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Antibiotic resistance & pathogen profile in ventilator-associated pneumonia in a tertiary care hospital in India

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***Background & objectives*: Ventilator-associated pneumonia (VAP) is an important hospital-acquired infection with substantial mortality. Only a few studies are available from India addressing the microbiological aspects of VAP, which have been done with small study populations. This study was carried out in the intensive care units (ICUs) of a tertiary care hospital to assess the profile of pathogens and to determine the pattern of antimicrobial resistance.**

***Methods*: This was a retrospective study of clinically suspected cases of VAP. Over a three year period, a total of 247 cases in 2011, 297 in 2012 and 303 in 2013 admitted in ICUs on mechanical ventilation with clinical evidence of VAP were included in our study. The endotracheal aspirate samples from these suspected cases were subjected to quantitative culture technique, and colony count of $\geq 10^5$ colony forming units/ml was considered significant. Antimicrobial susceptibility test for the isolates was done.**

***Results*: VAP rates of 44.1, 43.8 and 26.3 were seen in 2011, 2012 and 2013, respectively. In all the three years, non-fermentative Gram-negative bacilli were the predominant organisms, followed by *Pseudomonas* spp. and *Klebsiella* spp. *Staphylococcus aureus* exhibited a downwards trend in prevalence from 50.0 per cent in 2011 to 34.9 per cent in 2013. An increase in vancomycin-resistant enterococci was seen from 4.3 per cent in 2012 to 8.3 per cent in 2013, while methicillin resistance amongst the *S. aureus* crossed the 50 per cent mark in 2013. An increasing trend in resistance was shown by *Pseudomonas* spp. for piperacillin-tazobactam (PTZ), amikacin and imipenem (IPM). For the non-fermenters, resistance frequency remained very high except for IPM (33.1%) and polymyxin-B (2.4%).**

***Interpretation & conclusions*: Our findings show VAP as an important problem in the ICU setting. The incidence of multidrug-resistant pathogens was on the rise. The resistance pattern of these pathogens can help an institution to formulate effective antimicrobial policy. To have a comprehensive pan-India picture, multicentric studies are needed.**

Key words Antimicrobial resistance - multidrug resistance - pathogens - ventilator-associated pneumonia

Ventilator-associated pneumonia (VAP) is considered the second most common hospital-acquired infection associated with higher mortality and morbidity[1], and it develops when a patient is on mechanical ventilation for ≥48 h[2]. It has been found that 10-20 per cent of patients requiring mechanical ventilation develop associated pneumonia with a high mortality[3]. The VAP rate is measured as episodes per

1000 ventilator days and it varies widely in different regions, ranging from 4.4 cases/1000 ventilator days in the USA[4] to as high as 13-51/100 ventilator days in other parts of the world[5]. While the incidence is low in developed countries of the world, it continues to be unacceptably high in less-developed nations[6]. Thus, in a study from India, the incidence was found to be 30.7 and 15.8 per 1000 days in two different ICUs[7], while another report found it to be 53.2 per 1000 days[8].

The aetiologic agents of VAP include some of the common hospital pathogens such as *Pseudomonas* spp., *Acinetobacter* and other non-fermenters, members of the *Enterobacteriaceae* family, as well as Gram-positive pathogens such as staphylococci, and the fungal agent *Candida*[9,10]. The emergence of multidrug-resistant strains associated with VAP is linked to the excessive use of broad-spectrum antibiotics early in the intensive care settings in the economically developing countries[6].

Therefore, this study was undertaken to find the spectrum of pathogens and their antimicrobial resistance patterns over a period of three years in the intensive care units (ICUs) of a tertiary care hospital, and to determine the trends of infection.

Material & Methods

This was a retrospective, cross-sectional, descriptive study using data from laboratory records for the three consecutive years 2011, 2012 and 2013. The study was conducted at Sri Venkateswara Institute of Medical Sciences, Tirupati, India, which is a tertiary care referral hospital having a dedicated Respiratory Intensive Care Unit (ICU) and Medical ICU, apart from other ICUs for various medical and surgical subspecialities.

Only those patients who were on mechanical ventilation for more than 48 h and clinically suspected of having pneumonia were included. The total number of patients for the three years were 1159 in 2011, 903 in 2012 and 1022 in 2013, of whom 247, 297 and 303 had clinical and microbiological evidence of VAP, respectively, as per the modified Clinical Pulmonary Infection Score (CPIS)[11] of more than six.

The study protocol was approved by the institutional ethics committee.

Sample collection and microbiological analysis: The endotracheal aspirate samples were subjected to quantitative culture technique. A colony count of $\geq 10^5$ colony forming units (cfu)/ml was considered significant[12]. Any growth below this was considered as colonization or contamination. Identification of the isolates was done by standard biochemical tests[13], and antimicrobial susceptibility test was performed and interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines[14]. For the *Enterobacteriaceae* members and non-fermenters, the antibiotics used were amikacin (AK), ampicillin (AMP), amoxicillin-clavulanate (AXV), aztreonam (AZT), cefotaxime (CTX), cefepime (CPM), ceftazidime (CTZ), cefoperazone-sulbactam (CFS), chloramphenicol (CHL), ciprofloxacin (CIP), co-trimoxazole (COT), gentamicin (GEN), imipenem (IPM), piperacillin-tazobactam (PTZ), netilmicin (NET), polymyxin-B (PB) and tigecycline (TGC). For *Pseudomonas* isolates, AMP, AXV, cefoxitin (CXT), CHL, COT and TGC were excluded from the panel. For the Gram-positive pathogens, the panel included AMP, AXV, penicillin (PEN), CXT, COT, CIP, GEN, erythromycin (ERY), vancomycin (VAN) and linezolid (LIZ). CXT (30 µg) disc was used as a surrogate marker for determining methicillin resistance amongst the staphylococci. Control strains of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 were used for antimicrobial susceptibility tests. In selected instances, where CLSI guidelines for disc diffusion technique are not available, the following strategies were adopted: (*i*) for CFS, the CLSI interpretative guideline for cefoperazone was used; (*ii*) for PB, the recommendation of Galani *et al*[15] was adopted; (*iii*) and VAN susceptibility for *Staphylococcus* spp. and *Enterococcus* spp. were interpreted as per the British Society for Antimicrobial Chemotherapy guidelines[16].

Data analysis: All laboratory and clinical data were extracted from infection control committee records and database. The clinical data concerning the number of ventilated days, CPIS score and various clinical and radiological findings were obtained from the VAP registers kept and maintained at all the ICUs by the infection control committee and updated daily for surveillance records. Simple counts and percentages were analyzed.

Results

During the study period significant growth of pathogens (mono or polybacterial) was found in 247 of 1159 (21.3%) patients in 2011, 297 of 903 (32.9%) in 2012 and 303 of 1022 (29.6%) in 2013. The VAP rates

were 44.1, 43.8 and 26.3 per 1000 ventilator days in the three years, respectively.

The total numbers of Gram-negative organisms isolated in the three years from 2011 to 2013 were 578, 737 and 512, respectively. In all the three years, non-fermentative Gram-negative bacilli were the predominant organisms, followed by *Pseudomonas* spp. and *Klebsiella* spp. Although the relative frequency of most of these organisms remained more or less the same over the years, a small incremental decrease in the number of *Klebsiella* spp. (23.7% in 2011 to19.3% in 2013) and *E. coli* (14.9-11.5% during the same period) were noted over the years. Amongst the Gram-positive isolates, *S. aureus* exhibited a downward trend in prevalence from 50.0 per cent in 2011 to 34.9 per cent in 2013 (Table I).

The percentages of resistant strains of the members of *Enterobacteriaceae* family are shown in Table II. Very high frequency of resistance ranging from 45 to 100 per cent was exhibited by these organisms for AMP, AXV, CIP and for CTX throughout the study period. AK resistance showed a steady rise for *E. coli* and other *Enterobacteriaceae*, but for *Klebsiella* spp., a fall was seen initially. Except for *Enterobacter* spp., most others showed an increasing resistance for IPM, as well as PTZ and CFS; the three drugs most commonly used in ICU settings. Sporadic resistant strains were seen appearing for PB, which, apart from TGC, remained the last line of agents for IPM-resistant isolates. There was no isolate of *Enterobacteriaceae* family and non-fermenters resistant to TGC.

The resistance patterns of two important hospital pathogens, namely *Pseudomonas* spp. and non-fermentative Gram-negative bacilli (NFGNB, primarily *Acinetobacter* spp.) are shown in Table III. An increase in resistance was shown by *Pseudomonas* spp. for CFS, PTZ, AK and IPM. For the non-fermenters, resistance frequency remains very high for most of the antimicrobials, ranging from 40 per cent to >80 per cent, except for IPM (33.1%) and PB (2.4%).

Table IV gives the resistance pattern for the Gram-positive isolates. Methicillin resistance (using the surrogate marker CXT) amongst the *S. aureus* crossed the 50 per cent mark while for coagulase-negative staphylococci (CONS), it was >40 per cent. However, a small decrease in the frequency of resistance was observed for both *S. aureus* and CONS over the years. From 2012, a rise in VAN-resistant enterococci (VRE) was observed (from 4.3 to 8.3 per cent in 2013) although all the other Gram-positive isolates remained susceptible to VAN as well as LIZ.

Discussion

VAP remains a serious complication amongst critical care patients in any healthcare setting and its

Table I. Distribution of ventilator-associated pneumonia pathogens during the study period (2011-2013)

Organisms	2011	2012	2013
Gram-negative pathogens			
Escherichia coli	86 (14.9)	99 (13.4)	59 (11.5)
Klebsiella spp.	137 (23.7)	164 (22.2)	99 (19.3)
Enterobacter spp.	49 (8.5)	41 (5.6)	87 (16.9)
Other *Enterobacteriaceae*	29 (5.0)	74 (10.0)	27 (5.3)
Pseudomonas spp.	134 (23.2)	178 (24.1)	113 (22.1)
NFGNB	143 (24.7)	181 (24.5)	127 (24.8)
Total	578	737	512
Gram-positive pathogens			
Staphylococcus aureus	37 (50.0)	47 (40.2)	29 (34.9)
CONS	17 (22.9)	26 (22.2)	24 (28.9)
Streptococcus spp.	5 (6.8)	21 (17.9)	18 (21.7)
Enterococcus spp.	15 (20.3)	23 (19.7)	12 (14.5)
Total	74	117	85
Candida spp.	18	22	23

Figures in parentheses are percentages. CONS, coagulase-negative staphylococci; NFGNB, non fermentative Gram-negative bacilli

Table II. Antimicrobial resistance (in %) of members of *Enterobacteriaceae* family

Antimicrobial agents	*Escherichia* coli			*Klebsiella* spp.			*Enterobacter* spp.			Other *Enterobacteriaceae*		
	2011 (n=86)	2012 (n=99)	2013 (n=59)	2011 (n=137)	2012 (n=164)	2013 (n=99)	2011 (n=49)	2012 (n=41)	2013 (n=87)	2011 (n=29)	2012 (n=74)	2013 (n=27)
AMP	96.5	91.9	96.6	97.1	95.7	94.9	77.5	100	89.6	100	97.3	85.2
AXV	87.2	70.7	93.2	87.6	71.9	83.8	71.4	82.9	80.4	79.3	83.8	74.1
CTX	79.1	81.8	89.8	77.4	72.6	78.8	57.1	85.4	65.5	65.5	90.5	66.7
CTZ	32.5	20.2	25.4	31.4	27.4	31.3	22.4	41.5	41.4	41.4	36.5	37.0
CPM	27.9	20.2	25.4	33.6	29.9	30.3	22.4	43.9	39.1	20.7	41.9	44.4
CFS	11.6	22.2	18.6	26.3	30.5	32.3	20.4	34.1	31.0	17.2	40.5	18.5
PTZ	6.9	15.1	20.3	25.5	28.0	29.3	22.4	34.1	31.0	17.2	33.8	11.1
IPM	6.9	7.0	8.4	13.1	12.8	18.1	10.2	26.8	17.2	10.3	10.8	18.5
AK	15.1	25.2	30.5	37.2	30.5	23.2	28.6	48.8	43.7	34.5	59.4	66.7
GEN	34.9	32.2	47.4	56.9	36.6	39.4	38.8	51.2	56.3	58.6	54.0	74.1
NET	9.3	14.1	16.9	25.5	25.0	23.2	16.3	26.8	29.9	10.3	31.1	29.6
AZT	26.7	20.2	25.4	32.8	31.1	31.3	22.4	41.5	27.6	20.7	39.2	18.5
CHL	5.8	11.1	10.2	12.4	13.4	18.2	10.2	24.4	29.9	17.2	28.4	33.3
COT	76.7	67.7	72.8	77.4	66.5	74.7	48.9	75.6	64.4	72.4	50.0	77.8
CIP	82.5	78.8	84.7	53.3	45.1	52.5	42.8	63.4	56.3	72.4	71.6	70.4
PB	1.1	1.0	1.7	0	1.8	0	6.1	2.4	0	0	2.7	0

Please refer to Material & Methods section for full forms of antimicrobials

Table III. Antimicrobial resistance (in %) of *Pseudomonas* spp. and non-fermentative Gram-negative *Bacilli*

Antimicrobial agents	*Pseudomonas* spp.			NFGNB		
	2011 (n=134)	2012 (n=178)	2013 (n=113)	2011 (n=143)	2012 (n=181)	2013 (n=127)
AMP	NT	NT	NT	94.4	93.9	94.5
AXV	NT	NT	NT	84.6	81.8	85.0
CTX	40.3	33.1	30.9	69.2	81.2	88.9
CTZ	42.5	31.5	31.8	35.7	51.4	56.7
CPM	8.2	2.8	14.1	31.5	53.0	69.3
CFS	11.9	11.2	19.5	23.1	51.9	40.1
PTZ	8.2	7.9	15.9	18.2	39.2	55.9
IPM	8.2	6.2	13.3	18.9	16.0	33.1
AK	17.9	15.7	18.6	56.6	70.7	67.7
GEN	47.0	24.1	23.9	69.9	61.9	72.4
NET	43.3	20.2	15.9	12.6	38.7	45.7
AZT	8.2	3.4	12.4	32.2	51.4	51.9
CHL	NT	NT	NT	16.1	44.2	33.8
COT	NT	NT	NT	75.5	82.9	80.3
CIP	38.0	23.0	24.8	69.2	79.0	77.9
PB	8.2	5.6	5.3	2.1	1.6	2.4

Please refer to Material & Methods section for full forms of antimicrobials. NFGNB, non-fermentative Gram-negative bacilli; NT, not tested

Table IV. Antimicrobial resistance (in %) of Gram-positive pathogens

Antimicrobial agents	*Staphylococcus aureus*			CONS			*Streptococcus* spp.			*Enterococcus* spp.		
	2011 (n=37)	2012 (n=47)	2013 (n=29)	2011 (n=17)	2012 (n=21)	2013 (n=24)	2011 (n=5)	2012 (n=21)	2013 (n=18)	2011 (n=15)	2012 (n=23)	2013 (n=12)
AMP	94.6	82.9	89.6	94.1	85.7	83.3	Nil	28.6	27.7	53.3	34.8	66.7
AXV	83.8	85.1	62.1	82.3	57.1	62.5	Nil	4.8	11.1	40.0	8.7	16.7
GEN	59.5	34.0	51.7	47.8	38.1	37.5	NT	NT	NT	NT	NT	NT
COT	64.9	70.2	51.7	94.1	61.9	58.3	52.4	52.4	66.7	20.0	4.3	8.3
CIP	67.6	59.6	65.5	64.7	47.6	50.0	4.8	4.8	11.1	46.7	26.1	66.7
ERY	56.7	57.4	48.3	64.7	47.6	45.8	19.0	19.0	16.7	60.0	34.8	50.0
PEN	94.6	87.2	93.1	94.1	90.5	91.7	38.1	38.1	33.3	53.3	39.1	33.3
CXT	78.4	57.4	51.7	70.6	42.8	41.7	NT	NT	NT	NT	NT	NT
VAN	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	4.3	8.3
LIZ	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Please refer to Material & Methods section for full form of antimicrobials. CONS, coagulase-negative staphylococci; NT, not tested

incidence has been found to be highly variable not only amongst hospitals, but also in different ICUs in the same hospital. The infection rates seen in the present study were comparable to the figures of 35.1 and 37 per cent in two different studies from India[17,18]. The VAP rate per 1000 ventilator days is a commonly used parameter worldwide, and although its rate was quite low in the USA (4.4)[4], in various other countries, it was found to be high in the range of 13-51 per 1000 ventilator days[5]. In various studies from India, the VAP rates were 22.9 from Puducherry[7] and 26 and 27.77, respectively, from two separate studies from Mumbai[19,20]. In the initial two years of our study, the VAP rates were high (44.1 and 43.8 per 1000 ventilator days), but could be brought down to 26.3 per 1000 days in 2013.

Both Gram-positive and Gram-negative bacteria are implicated in VAPs, and ESKAPE organisms (*Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa* and *Enterobacter* spp.) constitute 80 per cent of the VAP epoisodes[21]. Contrary to this, in a large international study of the 606 cases of VAP, Gram-positive pathogens were the predominant isolates (72.8%) with methicillin-resistant *S. aureus* (MRSA) constituting 42.7 per cent[22]. This finding was in contrast to other reports, where Gram-negative bacteria (particularly *Pseudomonas* spp., *Acinetobacter* spp., *E. coli* and *Klebsiella* spp.) were the predominant isolates from VAP[23,24]. In our study, the Gram-negative bacilli were the principal isolates, and *Pseudomonas* spp., non-fermenters and *Klebsiella* spp. were the most common pathogens with almost similar proportion of frequency, and these findings were corroborated by other Indian studies[17,25].

The introduction of extended spectrum third-generation cephalosporins about three decades back resulted in mutations in both bla_{TEM} and bla_{SHV} genes which were mainly reported amongst the *Klebsiella* spp[26]. However, in the last 10 years, there has been a rise in the prevalence of CTX-M phenotype which has spread to *E. coli*[27]. In our study the resistance to CTX rose to 78.8 per cent in *Klebsiella* spp. and almost 90 per cent in *E. coli*. Although these inactivating agents can be inhibited by β-lactamase inhibitors, non-susceptibility to PTZ in CTX-M-producing *E. coli* and *Klebsiella* spp. was 27.4 and 38.1 per cent, respectively, in a European study[28]. The situation was similar in our setting where resistance to piperacillin-tazobactam was on the rise not only for the *Enterobacteriaceae* members but also for non-fermenters. A similar trend was shown for cefoperazone-sulbactam, which is another potent agent widely used in the ICU settings.

The incidence of IPM-resistant *Enterobacteriaceae* was highest amongst *Enterobacter* spp., and other members such as *Citrobacter* spp. and Proteus group. In *E. coli* and *Klebsiella* spp., a rising trend could be observed in our study. In the multinational SENTRY study (2007-2009), the overall carbapenem resistance in *Klebsiella* spp. was 5.3 per cent, while it was 0.3 per cent in *E. coli*[29]. Increasing IPM resistance was also shown by *Pseudomonas* spp. in our study and amongst the non-fermenters, 33.1 per cent of the strains became IPM resistant by 2013. In one Indian study, the figures were 40 and 37.5 per cent for *P. aeruginosa* and *Acinetobacter*

spp., respectively[17]. In Europe, the non-susceptibility rates for *P. aeruginosa* have been reported to increase to about 20 per cent for carbapenems, 25 per cent for aminoglycosides and about 8 per cent for PTZ[30]. In Spain, however, the situation was different for *Acinetobacter* spp., with resistance rates of 45 per cent for carbapenem, 70 per cent for PTZ, 35 per cent for AK and 40 per cent for CTZ[31]. These findings point to an increasing global emergence of highly resistant strains of *Pseudomonas* spp. and *Acinetobacter* spp. In such cases of carbapenem resistance, colistin/polymyxin B remains the last option, and in our centre, the polymyxin B resistance rates were 1.6-2.4 per cent for non-fermenters and 5.3-8.2 per cent for *Pseudomonas* spp. In Europe, 0.4-0.8 per cent of *Pseudomonas* spp. and *Acinetobacter* spp. has been found to be colistin resistant[32].

MRSA is another global problem with prevalence reaching up to 25-50 per cent in America, Australia and South Europe[33]. This study showed >50 per cent of *S. aureus* and >40 per cent of CONS isolates being methicillin resistant. One important finding of our study was the emergence of VRE in 4.3 per cent in 2012 rising to 8.3 per cent in 2013. This is alarming since the high prevalence of VRE has been linked to the emergence of VAN-resistant *S. aureus*[34].

In conclusion, the findings showed VAP as a problem in the ICU setting, with high percentage of multidrug-resistant pathogens. The resistance pattern of these pathogens along with their profile can help an institution to formulate effective antimicrobial policy for VAP based on evidence of the local scenario, along with the necessary infection control measures. Further, to have a comprehensive pan-India picture, multicentric studies with high number of patient population need to be initiated.

***Conflicts of Interest*:** None.

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