

Development of Antibigram Toolkit for Enhancing Empiric Antibiotic Selection in a Tertiary Care Hospital

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ABSTRACT

Background: A clinical microbiology laboratory at the hospital receives frequent reports on the antibiotic susceptibilities of localized bacterial isolates. Clinicians frequently utilize antibiograms to evaluate regional susceptibility rates, as a tool in choosing initial antibiotic therapy, and to track changes in resistance over time within a facility.

Objectives:

To develop an antibiogram using culture sensitivity data.

To help select empirical antibiotic therapy for suspected infection.

Methodology: A prospective study was conducted in 243 patients with positive culture sensitivity data in the department of microbiology at MVJ medical college and research hospital for a period of 6 months (February 2022-August 2022). Patients of either gender irrespective of the age, diagnosed with bacterial infection and having positive culture sensitivity data with or without comorbid conditions and pregnancy were included. In a case report form that was specially created, all the necessary patient information was gathered. The antibiogram displays the percentage susceptibility of the most frequently isolated bacteria in tabular form.

Results: In our study the most isolated organism was found to be *Klebsiella species* (30.86%), followed by *E. coli* (18.52%), *Staphylococcus aureus* (10.29%), and *Pseudomonas aeruginosa* (9.47%), which showed that *Klebsiella species* were susceptible to colistin (100%), Fosfomycin (89%) and showed 100% resistance towards PIP/TAZ and ampicillin. *E. coli* showed susceptibility towards meropenem (100%), nitrofurantoin (95.5%), imipenem (95%), and Fosfomycin (91.7%).

Conclusion: The study's high rates of resistance across both Gram-positive and Gram-negative isolates highlight the need for accurate anti-microbial sensitivity testing data collected through routine cultures, which can aid in the consistent development and dissemination of antibiograms and guide the use of antibiotics.

Keywords: Antibiogram, Antibiotic resistance, Gram-positive, Gram-negative, Antimicrobial therapy, Culture sensitivity.

INTRODUCTION

Hospitals regularly test bacterial infections for antibiotic susceptibility, and the results are compiled in a table called an antibiogram. Doctors utilise antibiograms to choose the best preliminary antibiotic therapy and significantly cut on unnecessary antibiotic use. Antibiograms can be used to undertake surveillance for the emergence of drug-resistant bacteria, update information on resistance to antibiotics over time, and identify areas where antimicrobial stewardship initiatives should concentrate their efforts (1). The employment of an antibiogram for the selection of an empirical antimicrobial therapy can be more effective if the causative bacteria is recognised before the susceptibility results. It is less effective when an infectious bacterium is unidentified but suspected because of the location and/or kind of infection. Since prescribers use the antibiogram as a tool when deciding on an empirical antibiotic regimen, it is crucial that it be a helpful and simple resource. Antibiograms do not present quantifiable data like MIC but do give binary measures of susceptibility instead (whether a pathogen is susceptible or not) (2).

Antibiograms more typically list the percentage of susceptible isolates or exhibit susceptibility in a colour scheme manner. The range of susceptibility of the studied organisms, as well as the dependability of using that antimicrobial-pathogen combination, are frequently displayed on color-coded antibiograms. The organisms have consistently proven susceptible, as indicated by the green colour, which represents a range of susceptibilities. The antimicrobial-pathogen combo is probably unreliable because the colour red denotes a spectrum of susceptibilities that are frequently near or less than 50% susceptible. The area with both green and red where the isolates are more inconsistently sensitive is represented by the colour yellow. The percentages represent the number of isolated samples that were discovered to be antibiotic-susceptible. These antibiograms typically include

captions that inform the reader of the criteria used to establish the assigned colour coding (3).

In 2016, the UN identified antibiotic resistance as "the greatest and most urgent global risk." Antimicrobial resistance is caused by the misuse and overuse of antibiotics. Antibiotic-resistant bacterial infections are prevalent and on the rise in India. Given that overuse and misuse of antibiotics is the primary cause of antibiotic resistance, antibiotic stewardship (AS) and AS programmes (ASP) are essential for promoting safe antibiotic usage (4).

Hospitals in India are documenting significant levels of drug-resistant to carbapenems and fluoroquinolones as well as growing resistance to colistin and other polymyxins as the usage of these drugs increases. According to reports, among the BRICS (Brazil, Russia, India, China, and South Africa) countries, India consumed the most antibiotics in 2010 and was responsible for 23% of the increase in antibiotic retail sales. With a continuous rise in resistance to the last-resort medications carbapenems and colistin, recent developments in antibiotic resistance in India are worrisome. Cephalosporin and broad-spectrum penicillin utilisation have increased between 2000 and 2015 (5).

The Antimicrobial Stewardship Program (AMSP) is a strategic approach to antibiotic stewardship that aims to reduce unnecessary antibiotic use and control AMR. AMSP implementation has been shown to decrease overuse of antibiotics and has lowered resistance rates in many nations. Antimicrobial use has decreased by 22–36% due to AMS programmes, which have also resulted in significant cost reductions (5).

MATERIALS & METHODS

STUDY DESIGN

This is a prospective study conducted from February 2022 till August 2022 in MVJ Medical College and Research Hospital, Bangalore. Study was conducted in 243 in-patients of either gender irrespective of the age, diagnosed with bacterial infection and

having positive culture sensitivity data with or without comorbid conditions and pregnancy were included in the study. Inclusion Criteria: Patients with positive culture sensitivity test and treated with antibiotics. Exclusion Criteria: Patients who are diagnosed with infection of non-bacterial origin. In a case report form that was specially created, all the necessary patient information was gathered. The percentage susceptibility of the most frequently isolated bacteria is presented in the antibiogram in a tabular form.

Statistical Analysis: All descriptive analysis will be summarized as mean +/- standard deviation, median, or interquartile range based on data normality. The relationship between categorical variables is determined using the Fisher exact test. Significant results are those with a P-value of less than 0.05 and a 95% confidence interval. All statistical analysis is performed using SAS 9.4 and GraphPad prism 9 software.

RESULT

TABLE 1: DEMOGRAPHIC CHARACTERISTICS.

S.NO	Parameter	No. of patients	No. of patients (%)	
1	Gender	Male	132	54.3
		Female	111	45.6
2	Age(years)	0-18	39	16
		19-64	124	51
		65-100	80	33
3	Department	Pediatrics	38	15.6
		Medicine	114	46.9
		Surgery	59	24.2
		Orthopaedic	13	5.3
		OBG	18	7.4
	Tuberculosis	1	0.411	

SPECIMEN DISTRIBUTION

Of the 243 specimens collected for the culture sensitivity test, blood (29 specimens, or 12%), urine (54 specimens, or 22%), Endotracheal Tube (ET) aspiration (10 specimens, or 4%), Umbilical Venous Catheter (UVC) tip (9 specimens, or 4%), sputum (58 specimens, or 24%), pus (64 specimens, or 26%), and tissues (5 specimens, or 2%), Ear swab (2 specimens, 1%), swab (4 specimens, 2%), vaginal swab (1 specimen, 0.45%), Peripherally Inserted Central Catheter (PIC) Line (1 specimen, 0.45%), pleural fluid (1 specimen, 0.45%), ascitic fluid (1 specimen, 0.45%), and bronchoscopy (4 specimens, 2%) were included in the study.

TYPES OF GRAM-POSITIVE AND GRAM-NEGATIVE ORGANISMS

A total of 243 organisms were isolated from culture sensitivity testing; 64 isolates were found to be Gram-positive species and 179

isolates were found to be Gram-negative species.

Klebsiella species were 75 out of 179 Gram-negative organisms, with 49 isolates being *Klebsiella pneumoniae* and 7 isolates being *Klebsiella oxytoca*, 45 isolates being *E.coli*, 1 isolate being *Gram-negative bacilli*, 5 isolates being *Fermenter gram-negative bacilli*, 23 isolates being *Pseudomonas species*, 16 isolates being *Pseudomonas aeruginosa* and 7 isolates are other *Pseudomonas species*; 2 isolates are *Enterobacter species*; 1 isolate is *Enterobacter cloacae*; 1 isolate is *Enterobacter aerogenes*; 9 isolates are *Citrobacter species*, with 6 isolates being *Citrobacter freundii* and 1 isolate being *Citrobacter koseri*; 2 isolates are *Acetobacter species*; 11 isolates are *Acinetobacter species*; 2 isolates are *Providencia species*; 2 isolates are *Proteus species*, one of which is *Proteus mirabilis*.

Out of 64 Gram-positive organisms, 1 isolate was *Streptococcus species*, 25 isolates were *Staphylococcus aureus*, 1 isolate was other Gram-positive bacilli, 13 isolates were *Methicillin-resistant coagulase negative staphylococcus (MRCNS)*, 6 isolates were *Methicillin-*

sensitive coagulase negative staphylococcus (MSCNS), 2 isolates were *Methicillin-sensitive Staphylococcus aureus*, 8 isolates were *Coagulase-negative Staphylococci (CoNS)*, 8 isolates were *Enterococcus species*.

TABLE 2: COMORBIDITY OF STUDIED CASES

Comorbidity	Number	Percentage
Present (Bacterial Infection+ other conditions)	173	71%
Absent (only Bacterial Infection)	70	29%
Total	243	100%

**ANTIBIOGRAM
GRAM-NEGATIVE ORGANISMS**

Klebsiella species and *Escherichia coli* were the most isolated Gram-negative bacteria, with 75 and 45 specimens respectively. *Klebsiella species* were susceptible to colistin (100%), Fosfomycin (89%),

nitrofurantoin (88.9%), imipenem (84%) and meropenem (78.3%) and showed 100% resistance towards PIP/TAZ and ampicillin. *E. coli* showed susceptibility to meropenem (100%), nitrofurantoin (95.5%), imipenem (95%), and Fosfomycin (91.7%) as shown in Table 3.

TABLE 3: GRAM-NEGATIVE ANTIBIOGRAM

GRAM NEGATIVE	NO. OF ISOLATES	FLUROQUINOLONES		CEPHALOSPORINS								AMINOGLYCOSIDE			NITROFURANTOIN	MACROLIDE	PENICILLIN		
		CIPROFLOXACIN	LEVOFLOXACIN	CEFAZOLIN	CEFEPIME	CEFOTAXIME	CEFOXITIN	CEFTAZIDIME	CEFTRIAZONE	CEFUROXIME	AMIKACIN	GENTAMICIN	TOBRAMYCIN	ERYTHROMYCIN			AMOXYCILLIN	AMPICILLIN	PENICILLIN
<i>KLEBSIELLA SPECIES</i>	75	73.3	33.3	19	39	47		27	40	30		79	68.3	65	88.9	100	71.4	0	50
<i>ESCHERICHIA COLI</i>	45	18		13	45	24		20	21	16		87.5	70	47.4	95.5		20	20	33
<i>OTHER GRAM-NEGATIVE BACILLI</i>	6	100		17	80	80		80	100	80		60	75	60			33	50	100
<i>PSEUDOMONAS SPECIES</i>	23	100	50	20	77	17		50	28.6	0		90.9	85.7	85.7	100		0		
<i>ENTEROBACTER SPECIES</i>	4*	0		50	25	0		25	0	0		75	75	75			0		
<i>CITROBACTER SPECIES</i>	9	100		14	40	80		40	67	33		100	80	50	67		0	50	
<i>ACETOBACTER SPECIES</i>	2*			0	50	50		0	0	0		50	0	50				0	
<i>ACINETOBACTER SPECIES</i>	11	50		0	27.3	44		22	0	13		70	80	44.4			33	100	
<i>PROVIDENCIA SPECIES</i>	2*			100	0	0		100	0	0		50	50	100					
<i>PROTEUS SPECIES</i>	2*			0	100	0		100	0	0		100	100	100				0	

Gram negative	No. of isolates	Lincosamide	Carbapenem		Tetracycline	Phosphonic acid	Polymyxin	Glycopeptide	Oxazolidinone	Beta-lactam	Others		
		Clindamycin	Imipenem	Meropenem	Doxycycline	Fosfomycin	Colistin	Vancomycin	Linezolid	Clavulanic acid	Amoxicillin/clavulanic acid	Piperacillin/tazobactam	Trimethoprim/sulfamethoxazole
<i>KLEBSIELLA SPECIES</i>	75	100	84	78.3	100	89	100		100	66.7	29	0	39
<i>ESCHERICHIA COLI</i>	45		95	100		91.7		100	100	33.3	38		21.7
<i>OTHER GRAM-NEGATIVE BACILLI</i>	6		80	75		100					50		100
<i>PSEUDOMONAS SPECIES</i>	23		66.7	71.4		100	100			0	0	58.8	33.3
<i>ENTEROBACTER SPECIES</i>	4*		100	100							50		
<i>CITROBACTER SPECIES</i>	9		100	100		100				0	50	100	100
<i>ACETOBACTER SPECIES</i>	2*		100	100			100				0		
<i>ACINETOBACTER SPECIES</i>	11		50	33.3			100			100	33	0	0
<i>PROVIDENCIA SPECIES</i>	2*		100	100							100		
<i>PROTEUS SPECIES</i>	2*		100	100						100	50		

	Resistant		Susceptible		Intermediate
	Not significant, because number of isolates tested against an antibiotic is ≤ 4				
*	Not significant because number of isolates ≤ 4				

GRAM-POSITIVE ORGANISMS
Staphylococcus aureus and *MRCNS* were the most isolated Gram-positive bacteria, with 25 and 13 specimens respectively. *Staphylococcus aureus* showed 100% susceptibility to nitrofurantoin, linezolid,

83.3% to doxycycline and 70.8% to TMP/SMX. *MRCNS* showed 100% resistance to penicillin and susceptibility ranges of 91.7% to doxycycline and 61.5% to TMP/SMX as shown in Table 4.

TABLE 4: GRAM POSITIVE ANTI BIOGRAM

Gram positive	No. Of isolates	Fluroquinolones		Cephalosporins							Aminoglycoside			Nitrofurantoin	Macrolide	Penicillin		
		Ciprofloxacin	Levofloxacin	Cefazolin	Cefepime	Cefotaxime	Cefoxitin	Ceftazidime	Ceftriaxone	Cefuroxime	Amikacin	Gentamicin	Tobramycin	Nitrofurantoin	Erythromycin	Amoxycillin	Ampicillin	Penicillin
<i>STAPHYLOCOCCUS AUREUS</i>	25	39					67				50		100	43.5			12	
<i>MRCNS</i>	13	42				00							100	25			0	
<i>MSCNS</i>	6	67				100							100	100			50	
<i>MSSA</i>	2*	100					100							50	0	0	50	
<i>CoNS</i>	8	50				00	50				100		100	14.3			16.7	
<i>ENTEROCOCCUS SPECIES</i>	8	50	0	00	100		100	100	50	100	66.67	50	33.3	0	0	60	40	
<i>STREPTOCOCCUS SPECIES</i>	1*										100					100	100	
<i>GRAM POSITIVE BACILLI</i>	1*	100																

Gram positive	No. of isolates	Lincosamide	Carbapenem		Tetracycline	Phosphonic acid	Glycopeptide	Oxazolidinone	Others			
		Clindamycin	Imipenem	Meropenem	Doxycycline	Fosfomycin	Vancomycin	Linezolid	Amoxicillin/ clavulanic acid	Piperacillin/ tazobactam	Trimethoprim/ sulfamethoxazole	Novobiocin
<i>STAPHYLOCOCCUS AUREUS</i>	25	52			83.3		100	100			70.8	100
<i>MRCNS</i>	13	50			91.7			100			61.5	100
<i>MSCNS</i>	6	50			83.3			100			100	75
<i>MSSA</i>	2*	0			50		100	100			100	
<i>CoNS</i>	8	14			85.7			100	0		42.9	
<i>ENTEROCOCCUS SPECIES</i>	8	100	100	100	100	100	100	83	0			
<i>STREPTOCOCCUS SPECIES</i>	1*						100	100				
<i>GRAM POSITIVE BACILLI</i>	1*								0			

	Resistant		Susceptible		Intermediate
	Not significant, because number of isolates tested against an antibiotic is ≤ 4				
*	Not significant because number of isolates ≤ 4				

DISCUSSION

The current study's main goals are to identify the most frequent bacterial isolates that cause infectious diseases in hospitals as well as determine the resistance and susceptibility rates for those bacteria in the hospital. Antibiotic resistance is currently on the rise, and as a result, each hospital must have its own antibiogram. This document is essential for establishing an antibiotic plan and is a crucial component of an antimicrobial stewardship program.

According to the inclusion criteria, 243 participants were enrolled in our study over a six-month period. Overall, the study's findings indicate that third-generation cephalosporin antibiotics are frequently used in hospitals and that this antibiotic class also has the highest rate of resistance.

In terms of gender distribution, the study's overall population was made up of 54% men and 46% women, and a comparable study by MS Dikkatwar et al., 2020 found that 66.6% of the population was male and 33.3% was female. Hence, the study data shows that males were more exposed to bacterial infections than females.

Our study shows that most of the Gram-negative organisms showed the highest resistance to cephalosporins, followed by penicillins and beta-lactams, and the highest susceptibility to carbapenems, aminoglycosides, Fosfomycin and colistin. Whereas gram-positive organisms showed the highest resistance to penicillins and erythromycin and the highest susceptibility to nitrofurantoin, doxycycline, vancomycin and linezolid. One such study, performed by Mavis Pupopelle Dakorah et al. in 2022, also found ampicillin to have lowest susceptibility and amikacin to show highest susceptibility for gram-negative bacteria. Gentamicin had the highest susceptibilities among the Gram-positives, while clindamycin had the lowest susceptibilities.

Different samples were tested throughout our investigation for the isolation and identification of bacteria, and the majority of the microorganisms were isolated from urine (54), sputum (58), pus (64), and blood (29). In our study, *Klebsiella species* predominated in sputum, while *E. coli* was more frequently isolated in urine samples. According to a study of a similar nature done by MS Dikkatwar et al. in 2020, urine samples were the main cause of *E. coli* isolates.

According to our research, gram-negative bacteria outshone gram-positive bacteria in the total number of bacterial isolates, which is consistent with research by Litegebew Yitayeh et al. from 2021, which also found that gram-negative bacteria outshone gram-positive bacteria.

In our study, the most isolated organism was found to be *Klebsiella species* (30.86%), followed by *E.coli* (18.52%), *Staphylococcus aureus* (10.29%), and *Pseudomonas aeruginosa* (9.47%). Number of studies have reported varying range of *E.coli* isolation, while Mark Verway et al., 2022 observed most common isolation of *E. coli* (26.9%), *Staphylococcus aureus* (15.9%), *Klebsiella species* (8.2%), *Pseudomonas* (3.4%). Litegebew Yitayeh et al., 2021 observed most common isolation of *E.coli* (47.4%), *Klebsiella species* (7.4%), *Staphylococcus aureus* (10.4%).

Ceftriaxone (17%) was determined to be the antibiotic that was used most frequently throughout our investigation, followed by amikacin (10%) and amoxicillin + clavulanic acid (10%). During that time, 33 different antibiotics were prescribed to the research population. According to a 2017 study by Baidya S. et al., Ceftriaxone (36.3%), Amoxicillin + clavulanic Acid (13.5%), and Amikacin (10.7%) were the most frequently administered medications.

Our study showed that *Klebsiella species* were susceptible to colistin (100%),

Fosfomycin (89%), nitrofurantoin (88.9%), imipenem (84%), and meropenem (78.3%) and showed 100% resistance towards PIP/TAZ and ampicillin. *E. coli* showed susceptibility to meropenem (100%), nitrofurantoin (95.5%), imipenem (95%), and Fosfomycin (91.7%). *Pseudomonas aeruginosa* showed susceptibility to ciprofloxacin (100%), amikacin (90.9%), gentamicin (85.7%) and tobramycin (85.7%), whereas it showed 100% resistance to cefuroxime. *Staphylococcus aureus* showed the susceptibility ranges of 100%, 83%, 71% for linezolid, doxycycline and TMP/SMX respectively. The study conducted by Kenechi O. Nnamani et al., 2022 reported that *Staphylococcus aureus* showed 100% and 66.7% susceptibilities to linezolid and TMP/SMX respectively. MRCNS showed 100% susceptibility to linezolid and nitrofurantoin and 91.7% to doxycycline. *Staphylococcus aureus* showed 100% resistance to penicillin. A similar study was conducted by Brenna M. Roth et al., 2021 who found that imipenem, nitrofurantoin and PIP/TAZ showed the susceptibility ranges of 99.2%, 40.8% and 12.3% to *Klebsiella pneumoniae*. Nitrofurantoin and imipenem showed 72.1% and 99.3% susceptibility to *E. coli*, respectively. Ciprofloxacin, amikacin and gentamicin showed 71.2%, 88.9% and 71.5% susceptibility to *Pseudomonas aeruginosa*, respectively.

CONCLUSION

Improper use and overuse of antibiotics are the main reasons contributing to global antimicrobial resistance (AMR). The high rates of resistance in both Gram-negative and Gram-positive organisms discovered in our study indicated the requirement for accurate microbiology laboratory results that would be used to regularly and routinely develop, upgrade, and distribute antibiograms to guide the proper use of antibiotics.

Our study concluded that most organisms were isolated from male patients admitted to the general medicine department. Urine and sputum were the most prevalent specimens

collected from patients. Majority of the patients who were studied had diabetes mellitus as a comorbidity. Majority of the patients included in the study had diabetes mellitus as a comorbidity. Injection ceftriaxone was most commonly prescribed empiric antibiotic. *Klebsiella species* and *Escherichia coli* were the most isolated organism from culture sensitivity test.

The antibiogram was developed according to culture sensitivity data obtained from the hospital microbiology department and were classified and coloured based on CLSI guidelines. The generated antibiogram will be a crucial tool in the fight against AMR by promoting better antibiotic prescribing at all stages of health services. The increased level of resistance among the Gram-negative and Gram-positive isolates identified in the study highlights the requirement for accurate anti-microbial sensitivity testing data obtained via routine cultures that can contribute to the consistent and regular creation and promotion of antibiograms to guide appropriate antibiotic use. These results also confirm the need for AMR surveillance and monitoring both at public and private establishments to determine local occurrence and support an efficient AMS program.

LIMITATION

Antibiograms provide a binary measure of susceptibility but not quantitative results like Minimum inhibitory concentration (whether a pathogen is susceptible or not).

According to CLSI standards, a pathogen should be included on an antibiogram if 30 or more isolates are available. Due to the limited number of cultures acquired throughout the 6-month research period, if this guideline had been followed, no facility would have produced an antibiogram from the results of the available cultures. If four or more isolates of an organism were available, our microbiologist suggested including it. This could have affected how reliable the antibiograms were.

Only selected antibiotics were tested against an organism based on the disease or site of

infection by microbiology department of MVJ Hospital.

Declaration by Authors: I hereby declare that “Development of Antibiogram Toolkit for Enhancing Empiric Antibiotic Selection in A Tertiary Care Hospital” is our original work. It has not been previously published, nor has it been submitted for publication elsewhere in any format.

Ethical Approval: The MVJ Medical College and Research Hospital's ethics committee granted permission to conduct the research under the terms of Ethical Clearance No. MVJMC&RH/IEC-16/2022.

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