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Microbiological Characterization and Antibiotic Resistance Profile of Carbapenem-Resistant *Klebsiella pneumoniae* and Other Gram-Negative Pathogens Isolated from Clinical Samples in Pune, India

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Abstract:

Carbapenem-resistant Gram-negative bacteria, particularly carbapenem-resistant Klebsiella pneumoniae (CRKP), have emerged as major causes of healthcare-associated infections and are associated with high morbidity and mortality. The present study aimed to isolate, identify and characterize Gram-negative bacterial pathogens from clinical specimens and to determine their biochemical characteristics and antimicrobial susceptibility patterns with special reference to carbapenem resistance. A total of 17 clinical samples collected from diagnostic laboratories and hospitals in Pune between January and May 2022 were processed using standard microbiological procedures. The isolates were identified by morphological, cultural and biochemical methods, followed by antimicrobial susceptibility testing using the Kirby–Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute guidelines. Out of 17 samples, 8 were culture-positive, yielding five different Gram-negative bacterial species, namely Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella typhi and Escherichia coli. The findings demonstrated a high level of resistance to β-lactam and extended-spectrum β-lactamase group antibiotics, with a concerning trend of reduced susceptibility to carbapenems. The study highlights the growing burden of carbapenem resistance among clinically important Gram-negative pathogens and emphasizes the urgent need for continuous surveillance and rational antibiotic use.

Keywords: Carbapenem Resistance, Klebsiella Pneumoniae, ESBL, Antimicrobial Susceptibility, Gram-Negative Bacteria, Nosocomial Infection

Introduction:

The continuous rise of antimicrobial resistance represents one of the most serious threats to modern healthcare. Among Gram-negative pathogens, *Klebsiella pneumoniae* is a clinically significant opportunistic bacterium frequently associated with hospital-acquired infections such as urinary tract infections, respiratory tract infections, bloodstream infections and surgical-site infections. The organism commonly colonizes the gastrointestinal tract, skin and nasopharynx and can cause severe disease in immunocompromised and critically ill patients. The emergence of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) has markedly limited therapeutic options. Carbapenems were originally considered the drugs of last resort for infections caused by extended-spectrum β-lactamase-producing organisms. However, the rapid dissemination of carbapenemase-encoding genes, often carried on transferable plasmids,

has resulted in widespread resistance. CRKP infections are associated with prolonged hospitalization, increased treatment costs and high mortality rates, particularly in intensive care units. The situation is further complicated by the frequent co-existence of resistance to aminoglycosides, fluoroquinolones and cephalosporins. In addition to *Klebsiella pneumoniae*, other Gram-negative pathogens such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus* spp. and *Salmonella* spp. also contribute substantially to the burden of healthcare-associated infections. Continuous monitoring of antimicrobial resistance patterns and laboratory-based identification of resistant pathogens are essential to support effective infection control policies. Therefore, the present study was designed to investigate the distribution of Gram-negative pathogens in clinical samples and to evaluate their biochemical characteristics and antimicrobial resistance profiles, with particular emphasis on carbapenem resistance.

Aim and Objectives:

Aim:

To isolate and characterize Gram-negative bacterial pathogens from clinical samples and to determine their antimicrobial susceptibility patterns with special emphasis on carbapenem resistance.

Objectives

1. To collect and process clinical samples obtained from diagnostic laboratories and hospital settings.
2. To isolate and identify pathogenic Gram-negative bacteria using standard microbiological methods.
3. To characterize the isolates based on morphological, cultural and biochemical properties.
4. To evaluate the antibiotic susceptibility and resistance patterns of the isolated organisms.
5. To screen and identify carbapenem-resistant and extended-spectrum β-lactamase producing organisms

Materials and Methods:

Study Design and Sample Collection:

A laboratory-based cross-sectional study was carried out between January and May 2022. A total of 17 clinical samples, including blood, urine, saliva, sputum and wound swabs, were collected from diagnostic laboratories and hospital facilities in Pune. Samples were transferred aseptically in nutrient broth and processed immediately.

Enrichment and Isolation:

Each clinical sample was inoculated into 0.5 mL of nutrient broth and incubated at 37 °C for 24 hours for enrichment. Following incubation, enriched cultures were streaked onto nutrient agar plates and incubated at 37 °C for 24 hours. Distinct colonies were selected for further characterization.

Preservation of Isolates:

Pure cultures were maintained on nutrient agar slants and stored at 4 °C for routine laboratory use.

Morphological Characterization:

Colony morphology including size, margin, elevation, pigmentation and opacity was recorded. Gram staining was performed for microscopic examination following standard procedures.

Biochemical Characterization:

All isolates were subjected to conventional biochemical tests, including:

- Sugar fermentation tests (glucose, lactose and mannitol)
- Indole test
- Methyl red test
- Voges–Proskauer test
- Citrate utilization test
- Catalase test
- Coagulase test
- Oxidase test
- Urease test
- DNase test
- Haemolysis on blood agar

The results were interpreted according to standard bacteriological manuals.

Cultural characterization on selective and Differential Media

Selected isolates were inoculated onto appropriate selective and differential media such as MacConkey agar, XLD agar, EMB agar, cetrimide agar and blood agar. Plates were incubated at 37 °C for 24 hours and characteristic colony appearances were recorded.

Antimicrobial Susceptibility Testing:

Antibiotic susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar. Bacterial inocula were standardized to match 0.5 McFarland turbidity standards. After incubation at 37 °C for 18–24 hours, the zones of inhibition were measured and interpreted according to the guidelines prescribed by the Clinical and Laboratory Standards Institute.

Results:

Sample Distribution and Isolation Rate:

Out of 17 clinical samples processed, 8 samples yielded bacterial growth. Five different Gram-negative bacterial species were isolated

Table 1 Data of Collection of Sample

Sr.No	Sample	Organism	+ve/-ve
1	Sample 1	K.pneumoniae,P. Vulgaris	+ve
2	Sample 2	N.A	-ve
3	Sample 3	P.aeruginosa,S.typhi,E.coli	+ve
4	Sample 4	N.A	-ve
5	Sample 5	S.tphi,E.coli	+ve
6	Sample 6	N.A	-ve
7	Sample 7	N.A	-ve
8	Sample 8	K.pneumoniae,Paeruginosa,S.typhi	+ve
9	Sample 9	N.A	-ve
10	Sample 10	P.aeruginosa,S.typhi,E.coli	+ve
11	Sample 11	N.A	-ve
12	Sample 12	N.A	-ve
13	Sample 13	P.aeruginosa,S.typhi,E.coli	+ve
14	Sample 14	N.A	+ve
15	Sample 15	P.aeruginosa,S.typhi	+ve
16	Sample 16	N.A	-ve
17	Sample 17	S.Typhi,P.aeruginosa	+ve

Frequency Distribution of Isolates:

The identified bacterial species were:

- *Klebsiella pneumoniae*
- *Pseudomonas aeruginosa*
- *Proteus vulgaris*
- *Salmonella typhi*
- *Escherichia coli*

Among the isolates, *Salmonella typhi* and *Klebsiella pneumoniae* represented a

significant proportion of the recovered pathogens.

Morphological and Microscopic Characteristics:

All isolates were Gram-negative rods. Colony morphology varied from opaque to translucent with smooth to mucoid appearance depending on the organism

Table 2 Colony Characteristics of the Isolate

Sr. No	Character	Isolate K	Isolate P.v	Isolate P.a	Isolate S	Isolate E
1	Size	2mm	1-2mm	2mm	2-3mm	1-2mm
2	Shape	Circular	Circular	Circular	Circular	Circular
3	Colour	Greyish white	Greyish white	Greyish white	Greyish white	Greyish white
4	Margin	Entire	Entire	Entire	Entire	Entire
5	Elevation	Convex	Convex	Raised	Convex	Convex
6	Opacity	Translucent	Translucent	Translucent	Opaque	Translucent
7	Consistency	Smooth	Smooth	Smooth	Smooth	Smooth
8	Gram Nature	Gram Negative	Gram Negative	Gram Negative	Gram Positive	Gram Negative
9	Motility	Non motile	Non motile	Motile	Motile	Motile

Biochemical Characterization:

The Results of Biochemical Characteristics are as follows.

Table 3. Biochemical Characteristics of the Isolate

Sr. No	Test	IsolateK	Isolate P.v	IsolateP.a	IsolateS	Isolate E
1	Indole	-ve	-ve	-ve	-ve	+ve
2	Methly Red	-ve	+ve	-ve	+ve	+ve
3	Vogus proskaur	+ve	-ve	-ve	-ve	-ve
4	Citrate	+ve	-ve	+ve	-ve	-ve
5	Catalase	+ve	+ve	+ve	+ve	+ve
6	Oxidase	-ve	-ve	+ve	+ve	+ve
7	Coagulase	+ve	+ve	-ve	+ve	+ve
8	Urease	+ve	+ve	-ve	-ve	-ve
9	Dnase	-ve	-ve	+ve	-ve	-ve
`10	Hemolysin	Hemolysin	Hemolysin	Hemolysin	Hemolysin	Hemolysin
11	Glucose	+ve	+ve	-ve	+ve	+ve
12	Lactose	+ve	-ve	-ve	-ve	+ve
13	Mannitol	+ve	-ve	-ve	+ve	+ve

Cultural Characteristics:

Distinct growth patterns were observed on selective and differential media. *Klebsiella pneumoniae* produced mucoid lactose-fermenting colonies on MacConkey agar. *Escherichia coli* produced characteristic metallic sheen colonies on EMB agar, while *Salmonella typhi* formed red colonies with black centres on XLD agar. *Pseudomonas aeruginosa* produced pigmented colonies on cetrimide agar.

Table 4 Cultral Characteristics of the Isolate

Name of media selective and differential media	Isolate K	Isolate P.v	IsolateP.a	Isolate S	Isolate E
MacConkey Agar	Pink Colonies	N.A.	N.A.	N.A.	N.A.
Eosin Methylene Blue Agar (EMB)	N.A.	Colorless colonies	N.A.	N.A.	Green Metalic sheen
Xylose Lysin Deoxycholate Agar (XLD)	N.A.	N.A.	N.A.	Red with black center	N.A.
Pseudomonas isolation Agar	N.A.	N.A.	Greenish blue	N.A.	N.A.

Antimicrobial Susceptibility Pattern:

All isolates were tested against selected groups of antibiotics. A high level of resistance was observed against extended-spectrum β-lactam antibiotics. Reduced susceptibility and resistance to carbapenem group antibiotics were detected among the *Klebsiella pneumoniae* isolates, indicating the presence of carbapenem-resistant strains. The overall resistance pattern suggested a multidrug-resistant profile among most of the recovered pathogens.

Sr. No	Name of the Group	Name of Antibiotics	*K. pneumoniae*	*P.vulgaris*	*P. aeruginosa*	*S.typhi*	*E.coli*
1.	Cephalosporin	Cefadroxil	S	R	S	R	R
		Cefuroxime	R	R	R	R	R
		Cefixime	S	R	S	S	S
2.	Fluroquinolones	Moxifloxacin	R	R	R	R	S
		Norfloxacin	R	R	R	R	R
		Levofloxacin	S	S	S	S	S
		Ofloxacin	R	R	S	R	R
		Ciprofloxacin	S	R	S	S	R
3.	Aminiglycoside	Gentamycin	R	R	R	R	R
		Kanamycin	R	R	R	R	R
4.	Carbapenam	Imipenum	R	R	R	R	R
	- Lactan	Aztreonzm	R	S	S	S	R
		Amoxicillin	R	R	R	R	R

Discussion:

The present investigation demonstrates the presence of clinically significant Gram-negative pathogens in samples collected from hospital and laboratory settings. *Klebsiella pneumoniae* was identified as one of the predominant organisms, supporting its well-established role as a major cause of healthcare-associated infections, particularly urinary tract and respiratory tract infections. The biochemical and cultural characteristics observed in this study were consistent with established descriptions of the isolated organisms. The high prevalence of resistance to β-lactam antibiotics observed among the isolates is a matter of serious concern. The widespread use of extended-spectrum cephalosporins and other broad-spectrum antibiotics is likely to have contributed to the selection and persistence of resistant strains.The detection of carbapenem-resistant *Klebsiella pneumoniae* further emphasizes the critical challenge faced in the treatment of severe infections. The study findings also indicate that

resistance is no longer limited to *Klebsiella pneumoniae* alone but is increasingly observed among other Gram-negative pathogens, including *Escherichia coli* and *Pseudomonas* species. The observed resistance patterns strongly suggest that previous exposure to multiple antibiotic classes may be an important factor contributing to therapeutic failure and increased mortality. Continuous monitoring of resistance trends and strict adherence to antimicrobial stewardship practices are therefore essential to curb the further spread of resistant organisms.

Conclusion:

The present study confirms the increasing occurrence of multidrug-resistant Gram-negative bacteria in clinical samples collected from healthcare settings in Pune. *Klebsiella pneumoniae* was identified as a major pathogen showing resistance to multiple antibiotic groups, including extended-spectrum β-lactams and carbapenems. The emergence of carbapenem resistance among Gram-negative bacteria represents a serious public health concern, as treatment options are becoming progressively limited. The study highlights the urgent need for routine surveillance of antimicrobial resistance, strict infection control measures and rational use of antibiotics to prevent further dissemination of carbapenem-resistant organisms.

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