Original Article

Antimicrobial resistance pattern in aerobic bacteria isolated from endotracheal aspirate in ventilator-associated pneumonia: Ten years observation from a tertiary care hospital

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Abstract

Background and Aims: Ventilator-associated pneumonia (VAP) is a nosocomial infection associated with high morbidity and mortality. This study was undertaken to monitor the trend of the demographical details, comorbid conditions, bacterial etiological agents, and their antibiogram causing VAP in adults in the year 2008, 2013 and 2018.

Material and Methods: A retrospective study conducted at the Department of Microbiology, Hospital Infection control and Quality Control at a tertiary care teaching hospital. All the adult patients with more than 48 h of the mechanical ventilator with endotracheal intubation with Clinical Pulmonary infection Score >6 with suspicion of VAP were included in the study at a difference of 5 years, i.e., 2008, 2013, and 2018.

Results: A total of 338 patients were included in the study, of which males accounted for more than two-third of the patients studied. Nearly 45% of the patients belonged to geriatric (>60 years) age group. The most common comorbid conditions were chronic obstructive pulmonary disease, hypertension and diabetes mellitus. Among the gram-negative isolates, *Klebsiella pneumoniae*, *Acinetobacter* species, and *Pseudomonas aeruginosa* were the most common. There is an emergence of resistance to most commonly administered antimicrobial agents like aminoglycosides, levofloxacin, piperacillin/tazobactum, and carbapenems during the study period.

Conclusion: This is a ten-year study on the antibiotic resistance pattern of organisms causing VAP. As far as the authors are aware, this is the first study addressing the pattern of change in drug resistance in the organisms causing VAP over a decade. The emergence of multi-drug resistant (MDR) MDR pathogens, especially in intensive care unit (ICU), is a great concern for the intensivist and infection control physicians. Preventive measures need to be undertaken to control the spread of these pathogens to the patients in the ICU.

Keywords: *Acinetobacter*, antimicrobial resistance, MDR, VAP

Introduction

Nosocomial pneumonia, the second most common hospital-associated infection, with high morbidity and mortality is the most serious nosocomial infection. Ventilator-associated pneumonia (VAP), a type of nosocomial pneumonia, occurs in a patient on a mechanical ventilator (MV) for more than 48 h after intubation or tracheostomy.[1] Even though well-engineered

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devices, better supportive care, and advanced antimicrobial agents are available, VAP continues to be the leading cause of morbidity and mortality in intensive care units (ICUs), with crude mortality rate arranging from 30 to 70%.[2] It also increases the cost of treatment by an increase of the length of stay in ICUs and hospitals, high-end antimicrobial therapy, and other supportive care.[3] Patients with comorbid conditions like chronic obstructive pulmonary disease, organ failure, diabetes mellitus, hypertension/ischemic heart disease (IHD), cerebrovascular accidents are at high risk of developing VAP. The common etiological agents causing VAP are *Klebsiella pneumoniae*, *Acinetobacter* sp, *Pseudomonas aeruginosa*, and *Staphylococcus aureus (S. aureus)*, and they can vary depending on the patients and ICU setup.[4] Accurate diagnosis, identification of etiological agent, and timely start of antimicrobial therapy are very vital for the treatment of VAP.

VAP caused by multidrug resistant pathogens needs treatment with high-end, toxic, and costly antimicrobial agents. With the advent of rising antimicrobial resistance (AMR) among nosocomial pathogens, the monitoring of the change in the resistance pattern is very much essential. Early administration of appropriate empirical antimicrobial therapy plays a very crucial role in the treatment of the patient. The mortality rate can be as high as 75%; when there is a delay in appropriate empirical antimicrobial therapy, with delay of every hour, the survival rate may reduce by 8%.[5] Therefore, the pathogens causing infection in ICU, especially VAP, warrant close monitoring in change in pathogens and their antibiogram. So the appropriate empirical antimicrobial therapy can be initiated at the earliest and to reduce morbidity and mortality. This study was undertaken to monitor the trend of the demographical details, comorbid conditions, bacterial etiological agents, and their antibiogram causing clinically diagnosed VAP in adults between the years 2008 and 2018.

Material and Methods

A retrospective study was conducted at departments of Microbiology, Hospital infection, and quality control of a tertiary care teaching hospital following clearance from the Institutional ethics committee. All the adult patients, with more than 48 h of a mechanical ventilator with endotracheal intubation or tracheotomy with Clinical Pulmonary infection Score >6 with suspicion of VAP, were included in the study. We included patients with a difference of 5 years, i.e., 2008, 2013, and 2018 who were diagnosed with VAP were included in the study.

The medical records of the patients diagnosed with VAP with the bacterial growth of $>10^5$colony forming unit/ml were retrieved for data collection from the hospital information system. The details of the patients with VAP were collected, like demographic, bacteria isolated, and their antimicrobial susceptibility pattern excluding repeat isolates. The patient details were collected and classified by year of admission, gender, age wise (18–30, 31–45, 46–60, 61–75, and >75 years), comorbid conditions, and outcome of treatment. The respective Clinical and Laboratory Standard Institute (CLSI) guidelines[6] for antimicrobial susceptibility testing were used for calculating the AMR rate for gram-negative and gram-positive pathogens separately and analyzed for change in the pattern. The Microsoft Excel software was used to analyze the data and analysis was done by Z-test to compare the change in the data between the 2008–13, 2013–18, and 2008–2018. The P value <0.05 was considered to be significant.

Results

A total of 226, 184, and 206 patients were on Mechanical ventilation MV of which 100, 125, and 113 patients were included in the study in 2008–09, 2013–14, and 2018–19, respectively. Altogether 338 patients were included in the study, of which males accounted for more than two-third of the patients studied [Table 1]. Similarly, gender difference was observed in all three study years. Nearly, one-third of the patients belonged to the age group of 61–75 years and geriatric (>60 years) patients accounted for 45% of the patients included [Table 1]. Among the patients diagnosed with VAP, chronic obstructive pulmonary disease (COPD) (83, 24.56%), hypertension (66, 19.53%), and diabetes mellitus (57, 16.86%) were the three most common comorbid conditions [Table 1]. Nearly equal number of cases survived, around (40%) when compared to expired; meanwhile, 19% of the patients had left against medical advice. When the three groups were compared, there was a significant difference in the number of patients with VAP; there was a significant reduction in VAP cases in 2018 compared to 2013 [Table 1]. There was a significant difference in the incidence of VAP in the age group of 61–75 years as it increased in 2013 and 2018 as compared to 2008 as shown in Table 1. Similarly, comorbidity like hypertensive, renal failure, ischemic heart disease IHD, acute respiratory distress syndrome (ARDS) and Organo-phosphorous (OP) poisoning cases showed a significant difference in incidence among the three groups when compared [Table 1].

From the 338 patients included in the study, 397 pathogens were isolated. The gram-negative bacilli accounted for 377 (94.96%) and gram-positive cocci 20 (5.04%). Among the

Table 1: Demographic details of the VAP patients

	2008 *n* (%)	2013 *n* (%)	2018 *n* (%)	Total	*P*		
Total no. of patients on MV	226	184	206	616	2008–13	2008–18	2013–18
Total no. of patients diagnosed with VAP	100 (44.24)	125 (67.93)	113 (43.46)	338 (54.87)	0.000	0.028	0.009
Gender							
Female	32 (32)	35 (38)	38 (33.63)	105 (31.07)	0.515	0.801	0.348
Male	68 (68)	90 (72)	75 (66.37)	233 (68.93)	0.515	0.801	0.348
Age (in years)							
18–30	20 (20)	15 (12)	15 (13.27)	50 (14.79)	0.101	0.188	0.768
31–45	13 (13)	12 (9.6)	16 (14.16)	41 (12.13)	0.421	0.806	0.277
46–60	32 (32)	29 (23.2)	32 (28.32)	93 (27.51)	0.141	0.559	0.367
61–75	22 (22)	47 (37.6)	41 (36.28)	110 (32.54)	0.012	0.024	0.834
>75	13 (13)	22 (17.6)	9 (7.96)	44 (13.02)	0.345	0.230	0.028
Outcome							
Expired	46 (46)	51 (40.8)	38 (33.63)	135 (39.94)	0.435	0.067	0.255
Improved	41 (41)	48 (38.4)	47 (41.59)	136 (40.24)	0.692	0.930	0.616
LAMA	13 (13)	26 (20.8)	28 (24.78)	67 (19.82)	0.126	0.031	0.465
Co-morbidity							
COPD	30 (30)	30 (24)	23 (20.35)	83 (24.56)	0.313	0.106	0.500
Hypertension	23 (23)	5 (4)	38 (33.62)	66 (19.53)	0.000	0.088	0.000
Diabetes mellitus	27 (27)	29 (23.2)	28 (24.78)	57 (16.86)	0.513	0.712	0.776
Renal failure	28 (28)	7 (5.6)	13 (11.5)	48 (14.2)	0.000	0.003	0.102
Pneumonia	12 (12)	12 (9.6)	23 (23.35)	47 (13.91)	0.563	0.102	0.020
Cerebrovascular accident	6 (6)	13 (10.4)	13 (11.50)	32 (9.47)	0.239	0.161	0.785
Ischemic heart disease	11 (11)	3 (2.4)	17 (15.04)	31 (9.17)	0.009	0.384	0.001
ARDS	11 (11)	2 (1.6)	9 (7.96)	22 (6.51)	0.003	0.449	0.020
Cancer	2 (2)	9 (7.2)	8 (7.08)	19 (5.62)	0.074	0.082	0.971
Organo-phosphorus poisoning	9 (9)	6 (4.8)	2 (1.77)	17 (5.03)	0.211	0.018	0.197
Tuberculosis	4 (4)	6 (4.8)	6 (5.31)	16 (4.73)	0.773	0.652	0.858
Aspiration pneumonia	2 (2)	3 (2.4)	8 (7.08)	13 (3.85)	0.840	0.082	0.087
Road traffic accident	6 (6)	3 (2.4)	2 (1.77)	11 (3.25)	0.172	0.107	0.735
Guillian Barre syndrome	3 (3)	4 (3.2)	1 (0.88)	8 (2.37)	0.932	0.258	0.215
Meningitis	1 (1)	3 (2.4)	3 (2.65)	7 (2.07)	0.431	0.376	0.900
Chronic liver disease	2 (2)	3 (2.4)	2 (1.77)	7 (2.07)	0.840	0.902	0.735
Dengue	1 (1)	3 (2.4)	3 (2.65)	7 (2.07)	0.431	0.376	0.900
H1N1	0	1 (0.8)	2 (1.77)	3 (0.89)	0.371	0.183	0.504

gram-negative isolates, *Klebsiella pneumoniae* (137, 34.5%), *Acinetobacter* species (107, 26.95%), and *Pseudomonas aeruginosa* (16.62%) were the most common [Table 2] and *S. aureus* (20, 5.04%) among gram-positive pathogens. The methicillin-resistant *S. aureus* (MRSA) isolation rate was 50% i.e., 10 out of 20 *S. aureus*. Even though, *Klebsiella pneumoniae* was the most common isolate, there was not much difference in its isolation rate (33.33% to 36.29%), while the doubling of isolation rate of *Acinetobacter* sp was from 18.12 to 36.29%. The isolation rate of *Pseudomonas aeruginosa*, *Escherichia coli*, and *Citrobacter* species declined during decade long time period [Table 2]. There was a significantly higher incidence of *Acinetobacter* sp in 2018 compared to 2008, reduced incidence was seen with *Pseudomonas* sp in 2018 compared to 2008 and 2013 [Table 2]. No significant difference was noted among fermentors like *Klebsiella sp* and *Escherichia coli*.

The study of the AMR among the gram-negative pathogens revealed that more than 75% of the isolates, were resistant to ampicillin and third-generation cephalosporins, and around 50–75% resistance was observed among gentamicin and β lactam-β lactamase inhibitor combination. The least resistance was observed among levofloxacin, amikacin, imipenem, and meropenem in decreasing order [Table 3]. A similar level of resistance was observed among the individual gram-negative pathogens, except in *Acinetobacter* sp, where a higher level of resistance was observed to high-level drugs like amikacin and carbapenems (imipenem and meropenem), as shown in Table 3. In the only gram-positive pathogen, i.e., *S. aureus*, 50% of the isolates were methicillin-resistant *S. aureus*. There was a high level of resistance to aminoglycosides and fluoroquinolones. We have not observed any resistance to anti-MRSA drugs like vancomycin, teicoplanin, and linezolid during the study period in VAP cases.

Table 2: Distribution of various pathogens isolated during the study period

Pathogen	Year			Total (%)	*P*		
	2008 (%)	2013 (%)	2018 (%)		2008–13	2008–18	2013–18
Klebsiella species	47 (34.06)	45 (33.33)	45 (36.29)	137 (34.51)	0.899	0.706	0.618
Acinetobacter species	25 (18.12)	37 (27.41)	45 (36.29)	107 (26.95)	0.068	0.001	0.126
Pseudomonas species	28 (20.29)	28 (20.74)	10 (8.06)	66 (16.62)	0.927	0.005	0.004
Escherichia coli	15 (10.87)	11 (8.15)	13 (10.48)	39 (9.82)	0.444	0.920	0.518
Citrobacter species	17 (12.32)	5 (3.7)	5 (4.03)	27 (6.80)	0.009	0.016	0.891
Methicillin-resistant *Staphylococcus aureus*	4 (2.9)	3 (2.22)	3 (2.42)	10 (2.52)	0.724	0.810	0.916
Methicillin-sensitive *Staphylococcus aureus*	1 (0.72)	6 (4.44)	3 (2.42)	10 (2.52)	0.053	0.265	0.375
Serratia species	1 (0.72)	0	0	1 (0.25)	0.323	0.343	-
Total	138	135	124	397			

The *Klebsiella* species showed increased resistance to amikacin (P = <0.001), levofloxacin (P = 0.002), β-lactam β-lactamase inhibitor combination (0.001), and carbapenems (<0.001), whereas gentamicin showed decreasing resistance from 2008 to 2018. The carbapenem resistance increased drastically from 20% (n = 25) in 2008 to 82.2% (n = 45) in 2018 ($P \leq 0.001$), and levofloxacin resistance from 20 to 62.22% among *Acinetobacter* species. There is significant increase in Carbapenem resistance among the *Pseudomonas aeruginosa* isolates to 40% (n = 10, P = 0.001), *Escherichia coli* 30.7% (n = 13, P = 0.028), *Citrobacter* sp 100% (n = 5, P = 0.000) in 2018, compared to the resistance rate detected in 2008 as shown in Table 3. Levofloxacin, which is mainly used for respiratory infection, as also shown an emergence in resistance among all gram-negative pathogen to around 60%. There was significant rise in resistance to amikacin, levofloxacin, piperacillin/tazobactum, and carbapenems over a decade among all gram-negative pathogens [Table 3]. No significant difference was observed in AMR patterns in *S. aureus* isolates across the decade.

Discussion

As per our literature search, this study is the first study exploring the epidemiology of VAP over a decade in the Indian Subcontinent. All these patients were clinically diagnosed using modified Clinical Pulmonary Infection Score (m-CPIS), rather than National Health Surviellance Network (NHSN) surveillance criteria. Among the 338 patients included, males constituted nearly two-third of the patients in all three years of study. The higher incidence in male patients is observed in various studied worldwide.[7-9] Most of the patients (45.5%) were from the geriatric age group, i.e., above 60 years as seen in studies conducted globally on VAP. This may be attributed to the occurrence of multiple comorbidity in patients with advancing age. Among the comorbidities noted in our patients, COPD, hypertension, and diabetes mellitus were the three most common ones, which are known to be non-modifiable host factors.[10] COPD is the most common predisposing factor for VAP.[11,12] The avian influenza (H1N1) which emerged in 2009 globally was observed to be a risk factor for patients to develop VAP in the patients of 2013 and 2018.

The flora causing VAP in ICU varies geographically and also varies from ICU to ICU in the same hospital. So the study of the flora of the ICU is very important for the early initiation of appropriate empirical antimicrobial therapy. *Klebsiella* sp was the most common pathogen to cause in all three years, but the incidence *Acinetobacter* species has steadily increased from 18.12 to 36.29% during the study period, which is observed in Asian hospitals.[7,8,13] However, in Western countries like USA, *S. aureus*, and *Enterobacterales* are the important pathogens causing VAP.[14,15] Various studies have shown different flora causing VAP, and also over time period, there is a change in flora and also higher incidence of AMR.[11,16]

The emergence of MDR *Acinetobacter* species in the ICU causing VAP is a major concern worldwide as it increases mortality, length of stay, and increases the cost of treatment.[7,8] A study of 162 Lebanese patients observed similar bacterial flora causing VAP compared to our study, but *Acinetobacter* species was among the commonest pathogens.[7] compared Klebsiella species in our study population. The incidence of *S. aureus* in VAP in our ICU was as low as 5%, but the isolation of MRSA among them is as high as 50%. Among gram-negative pathogens like *Klebsiella*, *Acinetobacter*, *Pseudomonas*, the AMR was high to third cephalosporins, gentamicin, and low to levofloxacin, beta lactam-beta lactamase inhibitor combinations. Least to no resistance was observed for carbapenems in 2008, among these isolates. But a decade later, in 2018, there was a very high level of resistance, i.e., up to 82% of resistance to carbapenems was observed among the Acinetobacter species. This high level of resistance to carbapenems is an issue of major concern for the intensivist and the infection control professionals in the ICU.[7,8] Even though the isolation of MRSA is 50% among

Table 3: Year-wise antimicrobial resistance pattern of pathogens isolated between 2008 and 2018

Pathogen isolated	Antibiotics	2008 % (*n*)	2013 % (*n*)	2018 % (*n*)	Total % (*n*)	*P*		
						2008–2013	2008–2018	2013–2018
Klebsiella (137)	3rd Cephalosporins	87.23 (41)	73.33 (33)	77.78 (35)	79.56 (109)	0.096	0.235	0.625
	Gentamicin	76.6 (36)	62.22 (28)	57.78 (26)	65.69 (90)	0.138	0.057	0.668
	Amikacin	10.64 (5)	40 (18)	51.11 (23)	33.58 (46)	0.002	<0.001	0.293
	Levofloxacin	29.79 (14)	55.56 (25)	62.22 (28)	48.91 (67)	0.014	0.002	0.522
	Piperacillin/Tazobactum	29.79 (14)	62.22 (28)	64.44 (29)	51.82 (71)	0.002	0.001	0.827
	Imipenem	2.13 (1)	31.11 (14)	46.67 (21)	26.28 (36)	<0.001	<0.001	0.134
	Meropenem	0 (0)	46.67 (21)	48.89 (22)	31.39 (43)	<0.001	<0.001	0.833
Acinetobacter (107)	Ampicillin	100 (25)	100 (37)	100 (45)	100 (107)	0.411	0.129	0.248
	3rd Cephalosporins	100 (25)	97.3 (36)	91.11 (41)	95.33 (102)	0.885	0.296	0.176
	Gentamicin	88 (22)	89.19 (33)	77.78 (35)	84.11 (90)	0.073	0.713	0.099
	Amikacin	60 (15)	81.08 (30)	64.44 (29)	69.16 (74)	0.000	0.001	0.196
	Levofloxacin	20 (5)	75.68 (28)	62.22 (28)	57.01 (61)	0.019	0.066	0.454
	Piperacillin/Tazobactum	68 (17)	91.89 (34)	86.67 (39)	84.11 (90)	<0.001	<0.001	0.663
	Imipenem	20 (5)	78.38 (29)	82.22 (37)	66.36 (71)	<0.001	<0.001	0.600
	Meropenem	20 (5)	86.49 (32)	82.22 (37)	69.16 (74)	0.411	0.129	0.248
Pseudomonas (66)	3rd Cephalosporins	14.29 (4)	67.86 (19)	70 (7)	45.45 (10)	<0.001	0.002	0.901
	Gentamicin	10.71 (3)	60.71 (17)	70 (7)	40.91 (27)	<0.001	0.001	0.604
	Amikacin	3.57 (1)	42.86 (12)	60 (6)	28.79 (19)	0.001	0.000	0.358
	Levofloxacin	14.29 (4)	32.14 (9)	40 (4)	25.76 (17)	0.119	0.095	0.656
	Piperacillin/Tazobactum	7.14 (2)	42.86 (12)	60 (6)	30.30 (20)	0.003	0.001	0.358
	Imipenem	0 (0)	32.14 (9)	40 (4)	19.70 (13)	0.002	0.001	0.656
	Meropenem	0 (0)	32.14 (9)	40 (4)	19.70 (13)	0.002	0.001	0.656
Escherichia coli (39)	Ampicillin	100 (15)	100 (11)	100 (13)	100 (39)	-	-	-
	3rd Cephalosporins	73.33 (11)	90.91 (10)	100 (13)	87.18 (34)	0.272	0.055	0.279
	Gentamicin	73.33 (11)	45.45 (5)	76.92 (10)	66.67 (26)	0.162	0.829	0.127
	Amikacin	13.33 (2)	36.36 (4)	38.46 (5)	28.21 (11)	0.181	0.138	0.917
	Levofloxacin	33.33 (5)	72.73 (8)	61.53 (8)	53.85 (21)	0.059	0.148	0.568
	Piperacillin/Tazobactum	26.67 (4)	54.55 (6)	61.53 (8)	46.15 (18)	0.162	0.074	0.732
	Imipenem	0 (0)	18.18 (2)	30.77 (4)	15.38 (6)	0.099	0.028	0.485
	Meropenem	0 (0)	27.27 (3)	30.77 (4)	17.95 (7)	0.042	0.028	0.853
Citrobacter (27)	Ampicillin	94.12 (16)	100 (5)	100 (5)	96.30 (26)	0.585	0.585	-
	3rd Cephalosporins	82.35 (14)	40 (2)	100 (5)	77.78 (21)	0.076	0.324	0.072
	Gentamicin	41.18 (7)	20 (1)	100 (5)	48.15 (13)	0.397	0.031	0.033
	Amikacin	0 (0)	20 (1)	60 (3)	14.81 (4)	0.074	0.003	0.233
	Levofloxacin	11.76 (2)	40 (2)	60 (3)	25.93 (7)	0.166	0.035	0.545
	Piperacillin/Tazobactum	11.76 (2)	20 (1)	100 (5)	29.63 (8)	0.642	0.001	0.033
	Imipenem	0 (0)	20 (1)	100 (5)	22.22 (6)	0.074	0.000	0.033
	Meropenem	0 (0)	20 (1)	100 (5)	22.22 (6)	0.074	0.000	0.033
Staphylococcus aureus (20)	Ampicillin	100 (4)	77.78 (7)	66.67 (4)	75 (15)	0.924	0.633	0.641
	3rd Cephalosporins	100 (4)	33.33 (3)	50 (3)	50 (10)	0.120	0.330	0.530
	Gentamicin	25 (1)	66.66 (6)	66.67 (4)	55 (11)	0.123	0.156	1.000
	Amikacin	25 (1)	33.33 (3)	50 (3)	35 (7)	0.606	0.330	0.530
	Levofloxacin	50 (2)	44.44 (4)	50 (3)	45 (9)	0.874	0.748	0.836

the S. *aureus* isolates, we have not observed an emergence of resistance to anti-MRSA drugs like vancomycin, linezolid, and teicoplanin in our ICU, which is reported in Western countries.[17] The flora causing VAP in ICU is dynamic and the monitoring change in their antibiogram is very much important for ID physicians to start appropriate empirical antimicrobial therapy among the patients in the initial few hours of admission.

Conclusion

In this study, attempt was made to understand the epidemiology of clinically diagnosed VAP in a tertiary care hospital over a decade. An increase in the incidence of VAP among males and geriatric patients with comorbid conditions like COPD, hypertension, and diabetes mellitus was prominent. Even though, *Klebsiella* species continues to be the most common

pathogen causing VAP during the study period, *Acinetobacter* species is emerging rapidly in ICU, especially the multidrug resistance strain. The emergence of high level of resistance to reserved high-end antimicrobial agents like beta lactam–beta lactamase inhibitor, aminoglycosides, and carbapenems in ICU is a matter of concern.

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Conflicts of interest
There are no conflicts of interest.

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