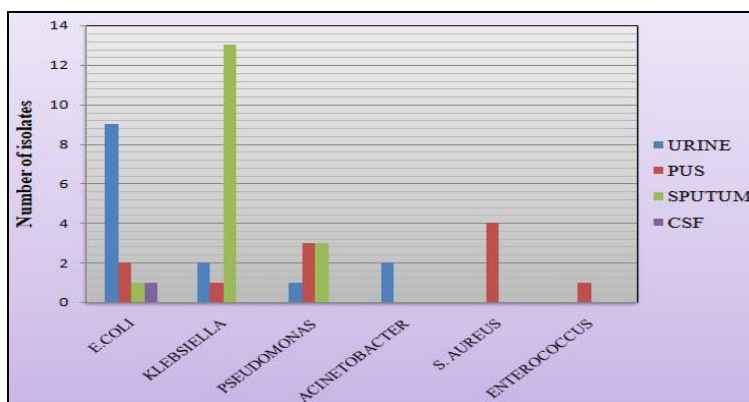


FIG. 4: NUMBER OF GRAM +VE AND -VE ORGANISMS FOUND AMONG VARIOUS SAMPLES

Antimicrobial Resistance Pattern: *E. coli* (69%) were resistant to ampicillin/sulbactam and cotrimoxazole. *Klebsiella* (75%) were resistant to

tetracyclin and ciprofloxacin, 68.75 % were resistant to ampicillin/sulbactam, ceftizoxime and ceftazidime & 62.5 % were resistant to

amoxicillin/clavulanic acid, cefuroxime, cefadroxil, gatifloxacin and norfloxacin. Pseudomonas (85.71 %) were resistant to tetracycline and chloramphenicol while 71.43 % were resistant to ciprofloxacin, ampicillin /sulbactam and amoxicillin/clavulanic acid. Acinetobacter 100% (n=2) were resistant to ampicillin /sulbactam, ceftizoxime, cefpirome, tetracycline, ciprofloxacin, norfloxacin, azithromycin, Teicoplanin,

chloramphenicol and co-trimoxazole. The gram positive *S. aureus* were 100 % (n=4) resistant to ciprofloxacin and 75 % were resistant to vancomycin and erythromycin. Enterococcus showed 100 % (n=1) resistance to amoxicillin, penicillin-G, cephalixin, cefotaxime, tetracycline, levofloxacin, roxithromycin, neomycin, lincomycin, clindamycin and co-trimoxazole **Table 1.**

TABLE 1: ISOLATED ORGANISM WISE ANTIBIOTICS' RESISTANCE PATTERN

Sr. no.	Gram negative				Gram positive		
	<i>E. coli</i> (%)	<i>Klebsiella</i> (%)	<i>Pseudomonas</i> (%)	<i>Acinetobacter</i> (%)	<i>S. aureus</i> (%)	<i>Enterococcus</i> (%)	
1	β-Lactam antibiotics						
	Ampicillin/Sulbactam	9 (69)	11 (68.75)	5 (71.43)	2 (100)	1 (25)	0 (0)
	Meropenem	3 (23)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)
	Piperacillin/Tazobactam	2 (15)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)
	Ticarcillin+Clavulanic Acid	5 (38)	6 (37.50)	1 (14.19)	1 (50)	0 (0)	0 (0)
	Aztreonam	3 (23)	2 (12.5)	0 (0)	1 (50)	0 (0)	0 (0)
	Piperacillin	1 (8)	4 (25)	3 (42.86)	0 (0)	0 (0)	0 (0)
	Amoxicillin+ Clavulanic Acid	3 (23)	10 (62.50)	5 (71.43)	0 (0)	1 (25)	0 (0)
	Cloxacillin	0 (0)	0 (0)	0 (0)	0 (0)	1 (25)	1 (100)
	Amoxicillin	0 (0)	0 (0)	0 (0)	0 (0)	2 (50)	1 (100)
2	Cephalosporin						
	Ceftizoxime	7 (54)	11 (68.75)	3 (42.86)	2 (100)	0 (0)	0 (0)
	Cephalexin	5 (38)	1 (6.25)	0 (0)	2 (100)	1 (25)	1 (100)
	Cefoperazone+sulbactam	4 (31)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)
	Cefpirome	7 (54)	1 (6.25)	0 (0)	2 (100)	0 (0)	0 (0)
	Cefotaxime	1 (8)	10 (62.5)	3 (42.86)	0 (0)	1 (25)	1 (100)
	Ceftriaxone	1 (8)	7 (43.75)	1 (14.19)	0 (0)	0 (0)	0 (0)
	Cefepime	0 (0)	5 (31.25)	2 (28.57)	0 (0)	0 (0)	0 (0)
	Cefuroxime	4 (31)	10 (62.5)	4 (57.14)	0 (0)	0 (0)	0 (0)
	Cefadroxil	3 (23)	10 (62.5)	4 (57.14)	0 (0)	0 (0)	0 (0)
3	Tetracyclines						
	Cefoperazone	0 (0)	8 (50)	1 (14.29)	0 (0)	0 (0)	0 (0)
	Ceftizidime	2 (15)	11 (68.75)	4 (57.14)	0 (0)	0 (0)	0 (0)
4	Quinolones						
	Tetracycline	6 (46)	12 (75)	6 (85.71)	2 (100)	1 (25)	1 (100)
	Oxytetracyclin	1 (8)	6 (37.5)	4 (57.14)	0 (0)	0 (0)	0 (0)
5	Macrolide						
	Doxycyclin	0 (0)	0 (0)	0 (0)	0 (0)	1 (25)	0 (0)
	Ciprofloxacin	4 (31)	12 (75)	5 (71.43)	2 (100)	4 (100)	0 (0)
	Sparfloxacin	5 (38)	1 (6.25)	0 (0)	1 (50)	0 (0)	0 (0)
	Gatifloxacin	4 (31)	10 (62.5)	3 (42.86)	1 (50)	0 (0)	0 (0)
	Norfloxacin	4 (31)	10 (62.5)	4 (57.14)	2 (100)	0 (0)	0 (0)
	Ofloxacin	2 (15)	1 (6.25)	0 (0)	2 (100)	0 (0)	0 (0)
	Nalidixic acid	1 (8)	9 (56.25)	3 (42.86)	0 (0)	0 (0)	0 (0)
6	Aminoglycosides						
	Levofloxacin	0 (0)	0 (0)	0 (0)	0 (0)	2 (50)	1 (100)
	Azithromycin	4 (31)	1 (6.25)	0 (0)	2 (100)	0 (0)	0 (0)
	Roxithromycin	0 (0)	0 (0)	0 (0)	0 (0)	2 (50)	1 (100)
6	Aminoglycosides						
	Clarithromycin	0 (0)	0 (0)	0 (0)	0 (0)	1 (25)	1 (100)
	Erythromycin	0 (0)	0 (0)	0 (0)	0 (0)	3 (75)	0 (0)
6	Aminoglycosides						
	Amikacin	3 (23)	4 (25)	0 (0)	1 (50)	0 (0)	0 (0)
	Gentamicin	1 (8)	3 (18.75)	0 (0)	0 (0)	1 (25)	1 (100)

7	Neomycin	0 (0)	0 (0)	0 (0)	0 (0)	2 (50)	1 (100)
						Glycopeptide	
8	Teicoplanin	5 (38)	1 (6.25)	0 (0)	2 (100)	0 (0)	0 (0)
	Vancomycin	0 (0)	0 (0)	0 (0)	0 (0)	3 (75)	0 (0)
9						Lincosamide	
	Lincomycin	0 (0)	0 (0)	0 (0)	0 (0)	1 (25)	1 (100)
10	Clindamycin	0 (0)	0 (0)	0 (0)	0 (0)	2 (50)	1 (100)
						Nitrobenzene	
10	Chloramphenicol	2 (15)	2 (12.5)	6 (85.71)	2 (100)	0 (0)	0 (0)
						Sulfonamide	
	Co-trimoxazole	9 (69)	6 (37.5)	4 (57.14)	2 (100)	2 (50)	1 (100)

The *E. coli* (n=9) from the urine samples (n=14) were resistant to ampicillin /sulbactam, ceftiprome (77.77 %) and co-trimoxazole, ceftizoxime (66.66 %). While the acinetobacter (n=2) from the urine samples were resistant to ampicillin /sulbactam, co-trimoxazole, ceftizoxime, chloramphenicol, cephalixin, tetracyclin, ciprofloxacin, norfloxacin, ofloxacin, azithromycin, ceftiprome and teicoplanin (100%). *Klebsiella* (n=2) were resistant to Chloramphenicol (100%).

The *Klebsiella* (n=13) from the sputum samples (n=17) were resistant to tetracyclin (84.61 %), ciprofloxacin, ofloxacin, ceftizoxime, cefadroxil, ceftizidime, amoxicillin/clavulanic acid (76.92 %) and ampicillin /sulbactam, cefotaxime, cefuroxime, norfloxacin, gatifloxacin, nalidixic acid (69.23 %). While pseudomonas (n=3) from the sputum samples were resistant to piperacillin, chloramphenicol, tetracyclin (100 %) and ampicillin/sulbactam, amoxicillin/clavulanic acid, ciprofloxacin, ofloxacin, ceftizidime (66.66 %).

The gram negative *Pseudomonas* (n=3) from the pus samples (n=11) were resistant to ampicillin /sulbactam, co-trimoxazole, chloramphenicol, ciprofloxacin, norfloxacin, ofloxacin, tetracycline, oxytetracyclin, cefuroxime, cefadroxil, nalidixic acid and amoxicillin/clavulanic acid (100%). While gram positive staphylococcus aureus (n=4) from pus samples were resistant to ciprofloxacin (100%), amoxicillin, vancomycin, erythromycin (75 %), and co-trimoxazole, levofloxacin, roxithromycin, neomycin, clindamycin, penicillin-G (50%).

DISCUSSION: Development of resistance to antibiotics is one of the significant problems encountered by the world in current era. The unavailability of efficacious antibiotics can challenge healthcare workers to tackle the infectious diseases and to manage the

complications, especially among immunosuppressed patients. The increasing prevalence of antimicrobial resistant bacteria might affect the ability to control infectious diseases by reducing treatment effectiveness and increasing mortality rates and healthcare costs. This study aimed to identify the prevalence of bacterial infections and their resistance against antimicrobial agents in tertiary care hospital of Gujarat, India.

Despite the lack of significant differences between isolated organisms across age groups, most of the causative organisms identified in present study were (39.5%) in the group aged 26-50 years than in other age groups. The similar results were also obtained in previous study of Divyashanthi CM *et al* and Balan K *et al* studies ^{7, 8}. Percentage of positive culture was higher among male (62.79 %) as compared to female (37.21%) patients which is in accordance with the previous Indian as well as abroad studies ^{2, 7, 10, 11}.

In this study, the most common causative agents were *Klebsiella pneumonia* (37.20%) and *E. coli* (30.23%) followed by pseudomonas (16.28%), *S. aureus* (9.30%), acinetobacter (4.65%) and enterococcus (2.32%) among forty three samples. These data are comparable to previous Indian studies ^{8, 9, 12}. Amongst all the cultures most common samples were of sputum (n=17). *Klebsiella* was major causative organism in the sputum samples. Second most common culture positive cases were of urine (n=14) and majority of them were having *E. coli* infections ^{13, 14}. While pus samples showed various gram positive as well as gram negative growth, among them *Staphylococcus aureus* and pseudomonas were major organisms respectively. Only one CSF sample showed positive culture growth of *E. coli*. From the sputum samples (n=17), the most common (76.47%) isolated bacterial pathogen was *Klebseilla* (n=13).

Among these *Klebsiella* isolates, most (84.61%) were resistant to tetracycline followed by 76.92% to ciprofloxacin, ofloxacin, ceftizoxime, cefadroxil, ceftizidime, amoxicillin/clavulanic acid and 69.23% to ampicillin /sulbactam, cefotaxime, cefuroxime, norfloxacin, gatifloxacin, nalidixic acid. Similar resistance pattern to tetracycline and cephalosporin was seen in previous studies^{15, 16, 17}.

While 100% pseudomonas were resistant to piperacillin, chloramphenicol, tetracyclin and 66.66% were resistant to ampicillin/sulbactam, amoxicillin/clavulanic acid, ciprofloxacin, ofloxacin, ceftizidime. Tetracycline resistance is alarming as it is a broad-spectrum antibiotic. Resistance to ampicillin/sulbactam, ceftizoxime, and ceftazidime is of particular concern because these antibiotics are often used for serious infections. Resistance to multiple antibiotics in *Klebsiella* in sputum samples indicates the potential for limited treatment options, especially for patients with compromised immune systems.

The most common causative agent of UTIs in this study was *E. coli* followed by *K. pneumonia* and *Acinetobacter*. These findings are in consistence with local as well as global epidemiological data. The most frequently identified bacteria in urinary isolates from female outpatients in the India was *E. coli* followed by *K. pneumonia* and globally it was *E. coli* and *Klebsiella* spp^{18, 19}. *E. coli* were mostly resistant to ampicillin /sulbactam, ceftazidime (77.77%) and co-trimoxazole, ceftizoxime (66.66%). A similar result was reported in a study of Malik *et al* and Dr. P. Sneka *et al* showing ampicillin/sulbactam and co-trimoxazole resistance^{20, 21}. While in contrast we found less fluorouinolone resistance as compared to the Malik *et al* study²⁰.

A systematic review results showed similar trends with regards to co-trimoxazole in India while fluoroquinolone and cephalosporin were showing higher resistance pattern as compared to our study²². These antibiotics will become unreliable and ineffective in treating *E. coli* infections in future. Resistance percentages nearing 77% and 66% for these drugs, respectively, indicate a dire situation, urging clinicians to reconsider their prescription practices and explore alternative treatments. Similarly, the *Acinetobacter* strains exhibited an

even more worrisome pattern of resistance, showing 100% resistance to a broad range of antibiotics. The resistance observed in *Acinetobacter* is particularly concerning due to its inherent ability to survive in hospital environments and cause infections in immune-compromised individuals which could lead to high mortality rates, especially in critically ill patients.

Most common gram negative organism found in pus is pseudomonas followed by *E. coli* and *Klebsiella*, pseudomonas was 100% resistant to ampicillin /sulbactam, co-trimoxazole, chloramphenicol, ciprofloxacin, norfloxacin, ofloxacin, tetracycline, oxytetracyclin, cefuroxime, cefadroxil, nalidixic acid, amoxicillin/clavulanic acid. While gram positive organisms found in pus are *Staph. aureus* followed by *Enterococcus*. *Staphylococcus aureus* displayed high resistance to ciprofloxacin (100%) and considerable resistance to vancomycin (75%). All above findings are in similar pattern with previous study except vancomycin which was not showing resistance in previous study²³.

Vancomycin is often a last-resort antibiotic. *Enterococcus* exhibited complete resistance (100%) to a wide range of antibiotics, including amoxicillin, penicillin-G, tetracycline, and others. This poses a significant threat as *Enterococcus* infections can be difficult to treat. The findings of this study present alarming implications for the management of urinary tract infections, as evidenced by the high resistance rates observed among both *E. coli* and *Acinetobacter* strains to multiple antibiotics. The resistance patterns observed in *E. coli* indicate a significant level of resistance to commonly prescribed antibiotics such as ampicillin/sulbactam, ceftazidime, co-trimoxazole, ceftizoxime.

Notably, the high resistance rates of *E. coli* to ceftazidime are concerning, as this antibiotic is often used as a last resort in the treatment of severe bacterial infections. The differences in resistance patterns among sample types indicate the need for tailored treatment strategies. For example, urine samples of *E. coli* and *Acinetobacter* exhibited notable resistance, while sputum and pus samples of *Klebsiella* and *Pseudomonas* had high resistance.

Implications: The antimicrobial resistance observed in present study has possible significant implications:

Limited Treatment Options: The growing resistance to multiple antibiotics reduces the efficacy of conventional treatment options.

Increased Healthcare Costs: Prolonged hospital stays and the need for stronger antibiotics can result in increased healthcare costs.

Patient Morbidity and Mortality: Patients with resistant infections may experience prolonged illness, increased complications and higher mortality rates.

Antibiotic Stewardship: Implementing antibiotic stewardship programs becomes essential to ensure the responsible use of antibiotics and to slow down the emergence of resistance.

CONCLUSION: The high levels of antimicrobial resistance observed in this study highlight the urgent need for a multifaceted approach to combat resistance, including antibiotic stewardship and the development of new antibiotics. These findings underscore the importance of continuous surveillance and research to adapt treatment strategies to an ever-evolving microbial landscape.

Limitation: The study did not determine the resistance detected was due to hospital-acquired or community acquired infection. The study did not show the trends of antibiotics resistance from year to year.

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CONFLICTS OF INTEREST: None

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