International Journal of Health Sciences and Research
[Indexed, Peer-Reviewed Journal]
Volume 16; Issue: 4; April 2026
Website: www.ijhsr.org
Original Research Article *ISSN: 2249-9571*

Cumulative Antibiogram and Antimicrobial Susceptibility Patterns of Pus Isolates Using WHONET 5.6 2025 in a Microbiology Department of Clinical Laboratory in Navsari, South Gujarat

Manoj A Kahar[1], PrashantKumar Jagubhai Patel[2], Dhvani Y Kahar[3], Nafisa Patel[4]

[1]Lab Director, Bhanumati Clinical Laboratory Navsari, Gujarat, India.
[2]Assistant Professor, Department of Pathology, Kiran Medical College, Surat, Gujarat, India.
[3]Student of M.Sc. in Microbiology, Naran Lala College of Professional & Applied Sciences, Navsari, Gujarat, India.
[4] Associate Professor, Department of Microbiology, Naran Lala College of Professional & Applied Sciences, Navsari, Gujarat, India.

Corresponding Author: Manoj A Kahar

DOI: https://doi.org/10.52403/ijhsr.20260423

ABSTRACT

Background: Pyogenic infections leading to pus formation are common in clinical practice. Increasing Antimicrobial Resistance (AMR) poses a major therapeutic challenge. Local cumulative antibiograms are essential for guiding empirical therapy.
Aims: To identify etiologies of pyogenic infections for 4 years (January 2022 to December1 2025) & to identify susceptibility pattern of pathogens isolated. To familiarize with WHONET software 2025 version 5.6 for preparing Antibiogram & to generate an Antibiogram for pus isolates which can guide empirical treatment.
Materials & Methods: A retrospective observational study was conducted analysing non-duplicate pus isolates from January 2022 to December 2025. Antimicrobial susceptibility data were processed using WHONET software 2025 version 5.6 following Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data Clinical & Laboratory Standard Institute Analysis (CLSI) M39 5th edition guidelines. Findings of present study were compared with published national & international studies.
Results: 264 out of 517 pus samples resulted in positive isolates giving a yield of 51.06% with 6 species being isolated; Gram negative bacteria were 184 (69.69%) & Gram positive bacteria were 80 (30.30%). The most common organism isolated was *Staphylococcus aureus* 80 (30.30%) followed by *Escherichia coli* 42 (15.90%). The least isolated organism was *Enterobacter spp.* 12.12%. The most sensitive antimicrobial for both Gram negative & Gram positive was Trimethopirm-sulfamethoxazole (Cotrimoxazole) & the susceptibility was for *E. coli* 73.7%, for *Enterobacter spp.* 90.9%, for *Klebsiella pneumoniae* 70%, for *Klebsiella spp.* 81.8%, & *Staphylococcus aureus* 95.8%.
Conclusion: This study confirms that the bacteria implicated in pyogenic infection forming pus are *S. aureus, E. coli, K. pneumoniae, Klebsiella spp., P. aeruginosa, Enterococcus spp.* These organisms have developed drug resistance to some important antimicrobials such as cefazolin, cephalosporins & fluoroquinolones. Appropriate & rational selection of

antimicrobials would limit the emerging drug resistance in the future with the use of local antibiograms.

Keywords: Antimicrobial resistance, WHONET, cumulative antibiogram, pus, CLSI M39.

INTRODUCTION

With growing Antimicrobial Resistance (AMR) worldwide, it is essential to monitor the emerging trends in AMR at the local facility level, which will support empirical therapy decision making, infection control interventions, and implementation of appropriate AMR containment strategies. Monitoring of AMR trends in health care facilities is commonly performed using an annual summary of susceptibility rates of common pathogenic organisms to various antimicrobial agents, known as a cumulative antibiogram report (1).Pyogenic infections are among the most commonly encountered bacterial infections in routine clinical practice and account for a significant proportion of specimens processed in diagnostic microbiology laboratories.

Irrational antibiotic use, empirical therapy without microbiological evidence, and inappropriate prescribing practices have accelerated the development of resistance, resulting in limited therapeutic options, prolonged illness, increased healthcare costs, and adverse clinical outcome (2,3). Antimicrobial susceptibility testing and cumulative antibiograms play a critical role in guiding empirical therapy and monitoring resistance trends. Local antibiograms are particularly important due to geographic and institutional variations in resistance patterns (4).

In India, AMR is a growing public health concern, with state-level surveillance programs such as Gujarat State Antimicrobial Resistance Surveillance Network (GUJSAR) emphasizing the need for reliable local data (5). Continuous surveillance of local antimicrobial susceptibility patterns is a must for combating emerging antimicrobial resistance. WHONET is an effective computerized microbiology laboratory data management and analysis program that can provide guidance for empiric therapy of infections, alert clinicians of trends of antimicrobial resistance, guide drug-policy decisions and preventive measures (6). The present study was conducted to analyse the prevalence of bacteria forming pus infection and antimicrobial susceptibility patterns of bacterial isolates from pus samples using WHONET software 2025 version 5.6 at a department of microbiology in clinical laboratory of Navsari district, South Gujarat, and to compare the findings with national and international studies.

Aims

1. To identify etiologies of pyogenic infections for 4 years (Jan 2022 to Dec 2025) & to identify susceptibility pattern of pathogens isolated.
2. To familiarise with the use of WHONET software 2025 version 5.6 for preparing cumulative antibiogram for pus isolates which can guide empirical treatment.

MATERIALS & METHODS

Study Design and Setting

A retrospective observational study was conducted in microbiology department of a standalone clinical laboratory in Navsari, South Gujarat, India. Routine antimicrobial susceptibility testing (AST) data generated over a four-year period (January 2022–December 2025) were analysed to assess local antimicrobial resistance patterns in pus isolates and support empirical therapy.

Culture & Isolation of Bacteria from Pus samples

Specimens for pus cultures were inoculated on appropriate bacterial culture media & isolates were identified & characterised by standard methods (such as colony morphology, haemolysis, Gram stain characteristics & appropriate biochemical test) (7–10). Appropriate Quality Control

measures were ensured by the laboratory using standard Internal Quality Control Protocols & participating in External Quality Control Assessment Program from Indian Association of Medical Microbiologists, New Delhi while performing culture & sensitivity tests.

Antimicrobial Susceptibility Test (AST)

AST was performed by Kirby-Bauer disk diffusion method following recommendations from Performance standards for antimicrobial disk susceptibility tests CLSI M02 14th ed. (11) . Bacterial suspensions were adjusted to 0.5 McFarland turbidity and inoculated onto Muller-Hinton agar plates. 1st tier Antimicrobial disks according to the Performance Standards for Antimicrobial Susceptibility Testing CLSI M100 guideline (12) were placed on the agar surface & plates were incubated at 37°C for 24 hours. Interpretive criteria (Susceptible, Intermediate or Resistance) were reported using CLSI M100 (12).

Data Source and Inclusion Criteria

AST records were obtained from laboratory information systems and microbiology registers. Clinically significant, non-duplicate bacterial isolates from pus samples were included. Repeat isolates from the same patient with identical susceptibility profiles were excluded in accordance with CLSI M39 5th ed. guidelines for cumulative antibiogram preparation (13). Only organisms with ≥30 isolates per organism during the defined study period were included in the cumulative antibiogram analysis.

Data Analysis Using WHONET software 2025 version 5.6

WHONET software 2025 version 5.6, developed by the WHO Collaborating Centre for Surveillance of Antimicrobial Resistance, is a computerized microbiology laboratory data management and analysis program designed to support antimicrobial resistance surveillance. The software enables standardized data entry, validation, and compatibility with CLSI M100 interpretative criteria (12). It facilitates analysis of antimicrobial susceptibility patterns, detection of resistance trends, and generation of cumulative antibiograms for selected pathogens. The WHONET Download and Installation Manual (14) provides detailed instructions for software installation, configuration, database setup, and data analysis procedures to ensure uniform reporting and surveillance practices.

Preparation of Cumulative Antibiogram

The cumulative antibiogram was prepared in accordance with CLSI M39 14th ed. guidelines. The percentage susceptibility was calculated by the following formula (13).

Percentage susceptibility = Number of isolates susceptible/Total number tested x 100

Isolates were further categorized based on their resistance profiles. An organism was considered as MDR when isolate was non-susceptible to at least one agent in three or more antimicrobial categories. Organisms were labelled as XDR when isolate was non-susceptible to at least one agent in all but two or fewer antimicrobial categories. Similarly an organism was considered as PDR when isolate was non-susceptible to all agents in all antimicrobial categories tested **(15)**.

Preparation of Antibiogram Using WHONET Software

WHONET software 2025 version 5.6 was downloaded from the official website (https://whonet.org) and installed using recommended steps by the official WHO website. Laboratory configuration, data entry, and analysis were performed as per WHONET guidelines **(14)**. Antibiograms were generated for organisms with ≥30 isolates, displaying percentages of susceptible, intermediate, and resistant

isolates recommended in CLSI M39 5th ed. guidelines **(13)**.

RESULT

In present study a total of 517 pus samples were received for culture & sensitivity in 4 years (Jan 2022 to Dec 2025). Out of 517 total received samples, 264 were positive for bacterial isolates, resulting in a yield of 51.06%, which is shown in the table no.1. Out of 264 positive samples 184 69.69% were Gram negative & 80 30.30% were gram positive isolates.

Table 1, Culture positivity rate & distribution of Gram positive & Gram negative isolates in pus samples in the present study

Variables	Frequency (n)	Percentage (%)
Total number of pus samples received	517	-
Total number of positive cultures	264	51.06%
Gram positive isolates	80	30.30%
Gram negative isolates	184	69.69%

The yield of Gram negative & Gram positive organisms out of 264 positive isolates are shown below as Table no, 2.

Table 2, Distribution of isolates in the present study from pus culture positive results

Bacterial group	Organisms	Number of isolates	Yield of Isolates
Gram positive isolates	Staphylococcus aureus	80	30.30%
Gram negative isolates	Escherichia coli	42	15.90%
	Pseudomona aeruginosa	38	14.39%
	Klebsiella pneumoniae	37	14.01%
	Klebsiella spp.	35	13.25%
	Enterobacter spp.	32	12.12%
		Total: - 264	

Cumulative antibiogram was prepared using WHONET software 2025 version 5.6. Tier 1 antimicrobials recommended by CLSI M100 were used for AST in the laboratory & same are shown in Antibiogram as Table no.3.

Table 3: - Antibiogram for Antimicrobial Susceptibility results for Pus isolates for Tier 1 Antimicrobial agents as per CLSI M 100

Organism	**Ampicillin**	**Cefazolin**	**Cefotaxime or ceftriaxone**	**Amoxicillin – clavulanate**	**Ampicillin – sulbactam**	**Gentamicin**	**Ciprofloxacin**	**Trimethopirm - sulfamethoxazole**	**Piperacillin – Tazobactam**	**Levofloxacin**	**Ceftazidime**	**Cefepime**	**Tobramycin**	**Azithromycin or Clarithromycin or Erythromycin**	**Clindamycin**	**Oxacillin Cefoxitin (surrogate for Oxacillin)**	**Doxycycline**	**Minocycline**	**Tetracycline**
Escherichia coli	13	0	0	33.4	65.2	43.5	4.3	73.7	82.6	0	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	0	0	7.1	58.6	56	64	4	70	64	28	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	7.7	-	46.2	23.1	46.2	46.2	15.4	-	-	-	-	-	-
Klebsiella sp.	13.6	0	20	48.5	63.6	63.6	9.1	81.8	72.7	22.7	-	-	-	-	-	-	-	-	-
Enterobacter sp.	5.9	0	5.9	28	82.4	58.8	5.9	90.9	82.4	23.5	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-	95.8	-	-	-	-	-	81.8	90.9	25.9	82.8	80	82.8

Note: (-) Indicates Not tested

In the present study, *Escherichia coli* showed the highest susceptibility to Piperacillin–Tazobactam 82.6%, while the least susceptibility was observed with Cefazolin, Cefotaxime/Ceftriaxone, and Levofloxacin 0%.

Klebsiella pneumoniae demonstrated maximum susceptibility to Trimethoprim–sulfamethoxazole 70%, whereas the least susceptibility was seen with Ampicillin and Cefazolin 0%.

Pseudomonas aeruginosa showed the highest susceptibility to Piperacillin–Tazobactam, Ceftazidime, and Cefepime 46.2% each, while the least susceptibility was observed with Ciprofloxacin 7.7%.

Klebsiella spp. exhibited the greatest susceptibility to Trimethoprim–sulfamethoxazole 81.8%, whereas the least susceptibility was noted with Cefazolin 0%.

Enterobacter spp. showed the highest susceptibility to Trimethoprim–sulfamethoxazole 90.9%, while the least susceptibility was observed with Cefazolin 0%.

Staphylococcus aureus demonstrated maximum susceptibility to Trimethoprim–sulfamethoxazole 95.8%, whereas the least susceptibility was seen with Cefoxitin 25.9%.

WHONET software includes a built-in function to generate bar & other charts showing the overall susceptibility of different isolates to various antimicrobials in a hospital or laboratory over a defined period. In the present study, the same analytical function of WHONET software was utilized to generate a bar graph depicting the overall susceptibility patterns of all 1st tier antimicrobials recommended in the CLSI M100 guidelines that were tested. The results are presented below as Figure No.1.

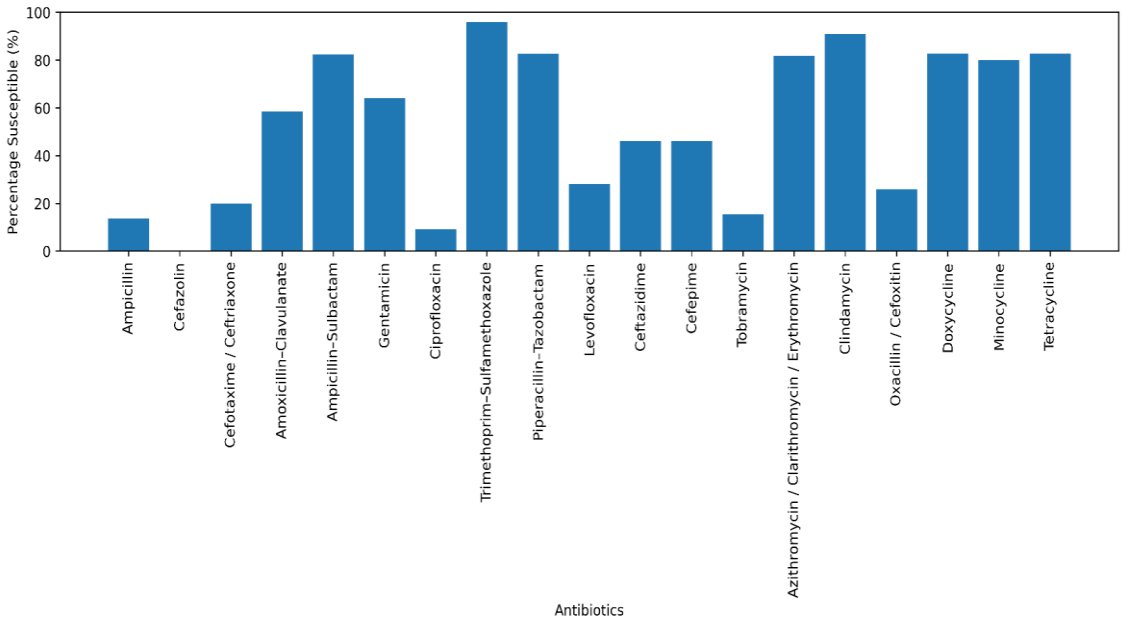

Figure 1: Bar Graph Showing Overall Susceptibility Pattern of First-Tier Antimicrobials as per CLSI M100 guidelines generated Using WHONET Software for pus isolates in the present study

DISCUSSION

Infections especially by bacteria create major health concern in outpatient departments as well as inpatient with its associated morbidity & mortality worldwide. Because of the time taken for performing traditional culture & sensitivity tests from clinical specimens, clinicians usually start antimicrobial treatment empirically. Antimicrobial Susceptibility Testing (AST) helps in decreasing the resistance of organisms. A bacterial isolate is termed as resistant when susceptibility rates fall below 80% (i.e., >20% resistance)

in a local antibiogram; for critical infections or high-risk patients, a stricter threshold of 90-95% susceptibility is often required, meaning resistance above 5-10% may make and antibiotic unsuitable for use (16).

Antimicrobial resistance (AMR) represents a major obstacle in the initiation of effective empirical antimicrobial therapy. AMR is primarily driven by two principal factors. *The first* is the use of antimicrobial agents, which exerts selective pressure by eliminating susceptible bacteria while allowing resistant organisms to survive and proliferate. *The second* is the presence of resistance genes within microorganisms. Resistance develops only when antimicrobial exposure occurs in organisms harbouring such resistance determinants, and continued antimicrobial use further facilitates the dissemination of resistant bacteria among hosts and across geographical regions **(10)**.

Bacterial resistance can be divided into two types. (I.) *Intrinsic resistance:* Antimicrobial resistance resulting from the normal genetic, structural, or physiologic state of a microorganism is referred to as intrinsic resistance. (II) *Acquired resistance:* Antibiotic resistance resulting from altered cellular physiology & structure caused by changes in a microorganism's genetic makeup is known as acquired resistance **(8)**. For any antimicrobial to be effective it must reach the site of infection, penetrate the bacterial cell & bind to specific cellular targets to inhibit growth or cause cell death. Their main modes of action include inhibition of cell wall synthesis, protein synthesis, nucleic acid synthesis, metabolic pathways &disruption of cell membrane. AMR possesses genetic determinants that allow survival under during exposure, & continued antimicrobial use promotes selection & spread of resistant strains (17).

A bacterial isolate is said to be resistant when susceptibility rates is lower than 80% (i.e. >20% resistance) in a local antibiogram. In critical infections or high-risk patients, a stricter threshold of 90–95% susceptibility is often required meaning resistance above 5–10% may make antibiotic unsuitable for use (16).

Skin & tissue infections leading to pus formation are important specimens received in a clinical microbiology laboratory. Accumulation of dead leucocytes and infectious agents commonly known as pus, results from infection by one of the pyogenic bacteria. The three main sources for infections are: *Environment* (exogenous microorganism in the air or those introduce by traumatic injury) *surrounding skin* and *endogenous sources* like mucous membrane of the gastrointestinal, Oropharyngeal and Genitourinary mucosa (18).

Bacterial isolates: -

In the present study, 264 out of 517 pus samples were culture positive, giving a yield of 51.06% as shown in table no.1. The yield rates of pus samples in present studies are compared with similar studies and shown below in table no. 04.

Table 4: - Comparison of yield in percentage of bacterial isolates in present study with other similar studies from pus samples

Study	Organisms	Total number of samples	Total Positive samples	Yield	Number of isolates	Yield of isolates
Jyoti Acharya et al. 2008 (19)	*Staphylococcus aureus*	3624	1838	50.70%	1051	51.52%
	Escherichia coli				492	24.12%
	Klebsiella pneumoniae				143	7%
	Pseudomonas aeruginosa				115	5.63%
	Enterococcus spp.				102	5%
	Acinetobacter calcoaceticus				56	2.74%
	Citrobacter spp.				38	1.86%
	Proteus spp.				18	0.88%
	Streptococcus spp.				11	0.54%
	Morganella morganii				7	0.34%

	Enterobacter spp.				5	0.25%
	Moraxella catarrahalis				1	0.05%
	Edwardsiella tarda				1	0.05%
	Salmonella Paratyphi A				1	0.05%
	Coagulase negative Staphylococcus				1	0.05%
Guta et al. 2014 (20)	*Staphylococcus aureus*	100	92	92%	45	25.40%
	Klebsiella spp.				32	18.1%
	Escherichia coli				30	16.9%
	Coagulase negative Staphylococcus				26	14.7%
	Pseudomonas aeruginosa				16	9%
	Proteus spp.				12	6.8%
	Streptococcus spp.				9	5.1%
	Citrobacter spp.				4	2.3%
	Enterobacter spp.				3	1.7%
Jaya Krishna Yakha et al. 2014 (21)	*Pseudomonas aeruginosa*	870	476	54.71%	106	22.26%
	Staphylococcus aureus				104	21.84%
	Escherichia coli				82	17.22%
	Acinetobacter spp.				49	10.29%
	Enterobacter spp.				47	9.87%
	Klebsiella spp.				45	9.45%
	Coagulase negative Staphylococcus				12	2.52%
	Non- Hemolytic Streotococci				12	2.52%
	Enterococcus spp.				6	1.26%
	Beta - Hemolytic streptococci				6	1.26%
	Proteus spp.				6	1.26%
	Citrobacter spp.				1	0.21%
Raghav Rao et al. 2014 (22)	*Staphylococcus aureus*	114	107	93.85	26	24.29%
	Pseudomonas aeruginosa				23	21.49%
	Escherichia coli				15	14.02%
	Klebsiella pneumoniae				13	12.15%
	Streptococcus pyogens				12	11.23%
	Staphylococcus epidermis				10	9.35%
	Proteus spp.				8	7.47%
Pandeya et al. 2018 (18)	*Staphylococcus aureus*	271	164	75.57%	53	32.31%
	Escherichia coli				34	20.73%
	Coagulase negative Staphylococcus				15	9.14%
	Klebsiella pneumoniae				15	9.14%
	Pseudomonas aeruginosa				10	6.09%
	Acinetobacter spp.				7	4.26%
	Citrobacter spp.				7	4.26%
	S. pyogens				4	2.43%
	P. spp				3	1.82%
	Enterococcus faecalis				12	7.31%
	K. oxytoca				2	1.2%
Rupinder Bakshi et al. 2024 (23)	*Escherichia coli*	5053	2126	42.07%	245	11.52%
	klebsiella pneumoniae				445	20.93%
	Pseudomonas aeruginosa				81	3.80%
	Acinetobacter Baumanii complex				335	15.75%
	Citrobacter spp.				13	0.61%
	Proteus spp.				14	0.65%
	Enterobacter spp.				9	0.42%
	Enterococcus spp.				337	15.85%
	Staphylococcus aureus				622	29.25%
	MRSA				25	1.17%

Present study	*Staphylococcus aureus*	517	264	51.06%	80	30.30%
	Escherichia coli				42	15.90%
	Pseudomonas aeruginosa				38	14.39%
	Klebsiella pneumoniae				37	14.01%
	Klebsiella spp.				35	13.25%
	Enterobacter spp.				32	12.12%

The yield of 51.06% in present study is comparable with Jyoti Acharya et al. (2008) (19) 50.70% & Jaya Krishna Yakha et al. (2014) (21) 54.71% as shown in table no.4. It is slightly lower than Pandeya et al. (2018) (18), who reported 60.50%. Higher culture positivity rates 92% were observed in Guta et al. (2014) (20) likely due to inclusion of clinically evident infected and surgical wound cases. Raghav Rao et al. (2014) (22) also has reported yield of 93.85% however, the authors have not explicitly mentioned the reasons of high culture positivity. In contrast, Rupinder Bakshi et al. (2024) (23) reported a slightly lower overall positivity rate of 42.07%.

In the present study, although Gram-negative organisms constituted 69.69% and Gram-positive isolates 30.30%, the single most predominant isolate was *Staphylococcus aureus* (30.30%). This is comparable with Jyoti Acharya et al. (2008) (19) 51.52%, Guta et al. (2014) (20) 25.40%, Raghav Rao et al. (2014) (22) 24.29%, and Pandeya et al. (2018) (18) 32.30%, where *S. aureus* was also the leading isolate.

Among Gram-negative organisms in the present study, the most common isolate was *Escherichia coli* 15.90%. This is comparable to Pandeya et al. (2018) (18), who reported *E. coli* 20.80% as the predominant Gram-negative isolate, and Jyoti Acharya et al. (2008) (19), where *E. coli* accounted for 24.12%. However, Jaya Krishna Yakha et al. (2014) (21) observed *Pseudomonas aeruginosa* 22.26% as the predominant Gram-negative organism, while Rupinder Bakshi et al. (2024) (23) reported higher prevalence of *Klebsiella pneumoniae* 20.93% among Gram-negative isolates.

The results of organisms isolated in present studies are consistent with the World Health Organization (WHO) Bacterial Priority Pathogens List 2024, which identifies *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, & *Pseudomonas aeruginosa* among the priority pathogens of global concern (24). The predominance of *Staphylococcus aureus* along with significant isolation of *E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* in the present study reflects the global importance of these organisms.

Antimicrobial Susceptibility:

In present study, the susceptibility for Gram-negative isolates were shown in Table No. 3.The susceptibility pattern of different isolates to various antimicrobial agents in present study is compared with various similar studies and is presented in table no.5.

Table 5: -Antibiogram from various studies compared with cumulative antibiograms of present study for pus sample

Study	organism	Ampicillin	Cefazolin	Cefotaxime or ceftriaxone	Amoxicillin – clavulanate	Ampicillin - sulbactam	Gentamicin	Ciprofloxacin	Trimethopirm - sulfamethoxazole	Piperacillin – Tazobactam	Levofloxacin	Ceftazidime	Cefepime	Tobramycin	Azithromycin or Clarithromycin or Erythromycin	Clindamycin	Oxacillin Cefoxitin (surrogate for Oxacillin)	Doxycycline	Minocycline	Tetracycline
Jyoti Acharya et al. 2008 (19)	*Staphylococcus aureus*	25.0	-	-	-	-	-	69.4	-	-	-	-	-	-	65.8	-	77.5	-	-	-
	Escherichia coli	27.9	-	52.3	-	-	69.5	49.6	-	-	-	-	-	-	-	-	-	-	-	-
	Pseudomonas aeruginosa	-	-	36.1	-	-	59.1	58.3	-	-	-	48.7	-	-	-	-	-	-	-	-
Guta et al. 2014 (20)	*Staphylococcus aureus*	-	-	64.4	-	-	80	-	-	-	-	-	-	-	-	-	-	-	-	-
	Klebsiella spp.	-	-	71.9	-	-	62.5	-	-	-	-	-	-	-	-	-	-	-	-	-
	Escherichia coli	-	-	66.7	-	-	100	-	-	-	-	-	-	-	-	-	-	-	-	-
	Coagulase negative Staphylococcus	-	-	50	-	-	50	-	-	-	-	-	-	-	-	-	-	-	-	-
	Pseudomonas aeruginosa	-	-	50	-	-	50	-	-	-	-	-	-	-	-	-	-	-	-	-
	Proteus spp.	-	-	33.3	-	-	50	-	-	-	-	-	-	-	-	-	-	-	-	-
	Streptococcus spp.	-	-	77.8	-	-	43.4	-	-	-	-	-	-	-	-	-	-	-	-	-
	Citrobacter spp.	-	-	50	-	-	100	-	-	-	-	-	-	-	-	-	-	-	-	-

	Enterobacter spp.	-	-	33.3	-	-	33.3	-	-	-	-	-	-	-	-	-	-	-	-
Jaya Krishna Yakha et al. 2014 (21)	*Staphylococcus aureus*	-		77.9	-	-	59.6	59.6	-	-	-	-	-	-	66.4	92.3	92.3	-	-	-
	Pseudomonas aeruginosa	-	-	-	-	-	46.2	48.1	-	47.2	-	26.4	-	-	-	-	-	-	-	-
	Escherichia coli	-	-	-	-	-	71.9	45.1	-	70.7	-	31.7	-	-	-	-	-	-	-	-
Raghav Rao et al 2014 (22)	*Staphylococcus aureus*	-	-	-	-	-	46.15	42.3	11.53	-	76.92	-	-	-	15.38	65.38	73.07	-	-	38.46
	Staphylococcus epidermis	-	-	-	-	-	40	60	50	-	90	-	-	-	30	70	80	-	-	40
	Streptococcus pyogens	-	-	-	-	-	66.66	58.33	75	-	83.33	-	-	-	33.33	50	66.66	-	-	41.66
	Escherichia coli	46.66	-	46.66	-	-	66.66	26.66	-	80	80	66.66	53.34	66.66	-	-	-	-	-	-
	Klebsiella spp.	46.15	-	46.15	-	-	61.53	30.76	-	76.92	76.92	61.53	46.15	61.53	-	-	-	-	-	-
	Proteus spp.	75	-	37	-	-	63	63	-	75	87	37	50	63	-	-	-	-	-	-
	Pseudomonas aeruginosa	52.17	-	39.13	-	-	-	-	-	86.95	-	39.13	52.17	-	-	-	-	-	-	-
Pandeya et al. 2017 (18)	*Escherichia coli*	-	-	29.4	-	-	67.6	17.6	26.5	50	-	-	11.8	-	-	-	-	-	-	14.7
	Pseudomonas aeruginosa	-	-	-	-	-	70	50	-	70	-	30	30	-	-	-	-	-	-	20
	Klebsiella pneumoniae	-	-	26.7	-	-	40	26.7	33.3	73.3	-	13.3	33.3	-	-	-	-	-	-	40
	S. aureus & CONS	-	-	-	-	-	92.5	39.6	32.1	-	-	-	-	-	37.3	-	-	-	-	52.8
	Enterococcus faecalis	66.7	-	-	-	-	66.7	41.7	-	-	-	-	-	-	50	-	-	-	-	100
Rupinder Bakshi et al. 2024 (23)	*Klebsiella pneumoniae*	-	27.69	-	27.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Acinetobacter baumanii		40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Escherichia coli		19.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Pseudomonas		6.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	aeruginosa																			
	Staphylococcus aureus	24.6	100	-	24.6	-	96	-	-	-	-	-	-	-	-	75.8	41.7	78.4	-	-
	Enterococcus faecalis	100	-	-	-	-	100	-	-	-	-	-	-	-	-	0	-	50	-	-
Present study	*Enterobacter spp.*	5.9	0	5.9	28	82.4	58.8	5.9	90.9	82.4	23.5	-	-	-	-	-	-	-	-	-
	Escherichia coli	13	0	0	33.4	65.2	43.5	4.3	73.7	82.6	0	-	-	-	-	-	-	-	-	-
	Klebsiella pneumoniae	0	0	7.1	58.6	56	64	4	70	64	28	-	-	-	-	-	-	-	-	-
	Klebsiella spp.	13.6	0	20	48.5	63.6	63.6	9.1	81.8	72.7	22.7	-	-	-	-	-	-	-	-	-
	Pseudomonas aeruginosa	-	-	-	-	-	-	7.7	-	46.2	23.1	46.2	46.2	15.4	-	-	-	-	-	-
	Staphylococcus aureus	-	-	-	-	-	-	-	95.8	-	-	-	-	-	81.8	90.9	25.9	82.8	80	82.8

Note: - (-) indicates Not tested.

For *E. coli*, Piperacillin-Tazobactam was 73.7%, which varies from other studies. Gentamicin was most effective for both Jyoti Acharya et al. (2008) **(19)** 69.5% & Guta et al. (2014) **(20)** 100%, while Raghav Rao et al. (2014) **(22)** reported highest susceptibility to Piperacillin-Tazobactam & Levofloxacin 80% each. Jaya Krishna Yakha et al. (2014) **(21)** 71.9% & Pandeya et al. (2018) **(18)** 67.6%, For Rupinder Bakshi et al. (2024) **(23)** Cefazolin 19.7% was only one antimicrobial tested out of the antibiotics tested in present study.

In the present study, *Escherichia coli* showed the least susceptibility to Cefazolin, Ceftriaxone, and Levofloxacin 0% each. In comparison, the least susceptibility in Jyoti Acharya et al. (2008) **(19)** was to Ampicillin 27.9%, in Guta et al. (2014) **(20)** to Ceftriaxone 66.7%, in Jaya Krishna Yakha et al. (2014) **(21)** to Ciprofloxacin 45.1%, in Raghav Rao et al. (2014) **(22)** to Ciprofloxacin 26.66%, in Pandeya et al. (2017) **(18)** to Cefepime 11.8%, and in Rupinder Bakshi et al. (2024) to Cefazolin 19.7%.

In present study, the most susceptible antimicrobial for *Klebsiella pneumoniae* was Trimethoprim-sulfamethoxazole (Cotrimoxazole) 70%. In comparison, study by Pandeya et al.(2018) **(18)** the most susceptibility was observed in Piperacillin-Tazobactam 73.3%. In the study by Rupinder Bakshi et al. (2024) **(23)** lower susceptibility was seen for Ampicillin 27.9%.

In the present study, *Klebsiella pneumoniae* showed the least susceptibility to Ampicillin and Cefazolin 0%. In comparison, the least susceptibility in Pandeya et al. (2017) **(18)** was to Ceftazidime 13.3%, in Raghav Rao et al. (2014) **(22)** to Ciprofloxacin 30.76%, and in Rupinder Bakshi et al. (2024) **(23)** to Cefazolin 27.69%.

In present study, *Klebsiella spp.* was also most susceptible to Trimethoprim-sulfamethoxazole 81%. When compared with other studies, Guta et al. (2014) **(20)** reported 76.92% susceptibility for Piperacillin-Tazobactam & Levofloxacin each as highest susceptibility while, the study by Raghav Rao et al. (2014) **(18)** reported highest susceptibility for Piperacillin-Tazobactam & Levofloxacin 76.92% for both of the antimicrobials.

In the present study, *Klebsiella spp.* showed the least susceptibility to Cefazolin 0%. In comparison, the least susceptibility in Guta et al. (2014) **(20)** was to Gentamicin 62.5%, and in Raghav Rao et al. (2014) **(22)** to Ciprofloxacin 30.76%.

For present study the most effective antimicrobial for *Pseudomonas aeruginosa* is Piperacillin- Tazobactam, Ceftazidime and Cefepime 46.2% each. This when compared with other according to Jyoti Acharya (2008) et al. **(19)** the most susceptible was Gentamicin 59.15%, in Guta (2014) et al. **(20)** the most susceptible was Ceftriaxone and Gentamicin 50% each, Jaya Krishna Yakha (2014) et al **(21)** the most susceptible antimicrobial was Ciprofloxacin 48.1%, in Raghav Rao (2014) et al. **(22)** it was Piperacillin- Tazobactam 86.95%, in Pandeya (2017) et al. **(18)** the most effective was antimicrobial was Gentamicin 70% and in Rupinder Bakshi (2024) et al the most susceptible was Cefazolin 6.9 %

In the present study, *Pseudomonas aeruginosa* demonstrated the lowest susceptibility to Ciprofloxacin (7.7%), When compared with other studies, higher susceptibility to Ciprofloxacin has been reported, such as 58.3% by Jyoti Acharya et al. (2008) **(19)**, 45.1% by Jaya Krishna Yakha et al. (2014) **(21)**, and around 50% by Pandeya et al. (2017) **(18)**, whereas more recent studies like Rupinder Bakshi et al. (2024) **(23)** have shown declining trends

In present study, for *Enterobacter spp.*, the most susceptible antimicrobial was Trimethoprim-sulfamethoxazole 90.9%. In comparison with study by Guta et al. (2014) (20), where 33.3% susceptibility was observed for Ceftriaxone & Gentamicin each.

In the present study, for *Staphylococcus aureus*, the most effective antimicrobial was Trimethoprim–Sulfamethoxazole 95.8% as

shown in Table no.3. In comparison, for *S. aureus*, Pandeya et al. (2018) (18) & Rupinder Bakshi et al. (2024) **(23)** reported Gentamicin as the most effective drug 92.5% and 96% respectively. Jaya Krishna Yakha et al. (2014) (21) observed highest susceptibility to Cefoxitin and Clindamycin 92.3%, while Jyoti Acharya et al. (2008) (19) reported better susceptibility to Cefoxitin 77.5%.

In the present study, for *Staphylococcus aureus*, the least effective antimicrobial was Cefoxitin 25.9%, indicating reduced susceptibility among isolates. In comparison, Jyoti Acharya et al. (2008) (19) reported low susceptibility to Ampicillin 25.8%, while Guta et al. (2014) (20) observed reduced susceptibility to Gentamicin 20% in *S. aureus*. Raghav Rao et al. (2014) (22) documented poor response to Erythromycin 15.38%, and Pandeya et al. (2018) (18) also reported lower susceptibility to macrolides 37.3%.

As stated in the study of Magiorakos et al. **(15)** *MDR*: Non-susceptible to ≥1 agent in ≥3 antimicrobial classes, *XDR*: Non-susceptible to ≥1 agent in all but ≤ 2 classes, *PDR*: Non-susceptible to all agents in all classes tested.

Based on this approach, *Escherichia coli* showed an extensive level of drug resistance *(XDR),* as it remained susceptible to only one antibiotic, Piperacillin–Tazobactam, while exhibiting resistance to most of the other tested drugs.

Both *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* demonstrated resistance to all the antimicrobial agents included in the study and were therefore categorized as pan drug resistant *(PDR)* organisms. On the other hand, *Enterobacter spp.* and *Klebsiella spp.* were found to be resistant to multiple classes of antibiotics but still retained susceptibility to a few agents, and hence were classified as multidrug resistant *(MDR).*

In the present study, *Staphylococcus aureus* showed low susceptibility to Cefoxitin 25.9%. Cefoxitin is used as a surrogate marker for detecting methicillin resistance **(12)** therefore, reduced susceptibility suggests the presence of Methicillin-Resistant *Staphylococcus aureus (MRSA)* among the isolates.

The primary mechanism for this resistance is the production of an altered penicillin-binding protein (i.e., PBP 2a), which renders all currently available beta-lactams essentially ineffective. Strains that carry the *mecA* gene, which encodes for PBP 2a, are referred to as MRSA **(8)**.

The reduced Cefoxitin susceptibility observed in the present study indicates a possible burden of MRSA in the study setting, highlighting the importance of continuous surveillance and infection control measures.

For clinical purposes, an isolate of *S. aureus* should be regarded as either sensitive or resistant to methicillin regardless of the underlying mechanism. In addition, it is important to appreciate that methicillin resistance implies resistance to all beta-lactam antibiotics, including the cephalosporins, even though disc sensitivity tests may show zones of inhibition **(10)**.

According to the World Health Organization (WHO) Bacterial Priority Pathogens List 2024, pathogens are categorized into critical, high, and medium priority groups based on their public health impact and resistance burden. In present study, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are placed under the critical priority group, while *Staphylococcus aureus* is included in the high priority group (24).

Among Gram-negative isolates (*Enterobacter spp., Escherichia coli, Klebsiella pneumoniae, Klebsiella spp.*), resistance to antimicrobial classes, particularly β-lactams & fluoroquinolones was observed. *Pseudomonas aeruginosa* demonstrated low susceptibility to antipseudomonal agents such fluoroquinolone & aminoglycoside activity.

In the present study, reduced to routinely used antimicrobials susceptibility observed among *Enterobacterales* and *Pseudomonas aeruginosa*, along with evidence of

methicillin resistance in *Staphylococcus aureus*, reflects this global resistance burden. These findings highlight the importance of continuous local surveillance, regular cumulative antibiogram preparation, and rational antimicrobial use to address the growing threat of antimicrobial resistance.

The differences in AST patterns in bacterial isolates in present study as compared with similar studies reinforces the need for antibiograms to be prepared by local microbiology departments which can guide the empirical treatment & help reduce the development of AMR

Limitations of present study

 This study lacks separated sampling from distinct hospital departments which may affect the Applicability of ward-level antimicrobial resistance surveillance.

 In present study, since samples were not limited to specific clinical entities such as post-operative infections, burns, or trauma-related wounds, disease-specific resistance patterns could not be independently evaluated

CONCLUSION

The four-year cumulative antibiogram of the present study from Navsari, South Gujarat analyses pus isolates using WHONET 5.6 (2025) in accordance with CLSI M39 guidelines. A culture positivity rate of 51.06% was observed, with Gram-negative organisms like *E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Klebsiella spp. & Enterobacter spp.* predominating, but individually Gram-positive *Staphylococcus aureus* was the most common isolated from pus sample followed by Gram-negative *Escherichia coli* and *Klebsiella spp*.

The present study shows a concerning level of antimicrobial resistance among the bacterial isolates. A significant proportion of isolates showed resistance to commonly used antibiotics, indicating limited treatment options.

Escherichia coli demonstrated extensive drug resistance, remaining susceptible to only a single effective agent. Both *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* exhibited pan drug resistance, showing resistance to all the antimicrobial agents tested.

Enterobacter spp. and *Klebsiella spp.* were found to be multidrug resistant, as they were resistant to several classes of antibiotics but still retained susceptibility to a few drugs.

In addition, *Staphylococcus aureus* isolates were identified as methicillin-resistant (*MRSA*), indicating resistance to beta-lactam antibiotics.

These findings highlight the growing burden of antimicrobial resistance in the region and emphasize the importance of regular cumulative antibiograms to guide rational empirical therapy and strengthen antimicrobial stewardship practices. In this context, the microbiology laboratory plays a vital role in precise pathogen identification, performing standardized antimicrobial susceptibility testing, detecting resistance patterns, and providing timely cumulative antibiogram reports, thereby supporting evidence-based treatment decisions and enhancing infection control and antimicrobial resistance surveillance.

Declaration by Authors

Ethical Approval: This study involved retrospective observational analysis of anonymised laboratory data without patient identifiers or direct patient interaction. As no intervention was involved, ethical committee approval was not required. The study adhered to the principles of the Declaration of Helsinki.

Acknowledgement: None

Source of Funding: None

Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

1. Sastry AS, Priyadarshi K, Deepashree R. *Essentials of antimicrobial stewardship*. 1st ed. New Delhi: Jaypee Brothers Medical Publishers; 2023.
2. World Health Organization. Global action plan on antimicrobial resistance [Internet]. Geneva: WHO; 2015 [cited 2026 Jan 30].

Available from: https://www.who.int/publications/i/item/9789241509763
3. Spellberg B, Bartlett JG, Gilbert DN. The future of antibiotics and resistance. N Engl J Med. 2013;368(4):299–302. doi:10.1056/NEJMp1215093
4. Hindler JF, Stelling J. Analysis and presentation of cumulative antibiograms: a new consensus guideline from the Clinical and Laboratory Standards Institute. Clin Infect Dis. 2007 Mar 15;44(6):867-73. doi: 10.1086/511864.
5. Gujarat State Antimicrobial Surveillance Network (GUJSAR). Semi-annual report (1 July–31 December 2024). Gandhinagar: GUJSAR; 2024.
6. Agarwal A, Kapila K, Kumar S. WHONET Software for the Surveillance of Antimicrobial Susceptibility. Med J Armed Forces India. 2009 Jul;65(3):264-6. doi: 10.1016/S0377-1237(09)80020-8.
7. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC. *Basic laboratory procedures in clinical bacteriology*. 2nd ed. Geneva: World Health Organization; 2003.
8. Tille PM. *Bailey & Scott's diagnostic microbiology*. 16th ed. St. Louis (MO): Elsevier; 2025.
9. Procop GW, Church DL, Hall GS, Janda WM. *Koneman's color atlas and textbook of diagnostic microbiology*. 7th ed. Burlington (MA): Jones & Bartlett Learning; 2020.
10. Collee JG, Fraser AG, Marmion BP, Simmons A. *Mackie & McCartney's practical medical microbiology*. 14th ed. New York: Churchill Livingstone; 1996.
11. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests. 14th ed. CLSI standard M02. Wayne (PA): CLSI; 2024.
12. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 35th ed. CLSI supplement M100. Wayne (PA): CLSI; 2025.
13. Clinical and Laboratory Standards Institute. Analysis and presentation of cumulative antimicrobial susceptibility test data (CLSI guideline M39). 5th ed. Wayne (PA): CLSI; 2022.
14. World Health Organization. WHONET: download, installation & user manuals [Internet]. Geneva: WHO; 2022 [cited 2026 Jan 30]. https://whonet.org/WebDocs/WHONET_2.Download_Installation_User_manuals.pdf
15. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012 Mar;18(3):268-81. doi: 10.1111/j.1469-0691.2011.03570. x.
16. ID Stewardship. Five important things to know about hospital antibiograms [Internet]. 2018 [cited 2026 Jan 30]. Available from: https://www.idstewardship.com/five-important-things-know-hospital-antibiograms/
17. Khameneh B, Diab R, Ghazvini K, Fazly Bazzaz BS. Breakthroughs in bacterial resistance mechanisms and the potential ways to combat them. Microb Pathog. 2016 Jun; 95:32-42. doi: 10.1016/j.micpath.2016.02.009.
18. Pandeya U, Raut M, Bhattarai S, Bhatt PR, Dahal PR. Bacteriological Profile and Antibiogram of Bacterial Isolates from Pus Samples in Tertiary Care Hospital of Kathmandu. TU J. Microbiol. [Internet]. 2018 Nov. 16 [cited 2026 Apr. 16];4:55-62. Available from: https://www.nepjol.info/index.php/tujm/article/view/21678
19. Acharya J, Mishra SK, Kattel HP, Rijal B, Pokhrel BM. Bacteriology of wound infection among patients attending a teaching hospital, Kathmandu, Nepal. J Nepal Assoc Med Lab Sci. 2008;9(1):76–81.
20. Guta M, Aragaw K, Merid Y. Bacteria from infected surgical wounds and their antimicrobial resistance in Hawassa University Referral Teaching Hospital, southern Ethiopia. Afr J Microbiol Res. 2014;8(11):1118–24. https://doi.org/10.5897/AJMR2013.6544
21. Yakha JK, Sharma AR, Dahal N, Lekhak B, Banjara MR. Antibiotic Susceptibility Pattern of Bacterial Isolates Causing Wound Infection Among the Patients Visiting B & B Hospital. Nepal Journal of Science and Technology [Internet]. 2015 Feb. 16 [cited

2026 Apr. 16];15(2):91-6. Available from: https://www.nepjol.info/index.php/NJST/article/view/12121

22. Raghav Rao DVMVSV, Basu R, Biswas DR. Aerobic bacterial profile and antimicrobial susceptibility pattern of pus isolates in a South Indian tertiary care hospital. IOSR J Dent Med Sci. 2014;13(3):59–62.
23. Bakshi R, Kaur K, Sharma P. Antibiogram and bacterial profile analysis of various clinical samples at a tertiary healthcare centre in the north-west region of Punjab. Int J Health Sci Res. 2024;14(12):180–90. DOI: https://doi.org/10.52403/ijhsr.20241221
24. Sati H, Carrara E, Savoldi A, Hansen P, Garlasco J, Campagnaro E, et al. The WHO bacterial priority pathogens list 2024: a prioritisation study to guide research, development, and public health strategies against antimicrobial resistance. Lancet Infect Dis. 2025;25(9):1033–43.

How to cite this article: Manoj A Kahar, Prashant Kumar Jagubhai Patel, Dhvani Y Kahar, Nafisa Patel. Cumulative antibiogram and antimicrobial susceptibility patterns of pus isolates using WHONET 5.6 2025 in a microbiology department of clinical laboratory in Navsari, South Gujarat. *Int J Health Sci Res.* 2026; 16(4):183-199. DOI: *https://doi.org/10.52403/ijhsr.20260423*

******