Section: Microbiology

Original Research Article

PREVALENCE AND ANTIMICROBIAL RESISTANCE PATTERN OF ESKAPE PATHOGENS ISOLATED FROM PUS SAMPLES IN TERTIARY CARE HOSPITAL, WESTERN GUJARAT, INDIA

Ekta Anilbhai Gondalia[1], Hiral M. Gadhavi[2], Hitesh K. Shingala[3], Krunal D. Mehta[4]

[1]3rd Year Resident Doctor, Department of Microbiology, Shree M.P. Shah Government Medical College, Jamnagar, India.
[2]Associate Professor, Department of Microbiology, Shree M.P. Shah Government Medical College, Jamnagar, India.
[3]Professor and Head, Department of Microbiology, Shree M.P. Shah Government Medical College, Jamnagar, India.
[4]Associate Professor, Department of Microbiology, Shree M.P. Shah Government Medical College, Jamnagar, India.

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Corresponding Author:
Dr. Krunal D. Mehta,
Associate Professor, Department of Microbiology, Shree M.P. Shah Government Medical College, Jamnagar, India.
Email: krunalm502@gmail.com

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ABSTRACT

Background: Antimicrobial resistance among ESKAPE pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.) represents a critical global health threat. Region-specific surveillance data from western Gujarat, India, remain scarce, particularly from pus specimens in tertiary care settings.

Materials and Methods: An observational study was conducted over 12 months (January–December 2025) in the Department of Microbiology, Western Gujarat, India. A total of 1,498 culture-positive pus specimens were included. ESKAPE pathogens were identified using standard microbiological procedures. Antimicrobial susceptibility testing (AST) was performed by the Kirby–Bauer disk-diffusion method on Mueller–Hinton agar, interpreted per CLSI 2025 breakpoints. MDR, XDR, and PDR were defined as per the Magiorakos et al. standardised definitions.

Results: Of 1,498 culture-positive pus specimens, 1,190 (79.4%) yielded ESKAPE pathogens. Pseudomonas aeruginosa was the most prevalent (35.0%), followed by Klebsiella pneumoniae (27.1%), Staphylococcus aureus (26.7%), and Acinetobacter baumannii (10.8%), Enterococcus faecium (4; 0.3%), and Enterobacter species (2; 0.2%). Among Staphylococcus aureus MRSA prevalence was 20.1%. ESBL production was detected in 75.5% of K. pneumoniae isolates, with carbapenem resistance in 15.5%. A. baumannii demonstrated 84.4% carbapenem resistance, with colistin retaining full susceptibility. Overall, 53.4% of isolates were MDR, 20.8% XDR, and no PDR isolates were detected. Gram-negative organisms collectively constituted 73.0% of all ESKAPE isolates.

Conclusion: This study demonstrates a high burden of MDR and XDR ESKAPE pathogens in pus specimens from a tertiary care hospital in western Gujarat. Carbapenem-resistant A. baumannii and ESBL-producing K. pneumoniae pose the greatest therapeutic challenge. These findings underscore the urgent need for robust antimicrobial stewardship programmes, evidence-based local antibiogram development, and stringent infection control measures.

Keywords: ESKAPE pathogens; pus specimens; antimicrobial resistance; MDR; MRSA.

INTRODUCTION

Antimicrobial-resistant bacteria constitute a major public health burden, accounting for approximately 1.27 million deaths annually worldwide directly attributable to antimicrobial-resistant bacteria, with a further 4.95 million deaths associated with bacterial resistance.[1] The World Health Organisation (WHO)

has placed six members of the ESKAPE group — Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp. — on its global priority pathogen list for which the development of new antimicrobial therapies is deemed critical.[2]

Pus and wound specimens represent one of the most common clinical specimens submitted to microbiology laboratories in tertiary care hospitals. Soft-tissue and wound infections caused by MDR ESKAPE pathogens are associated with prolonged hospitalisation, increased morbidity, treatment failure, and significant mortality.[3] The Gram-negative members of the group — particularly carbapenem-resistant A. baumannii (CRAB) and ESBL-producing K. pneumoniae — exploit outer membrane impermeability and enzymatic resistance mechanisms, rendering most available beta-lactam antibiotics ineffective.[4,5]

India is recognised as a global hotspot for antimicrobial resistance. The Indian Council of Medical Research (ICMR) AMR Surveillance Network has documented alarming increases in carbapenem-resistant Gram-negative organisms; however, region-specific data from western India, particularly Gujarat, remain sparse.[6] The National Action Plan on AMR (2017) emphasises the necessity of institution-level surveillance to enable targeted empirical therapy and support stewardship initiatives.[7]

Understanding the local ESKAPE pathogen burden and their resistance profiles from pus specimens is essential for establishing an evidence-based local antibiogram, guiding empirical antibiotic prescriptions, and informing infection control policies.

The present study, therefore, aims to determine the prevalence of ESKAPE pathogens, their antimicrobial susceptibility profile and burden of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) isolates in a tertiary care hospital in Western Gujarat, India.

MATERIALS AND METHODS

Study Design and Setting: An observational retrospective study was conducted in the Department of Microbiology, Western Gujarat, India. The study period spanned 12 months from January 2025 to December 2025.

Sample Size and Study Population: The study included 1,498 pus specimens that tested positive on culture from both inpatients and outpatients of all ages and genders during the study period. Of these, 1,190 isolates were identified as ESKAPE pathogens and included in the final analysis.

Inclusion and Exclusion Criteria

Inclusion criteria

Culture-positive isolates from pus specimens; samples from both in-patients and out-patients of all ages and genders; isolates with complete AST data.

Exclusion criteria

Isolates from culture-negative samples.

Sample Processing and Bacterial Identification

All pus specimens were processed according to standard microbiological procedures. Specimens were inoculated on Blood Agar and MacConkey Agar and incubated at 37°C for 24-48 hours. Colony morphology, Gram staining, and biochemical tests were employed for identification. Microsoft Excel was used for data entry and collation.

Antimicrobial Susceptibility Testing

AST was performed by the Kirby–Bauer disk-diffusion method on Mueller–Hinton agar.[8] Results were interpreted according to CLSI 2025 breakpoints.[9] Quality control strains used were Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853. Methicillin resistance in S. aureus (MRSA) was determined by the cefoxitin disk screen method [Figure 1]; vancomycin resistance in E. faecium (VRE) was detected by phenotypic method by disc diffusion; and colistin susceptibility was determined by broth micro dilution MIC, as disk diffusion is unreliable for this agent.[10]

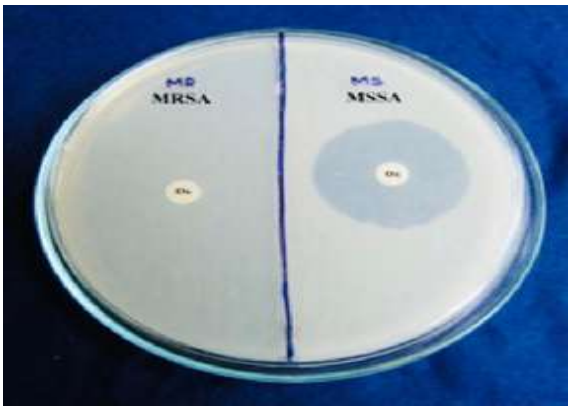

Figure 1: MRSA & MSSA by the cefoxitin screen method

Resistance Definitions

Multi-drug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antimicrobial categories. Extensive drug resistance (XDR) was defined as non-susceptibility to all but two or fewer antimicrobial categories. Pan-drug resistance (PDR) was defined as non-susceptibility to all available antimicrobial agents, as per the Magiorakos et al. standardised definitions.[11]

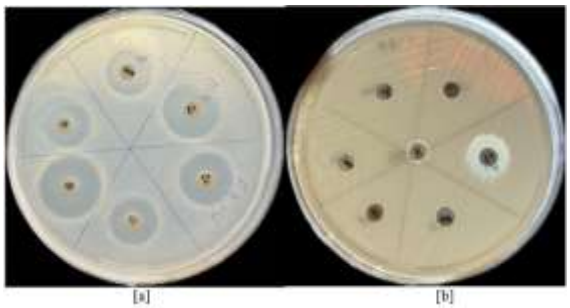

Figure 2: Drug Resistance Pattern-
2a: Organism Sensitive to all the drug categories,
2b: Organism sensitive to only one drug category (XDR)

Ethical Approval: All procedures in the current study were approved by the institute's ethics committee.

Statistical Analysis: The data were entered into an MS Excel spreadsheet. Data were summarised as frequencies and percentages.

RESULTS

Out of 1,498 culture-positive pus specimens received during the study period (January–December 2025), 1,190 yielded ESKAPE pathogens, which shows a prevalence of 79.4%.

Table 1: Prevalence of ESKAPE Pathogens

Total Positive Sample from Pus	ESKAPE Pathogen
1498	1190 (79.4%)

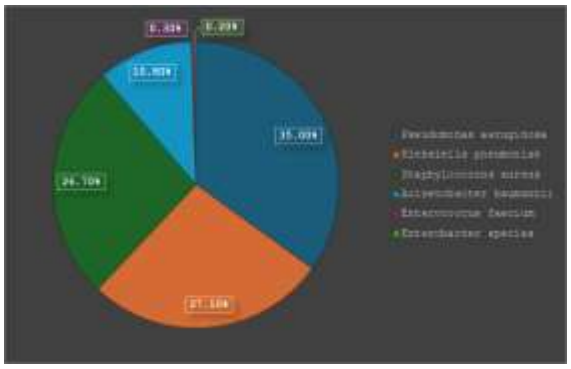

Figure 3: Distribution of ESKAPE Pathogens Isolated from Pus Specimens (January–December 2025)

Pseudomonas aeruginosa was the most common ESKAPE pathogen, accounting for 416 isolates (35.0%), followed by Klebsiella pneumoniae (322; 27.1%), Staphylococcus aureus (318; 26.7%), Acinetobacter baumannii (128; 10.8%), Enterococcus faecium (4; 0.3%), and Enterobacter species (2; 0.2%). Gram-negative organisms collectively constituted 73.0% of all ESKAPE isolates. [Figure 1]

Antimicrobial Susceptibility of Staphylococcus aureus: Of 318 S. aureus isolates, 64 (20.1%) were methicillin-resistant (MRSA) by phenotypic detection using the cefoxitin disk. High resistance was observed to macrolides (azithromycin, 42.9%), fluoroquinolones (ciprofloxacin, 38.4%), and gentamicin (23.6%), while linezolid and minocycline retained strong activity with resistance rates of only 6.6% and 0.9%, respectively.

Antimicrobial Susceptibility of Klebsiella pneumonia: Among 322 K. pneumoniae isolates, ESBL production was detected in 261 (75.5%), with high resistance to cephalosporins (ceftriaxone 76.7%, ceftazidime 74.2%), piperacillin/tazobactam (70.2%), fluoroquinolones (~52%), and trimethoprim-sulfamethoxazole (61.2%). Carbapenem resistance was noted in 15.5% of isolates, while 40.1% showed resistance to ceftazidime/avibactam, raising concern about the utility of this novel combination. Colistin remained fully active with 0% resistance among tested isolates.

Antimicrobial Susceptibility of Acinetobacter baumannii: All 128 A. baumannii isolates exhibited a highly resistant phenotype, complete resistance to ceftazidime (100%), resistance to carbapenems (84.4%), fluoroquinolones (~90%), and Trimethoprim-sulfamethoxazole (96.1%). Minocycline demonstrated relatively better activity with only 27.3% resistance, suggesting its potential role in combination therapy.

Antimicrobial Susceptibility of Pseudomonas aeruginosa: Among 416 P. aeruginosa isolates, notable resistance was observed to ceftazidime (49.8%), cefepime (47.0%), fluoroquinolones (~41%), and piperacillin/tazobactam (36.6%), while carbapenem resistance remained relatively lower at 17.3%. Resistance to ceftazidime/avibactam was only 19.3%, suggesting its potential therapeutic utility.

Enterococcus faecium: 4 E. faecium isolates were recovered. All were susceptible to vancomycin (100%) and linezolid (100%). Ciprofloxacin resistance was 25%, while 100% resistance was noted for chloramphenicol in the single isolate tested.

Enterobacter species: 2 E. cloacae isolates exhibited complete resistance to all beta-lactam antibiotics tested, including all cephalosporins and aztreonam. Both were susceptible to carbapenems, amikacin, gentamicin, ciprofloxacin, and levofloxacin.

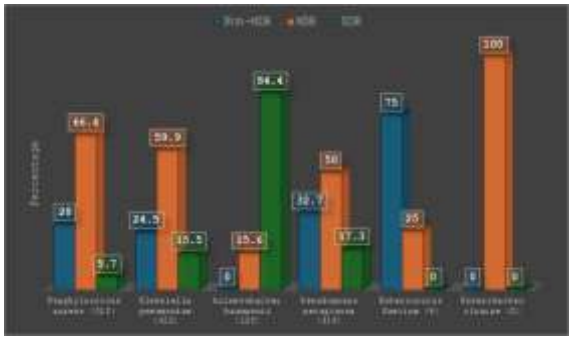

Figure 4: MDR / XDR Distribution Among ESKAPE Pathogens Isolated from Pus Specimens (n = 1,190).

Table 2: MDR / XDR Distribution Among ESKAPE Pathogens

Organism	Total Isolates (N)	Non-MDR n (%)	MDR n (%)	XDR n (%)	PDR n (%)
Staphylococcus aureus	318	89 (28.0%)	211 (66.4%)	18 (5.7%)	0 (0.0%)
Klebsiella pneumoniae	322	79 (24.5%)	193 (59.9%)	50 (15.5%)	0 (0.0%)

Acinetobacter baumannii	128	0 (0.0%)	20 (15.6%)	108 (84.4%)	0 (0.0%)
Pseudomonas aeruginosa	416	136 (32.7%)	208 (50.0%)	72 (17.3%)	0 (0.0%)
Enterococcus faecium	4	3 (75.0%)	1 (25.0%)	0 (0.0%)	0 (0.0%)
Enterobacter cloacae	2	0 (0.0%)	2 (100.0%)	0 (0.0%)	0 (0.0%)
Total	1190	307 (25.8%)	635 (53.4%)	248 (20.8%)	0 (0.0%)

The antimicrobial resistance profile of 1,190 isolates shows a high burden of multidrug resistance, with 53.4% (635 out of 1190) MDR and 20.8% (248 out of 1190) XDR, while only 25.8% (307 out of 1190) are non-MDR, and no PDR isolates were identified. The burden of XDR is largely driven by Acinetobacter baumannii (84.4%) and Pseudomonas aeruginosa (17.3%). The burden of MDR is largely driven by Staphylococcus aureus (66.4%) and Klebsiella pneumoniae (59.9%) [Figure 2]

Table 3a: Antimicrobial Category-wise Resistance Pattern (% Resistance) per ESKAPE Gram Positive Organisms

Antimicrobial Category	S. aureus (n=318)	E. faecium (n=4)
Aminoglycosides (Gentamicin 10 µg : S.aureus, Gentamicin 120 µg: E. faecium , Amikacin)	23.6%	50.0%
Fluoroquinolones (Ciprofloxacin, Levofloxacin)	38.4%	25.0%
Macrolides (Azithromycin)	42.9%	NA
Clindamycin	11.0%	NA
Tetracyclines	5.0%	0%
Oxazolidinones (Linezolid)	6.6%	0%
Nitrofurans (Nitrofurantoin)	11.1%	0%
Vancomycin	0%	0%

Table 3b: Antimicrobial Category-wise Resistance Pattern (% Resistance) per ESKAPE Gram Negative Organisms

Antimicrobial Category	K. pneumoniae (n=322)	A. baumannii (n=128)	P. aeruginosa (n=416)	E. species (n=2)
β-Lactam / β-Lactamase Inhibitors (Ceftazidime Avibactam)	80.7%	100%	19.3%	100%
Antipseudomonal Penicillin + BLI (piperacillin + Tazobactam)	70.2%	96.0%	36.6%	100%
Cephalosporins	77.6%	100%	49.8%	100%
Monobactams (Aztreonam)	71.6%	100%	27.2%	100%
Carbapenems (Imipenem, Meropenem, Ertapenem)	15.5%	84.4%	17.3%	0%
Aminoglycosides (Amikacin, Gentamicin)	38.2%	72.7%	58.3%	0%
Fluoroquinolones (Ciprofloxacin, Levofloxacin)	52.2%	90.6%	41.1%	0%
Tetracyclines	50.9%	100%	NA	50.0%
Colistin	0%	-	0%	0%

The antimicrobial category-wise resistance profile demonstrates markedly high resistance across most organism–drug combinations. The findings indicate a severely limited antimicrobial available, particularly against A. baumannii and K. pneumoniae. [Table 3a, 3b]

DISCUSSION

This study from a tertiary care hospital in western Gujarat provides important local data on the prevalence and antimicrobial resistance patterns of ESKAPE pathogens isolated from pus specimens over a 12-month period. The recovery of 1,190 ESKAPE isolates from 1,498 culture-positive pus samples (79.4%) highlights the dominant role of these organisms in wound and soft-tissue infections at our institution, consistent with the study by Bazira et al. (2025),[12] and Benkő et al. (2020),[13] having ESKAPE prevalence of 84.1% and 72.22%, respectively.

Study	Prevalence of ESKAPE pathogen
Present Study	79.4
Bazira et al. (2025),[12]	84.1
Benkő et al. (2020),[13]	72.22

P. aeruginosa constituted the most prevalent ESKAPE pathogen in this study (35.0%), a rate appreciably higher than the 25.38% reported by Pasha et al. (2024),[14] from a South Indian tertiary centre. This comparatively higher prevalence likely reflects the complex wound profile and case-mix of our tertiary referral centre in western Gujarat, combined with the intrinsic resistance characteristics and environmental persistence of the organism.

K. pneumoniae accounted for 27.1% of ESKAPE isolates in the present study, consistent with the 18.7% reported by Singh et al. (2025),[15] from a postoperative wound infection cohort in India. ESBL production was detected in 75.5% of K. pneumoniae isolates, comparable to the 73.1% reported by Ejaz et al. (2024).[16] Carbapenem resistance (15.5%) was similar to the 19.6% documented by Alotaibi et al. (2026),[18] from a Middle Eastern tertiary centre. The variation in prevalence across Indian centres likely reflects differences in ward distribution, patient case-mix, and local antimicrobial selection difference; the high ESBL burden combined with an escalating

carbapenem resistance rate underscores the urgent need for stewardship intervention before further resistance progression occurs.

Study	ESBL Production in K. pneumonia
Present Study	75.5
Ejaz et al. (2024),[16]	73.1
Study	**Carbapenem Resistance K. pneumoniae**
Present Study	15.5
Alotaibi et al. (2026),[18]	19.6

A. baumannii constituted 10.8% of ESKAPE isolates, comparable to the 13% reported by Muhinda et al. (2025),[19] from a Rwandan tertiary hospital. Carbapenem resistance in our study (84.4%) was consistent with the 82.5% documented by Susanna et al. (2026).[20] Colistin remains the last-resort agent for CRAB and showed 100% susceptibility. Minocycline demonstrated activity against 53.1% of isolates, making it a potential component of combination regimens for XDR A. baumannii infections.

Study	Carbapenem Resistance in A. baumannii
Present Study	84.4
Susanna et al. (2026),[20]	82.5

S. aureus accounted for 26.7% of ESKAPE isolates in the present study, closely mirroring the 26.92% reported by Muhinda et al. (2025),[19] from Rwanda. The MRSA prevalence of 20.1% in the present study is consistent with the 19.6% reported by Benkő et al. (2020).[13] Linezolid retained strong activity (93.4% susceptibility), and all isolates were presumptively vancomycin-susceptible.

Study	S. aureus
Present Study	26.7
Muhinda et al. (2023),[19]	26.92
Study	**MRSA**
Present Study	20.1
Benkő et al. (2020),[13]	19.6

Among K. pneumoniae isolates, the MDR rate in our study was 59.9%, consistent with the 45.7% MDR documented by Padmaja et al,[21] (2025) from an Indian ICU-based study.

For P. aeruginosa, MDR was 50.0% in our study, similar to 44.0% by Padmaja et al,[21] (2025) while the XDR rate of 17.3% was consistent with the 15.53% reported by Mirzaei et al. (2020).[22]

A. baumannii demonstrated 15.6% MDR with 84.4% XDR in our study, similar to 25.9% MDR by Padmaja et al,[21] (2025) and 73.13% XDR reported by Mirzaei et al. (2020),[22] from northeast Iran. The predominance of XDR over MDR in A. baumannii in our study reflects the near-pan-resistant phenotype of this organism in our setting, where only colistin and minocycline retained meaningful activity.

Study	MDR in K. pneumoniae
Present Study	59.9
Padmaja et al. (2025),[21]	45.7
Study	**MDR in Pseudomonas aeruginosa**
Present Study	50
Padmaja et al. (2025),[21]	44
Study	**XDR in Pseudomonas aeruginosa**
Present Study	17.3
Mirzaei et al (2020),[22]	15.53
Study	**MDR in A. baumannii**
Present Study	15.6
Padmaja et al. (2025),[21]	25.9
Study	**XDR in A. baumannii**
Present Study	84.4
Mirzaei et al. (2020),[22]	73.13

The dominance of Gram-negative ESKAPE pathogens (73.0%) in pus specimens from our centre reflects the broader Indian epidemiological trend and underscores the need for rational use of carbapenems and last-resort agents such as colistin, polymyxin B, and tigecycline. The WHO's ESKAPE priority list was created precisely because of the critical nature of these organisms in hospital infections.[2] Our findings align with national AMR surveillance data showing increasing MDR organisms, reinforcing the call for robust antibiotic stewardship programmes (ASPs) at the institutional level.

Strengths: This study provides one of the first dedicated analyses of ESKAPE pathogen profiles from pus specimens specifically in western Gujarat, filling an important regional data gap. The large sample size (1,190 ESKAPE isolates from 1,498 culture-positive specimens) and comprehensive AST covering a broad antibiotic panel strengthen the validity of the resistance profiles generated. Data capture over a full 12-month calendar period mitigates seasonal bias. All isolates were tested using the standardised CLSI 2025 guidelines, ensuring comparability with national and international studies.
Limitations: As a single-centre retrospective study, the findings may not be fully generalisable to other regions of Gujarat or India. The study was restricted to pus specimens, so ESKAPE resistance patterns may differ in other specimen types such as blood, urine, or respiratory samples. MDR/XDR/PDR classification was based on available antibiotic panels and may be an underestimate where full panel testing was not performed. The study did not include molecular characterisation of resistance genes (e.g., OXA carbapenemases, NDM, KPC, mecA) or patient-level epidemiological data such as prior antibiotic exposure, co-morbidities, or hospital ward distribution, which limits mechanistic inference. Additionally, colistin susceptibility testing by the disk-diffusion method for Pseudomonas is unreliable; BMD confirmation was performed only for a limited subset of isolates.

CONCLUSION

This observational study demonstrates a high burden of MDR ESKAPE pathogens from pus specimens at a tertiary care centre in western Gujarat, India. Pseudomonas aeruginosa and Klebsiella pneumoniae were the most prevalent organisms. ESBL production was dominant among Gram-negative ESKAPE members, and carbapenem-resistant A. baumannii represents a critical threat, with colistin and minocycline remaining as limited therapeutic options. The MRSA rate of 20.1% warrants continued surveillance and strict infection control. These findings provide an evidence base for institutional antibiogram development, empirical antibiotic protocol revision, and targeted antimicrobial stewardship. Multicentre prospective studies with molecular epidemiology are essential to fully characterise the AMR landscape in western India.

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