



## ANTIBIOTIC SUSCEPTIBILITY PATTERN OF BACTERIA ISOLATED FROM PATIENTS WITH VENTILATOR ASSOCIATED PNEUMONIA IN INTENSIVE CARE UNITS IN A TERTIARY CARE HOSPITAL

### Microbiology

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### ABSTRACT

**Background:** Ventilator-associated pneumonia (VAP) is one of the most important hospital-acquired infections. Emergence of antibiotic resistance is a major public health problem worldwide. VAP is considered as one of the leading causes of morbidity and mortality in intensive care units (ICUs). **Aim:** Identification of the bacterial etiological agents associated with Ventilator Associated Pneumonia (VAP) and the antibiotic susceptibility pattern of the isolates. **Material and methods:** This study was conducted in the Department of Microbiology, RNT Medical College and Associated Hospitals, Udaipur, Rajasthan over a period of six months from May 2024 to October 2024. Endotracheal secretions and Bronchoalveolar lavage samples received in Clinical Microbiology Laboratory were taken up for the study. Antibiotic susceptibility testing was done for culture-positive cases by Kirby-Bauer disk diffusion method as per CLSI 2025. **Results:** Total 54 cases were found to be culture positive. 01 was gram positive cocci and 53 were gram negative bacilli. The predominant bacteria such as *Pseudomonas* sp., *Acinetobacter* sp., *Klebsiella* sp. were found to be most sensitive to Levofloxacin and Imipenem and most resistance to Ampicillin and Piperacillin + Tazobactam. **Conclusion:** Multi Drug Resistant (MDR) microbes causing VAP are on the increase. The antibiotic resistance pattern of these isolates will aid clinicians in selecting the appropriate antimicrobial agents. Hence, it can lead to decreased mortality and morbidity due to life-threatening VAP.

### KEYWORDS

Ventilator-associated pneumonia, Antibiotic susceptibility, Intensive care unit, Hospital acquired infection.

### INTRODUCTION

Ventilator associated pneumonia refers to development of parenchymal lung infection in a patient who has undergone intubation and received mechanical ventilation for > 48 hours<sup>1</sup>. It can be of two types. Early-onset VAP, defined as occurring within the first 4 days of mechanical ventilation, usually carries a better prognosis, and is more likely to be caused by antibiotic sensitive bacteria. Late onset VAP occurring 5 days or more after mechanical ventilation is more likely to be caused by multidrug resistant (MDR) pathogens, and is associated with increased patient mortality and morbidity.<sup>6</sup> Common causative pathogens of VAP include gram-negative bacteria like *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Citrobacter* species, *Enterobacter* species and *Escherichia coli* and gram-positive bacteria like *Staphylococcus aureus*.<sup>7</sup> The causes of VAP are different among different patient populations and also in different type of Intensive care units. Thus, VAP poses grave complications in endotracheally intubated patients in ICUs worldwide. It leads to adverse clinical outcomes and increase in healthcare costs.<sup>9</sup>

### MATERIAL AND METHODS

This study was conducted in the Department of Microbiology, Ravindra Nath Tagore (RNT) Medical College and Associated Hospitals, Udaipur, Rajasthan over a period of six months from May 2024 to October 2024. This is a hospital based cross sectional study. Ethical approval was obtained from the institutional ethical committee before commencement.

### Inclusion Criteria:

Endotracheal secretions and BAL samples received in Clinical Microbiology Laboratory from various patients in ICUs for >48 hours who are receiving treatment from associated hospital of RNT Medical College including MBGH during the study duration of 6 months will be included for the study.

### Exclusion Criteria

1. Samples received in Clinical Microbiology Laboratory according to rejection criteria like incomplete data, insufficient volume and unacceptable specimens etc.
2. Repeated samples from same patient received within 24 hours.
3. Samples showing normal pharyngeal flora in growth.

Data will be collected from the requisition forms receive from different ICUs. The samples were processed as per protocol using standard microbiological techniques. Bacteriological media such as Blood agar, Chocolate agar, MacConkey agar by semi-quantitative culture technique by using standard bacteriological loop. Significant growth was considered when >10<sup>5</sup> colonies for ETA and >10<sup>6</sup> colonies for BAL<sup>5</sup>.

Bacterial isolates were identified based on colony characters and biochemical parameters. The antibiotic susceptibility profile was determined for these isolates by Kirby-Bauer disc diffusion method as per Clinical and laboratory Standards Institute (CLSI) 2025 guidelines. Freshly grown bacteria are suspended in 5mL sterile saline to make bacterial inoculums. The surface of Mueller Hinton agar plates was streaked with a sterile cotton swab. The antibiotic susceptibility pattern was tested using filter paper discs containing a specific concentration of antimicrobial drugs.

### RESULT

During the study period, out of 89 clinically VAP suspected Cases and number of culture positive cases was found to be 54 giving the culture positivity rate of 11.7%. Out of the total 54 majority of the cases were male 40 (74.07%) and female 14 (25.93%). Organism isolated were *Pseudomonas* sp., *Acinetobacter* sp., *Klebsiella* sp., *Citrobacter* sp. and *Escherichia coli* 15,13,11,08, and 04 respectively with the percentage of 27.77, 24.07, 20.37,14.81 and 7.40. *Enterobacter* species and *Staphylococcus aureus* were found 02 and 01 with the percentage of 3.70 and 1.85.

Out of the total culture confirmed cases, 01 (1.85%) was gram positive cocci and 53 (98.14%) were gram negative bacilli.

**Table 1. Bacterial Isolates in Cultures of Confirmed VAP Cases (N=54)**

Type of organism	Organism Isolated	Percentage
<i>Pseudomonas</i> species	15	27.77
<i>Acinetobacter</i> species	13	24.07
<i>Klebsiella</i> species	11	20.37
<i>Citrobacter</i> species	08	14.81
<i>Escherichia coli</i>	04	7.40
<i>Enterobacter</i> species	02	3.70
<i>Staphylococcus aureus</i>	01	1.85
Total	54	100.0

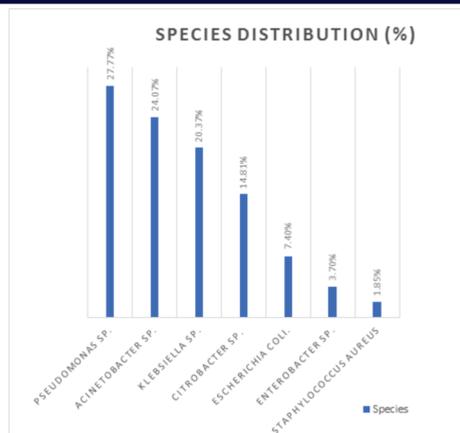


Fig 1: Species Distribution

The isolated Gram-negative bacteria were found to be most sensitive to Levofloxacin (45.28%) followed by Imipenem (43.40%), Gentamicin (39.62%) and Amikacin (39.62%) and Similarly Gram-negative bacteria shows maximum resistance to Ampicillin (100%) followed by Piperacillin + Tazobactam (77.36%), Tetracycline (71.05%) and Ceftazidime (67.92%).

Table 2. Antibiotic Sensitivity Pattern of the Gram Negative Bacterial Isolates (N=53)

Drug	S	S%	I	I%	R	R%
Piperacillin-Tazobactam	10	18.87	2	3.77	41	77.36
Ampicillin*	0	0	0	0	38	100
Meropenem	16	30.18	4	7.54	33	62.26
Gentamicin	21	39.62	0	0	32	60.38
Amikacin	21	39.62	0	0	32	60.38
Tetracycline*	11	28.94	0	0	27	71.05
Cotrimoxazole*	15	39.47	0	0	23	60.52
Ceftazidime	16	30.18	1	1.88	36	67.92
Imipenem	23	43.40	10	18.87	20	37.74
Levofloxacin	24	45.28	13	24.52	16	30.18

\*These drugs are not used in Pseudomonas sp. AST

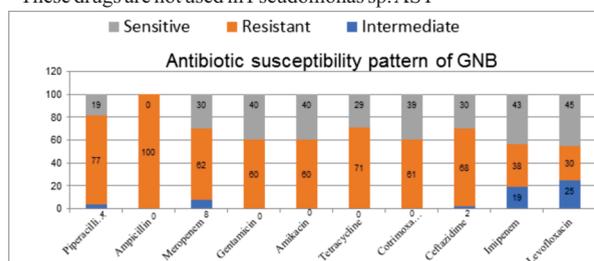


Fig 2: AST of Gram-negative Bacteria

The only isolated Gram-positive bacteria found to be sensitive to Linezolid, Ampicillin, Cefoxitin and Teicoplanin and resistance to Erythromycin, Gentamicin, Amikacin, Doxycycline, Ciprofloxacin, Azithromycin and Clindamycin. Pseudomonas sp. shows maximum sensitivity to Levofloxacin (73.33%) followed by Piperacillin + Tazobactam (66.67%), Imipenem (66.67%) and Aztreonam (66.67%) and shows maximum resistance to Ceftazidime (53.33%) followed by Meropenem (40%). Acinetobacter sp. shows maximum sensitivity to Tetracycline (46.15%) and Cotrimoxazole (46.15%) followed by Levofloxacin (38.46%) and shows maximum resistance to Ampicillin (100%) followed by Meropenem (92.31%) and Piperacillin + Tazobactam (92.31%) and Klebsiella sp. shows maximum sensitivity to Cotrimoxazole (36.36%), Imipenem (36.36%), Ceftazidime (36.36%), amikacin (36.36%) and gentamicin (36.36%) and shows maximum resistance to Piperacillin + Tazobactam (100%) followed by Ampicillin (90.9%) and Tetracycline (72.73%).

DISCUSSION

In the present study, incidence was 60.6%. On comparison with other studies, in Tanavi Chaudhari et al (2023)<sup>10</sup> incidence was 56% while in Shamataj Kattalger Razak et al. (2018)<sup>2</sup> incidence was 72% which was higher than this study. In this study majority of the cases were male 74.07% and female 25.93%. Other studies like in Tanavi Chaudhari et

al (2023)<sup>10</sup> incidence in male was 57.14% while in female 42.86%. And in Anuradha de et al (2018)<sup>8</sup> VAP was more common in male 60%, than in female 40%. In this study, organism most commonly isolated were Pseudomonas sp. 27.77% Acinetobacter sp. 24.07% followed by Klebsiella sp. 20.37%. In the study by Yogendra Kumar Tiwari et al. (2019)<sup>4</sup> Acinetobacter sp. was most common organism isolated (33.33%) followed by Pseudomonas sp. (26.98%) and Klebsiella sp. (17.46%). While in Gupta et al. (2011)<sup>11</sup> most commonly isolated were Pseudomonas species (30%) followed by Klebsiella sp. (23.3%) and Acinetobacter sp. (20%).

This study reported 98.14% were Gram negative bacilli and only 1.85% Gram positive cocci. This is consistent with the result of study by Eshwar Rajesh et al. (2021)<sup>7</sup> 97.97% were gram-negative bacilli and 2.02% were gram-positive cocci. Similarly, in Anuradha de et al. (2018)<sup>8</sup> gram-negative bacilli predominated (96.3%) over gram-positive cocci (3.7%). In this study Gram negative bacteria shows maximum sensitivity to Levofloxacin (45.28%) followed by Imipenem (43.40%), Gentamicin (39.62%) and Amikacin (39.62%) and most resistance to Ampicillin (100%) followed by Piperacillin + Tazobactam (77.36%), Tetracycline (71.05%) and Ceftazidime (67.92%). Study by Shamataj Kattalger Razak et al. (2018)<sup>2</sup> reported that gram-negative isolates showed highest sensitivity for Imipenem (49.9%) and were maximum resistant for Ampicillin (100%) followed by Ciprofloxacin (70%) and Ceftazidime (66.32%) while in Eshwar Rajesh et al. (2021)<sup>7</sup> gram-negative isolates were susceptible to Amikacin (52.27%) followed by Imipenem (50%) and Gentamicin (38.63%) and were maximum resistant for Ceftazidime (68.19%) followed by Ciprofloxacin (63.64%) and Piperacillin-tazobactam (38.64%) and study by Neelima Ranjan et al. (2014)<sup>7</sup> reported that gram-negative isolates showed highest sensitivity for Meropenem (86.56%) followed by Piperacillin + Tazobactam (65.67%) and were maximum resistant for Ceftazidime (64.17%), Co-trimoxazole (61.19%), Ciprofloxacin (55.22%) followed by Tetracycline (50.74%) and Amikacin (47.76%).

Only Gram-positive bacteria was most sensitive (100%) to Linezolid, Ampicillin, Cefoxitin and Teicoplanin and resistance (100%) to Erythromycin, Gentamicin, Amikacin, Doxycycline, Ciprofloxacin, Azithromycin and Clindamycin. Similar findings were found in Eshwar Rajesh et al. (2021)<sup>7</sup> gram-positive isolates were susceptible (100%) to Linezolid, Cefoxitin and Tetracycline and resistant (100%) to Ampicillin and Erythromycin.

In this study Pseudomonas sp. shows maximum sensitivity to Levofloxacin (73.33%) followed by Piperacillin + Tazobactam (66.67%), Imipenem (66.67%) and Aztreonam (66.67%) and shows maximum resistance to Ceftazidime (53.33%) followed by Meropenem (40%). In study by Ankita Patel et al. (2015)<sup>3</sup> showed maximum sensitivity (57.14%) to Amikacin and Gentamicin and showed (85.71%) resistant to Aztreonam, Piperacillin + Tazobactam and Ceftazidime followed by Ciprofloxacin (57.14%). While in Yogendra Kumar Tiwari et al. (2019)<sup>4</sup> 44.44% isolates were sensitive to Imipenem and Meropenem and 55.55% isolates were resistant to Piperacillin + Tazobactam and Levofloxacin.

In this study Acinetobacter sp. shows maximum sensitivity to Tetracycline (46.15%) and Cotrimoxazole (46.15%) followed by Levofloxacin (38.46%) and shows maximum resistance to Ampicillin (100%) followed by Meropenem (92.31%) and Piperacillin + Tazobactam (92.31%). Study by Yogendra Kumar Tiwari et al. (2019)<sup>4</sup> showed maximum sensitivity to Levofloxacin (85.71%) followed by Ceftazidime (66.66%) and showed maximum resistance to Meropenem (52.38%) followed by Piperacillin + Tazobactam (52.38%). In Anuradha de et al. (2018)<sup>8</sup> maximum sensitive to Tetracycline (94.70%) and showed maximum resistant to Piperacillin + Tazobactam (97.40%) followed by Imipenem (52.20%).

In this study Klebsiella sp. shows sensitivity (36.36%) to Cotrimoxazole, Imipenem, Ceftazidime, amikacin and gentamicin and shows maximum resistance to Piperacillin + Tazobactam (100%) followed by Ampicillin (90.9%) and Tetracycline (72.73%). Study by Yogendra Kumar Tiwari et al. (2019)<sup>4</sup> showed maximum sensitivity to Meropenem (76.92%) followed by Imipenem (69.23%), Levofloxacin (69.23%) and Ceftazidime (69.23%) and no maximum resistance to any of the drug. While in Anuradha de et al. (2018)<sup>8</sup> showed maximum sensitive to Imipenem (66.70%) and showed maximum resistant to Piperacillin + Tazobactam (100%) and Ciprofloxacin (100%) followed by Amikacin (66.70%).

## CONCLUSION

Ventilator Associated Pneumonia is a subgroup of Hospital Acquired Pneumonia and is associated with increased patient mortality and morbidity. Multi Drug Resistant (MDR) microbes causing VAP are on the increase. The antibiotic resistance pattern of these isolates will aid clinicians in selecting the appropriate antimicrobial agents. Hence, it can lead to decreased mortality and morbidity due to life-threatening VAP.

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