

Role of intensive care unit environment and health-care workers in transmission of ventilator-associated pneumonia

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Abstract

Background: *Pseudomonas aeruginosa* and *Acinetobacter baumannii* have been reported to cause outbreaks of ventilator-associated pneumonia (VAP) in several studies. The high prevalence of these pathogens prompted us to study the different strains of these pathogens prevailing in our intensive care units (ICUs) and determine the role of ICU environment and health-care workers (HCWs) in the transmission of infection.

Methodology: A prospective study was performed over a period of 15 months in two ICUs of Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Pondicherry, India. Surveillance samples were collected from the HCWs and the ICU environment. Quantitative antibiogram typing and PCR-RFLP were used for comparison of the isolates from the surveillance samples and VAP patients.

Results: *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were the most common potential VAP pathogens isolated from the surveillance cultures. Eight strains of *Pseudomonas aeruginosa* were present in our ICUs, but multi-drug resistant (MDR) strain 2 and strain 4 were the most prevalent strains. Six strains of *Acinetobacter baumannii* were found in our ICUs, of which MDR strain 1 and strain 3 were the most common. The strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* observed in the VAP patients were also found in the ICU milieu. Only one HCW was found to be the carrier of a *Pseudomonas aeruginosa* strain present in a VAP patient.

Conclusions: The ICU environment was observed to be the potential reservoir for VAP pathogens; therefore, strict adherence to environmental infection control measures is essential to prevent health-care-associated infections.

Key words: ventilator-associated pneumonia, ICU environment, health-care workers, multi-drug resistant pathogens, quantitative antibiogram typing, RFLP

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Introduction

Ventilator-associated pneumonia (VAP), defined as pneumonia occurring more than 48 hours after the initiation of endotracheal intubation and mechanical ventilation (MV), is a common nosocomial infection in intensive care units (ICUs), with an incidence ranging from 6% to 52% and even reaching 76% in some specific settings [1,2]. Several studies have shown that critically ill patients are at high risk for getting such nosocomial infections [3,4]. VAP continues to be a major cause of morbidity, mortality and increased financial burden in ICUs [5,6].

Pseudomonas aeruginosa and *Acinetobacter baumannii* are the most common pathogens causing VAP [7]. VAP caused by these organisms are often associated with high morbidity and mortality as they are often multi-drug resistant [8]. Outbreaks of VAP

caused by *Pseudomonas aeruginosa* and *Acinetobacter baumannii* have been reported in several studies [9,10]. Health-care workers (HCWs), contaminated equipment, and the ICU environment have been implicated in these outbreaks [9,10]. These pathogens usually survive in the ICU environment and equipment and transmit infection [9,11]. Cross-transmission can also occur from patient to patient via hands of the health-care personnel [9].

In a study done in an intensive care unit in a tertiary hospital in North India, 34.1% of 182 patients had one or more nosocomial infections [12]. Similarly, in another study involving ICUs of seven Indian cities, central venous catheter-related bloodstream infection rate was 7.92 per 1,000 catheter-days; the ventilator-associated pneumonia (VAP) rate was 10.46 per 1,000 ventilator-days; and

the catheter-associated urinary tract infection rate was 1.41 per 1,000 catheter-days [13]. Despite the high prevalence of nosocomial infections in India, there are not many studies on surveillance of ICUs for potential pathogens. However, in a study from North India, surveillance cultures performed to detect the source of *Stenotrophomonas maltophilia* causing empyema in a patient revealed the presence of the same isolate with the same sensitivity pattern in the povidone iodine used in the ICU [14]. In an earlier study, we reported a very high prevalence of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* among the VAP patients [7]. The high prevalence of these pathogens in our ICUs and the paucity of literature on ICU surveillance in India prompted us to study the different strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* prevailing in our ICUs and determine the role of the ICU environment and health-care workers in the transmission of VAP.

Methods

Study design and Setting

A prospective study was conducted during a 15-month period from October 2006 to December 2007 in the medical intensive care unit (MICU) and the critical care unit (CCU) of Jawaharlal Institute of Post-graduate Medical Education and Research (JIPMER), an 860-bedded tertiary care hospital and Institution of National Importance in India.

There are eight beds separated by curtains in each of these ICUs with adequate space for movement of staff and equipment. A total of eight HCWs (two doctors, three nurses and three nurses' aides) are posted in each of these ICUs.

Study population and surveillance samples

During the study period samples were taken from the 16 HCWs posted in the ICUs on four different occasions from their throats, noses and hands after obtaining informed consent. Environmental samples were collected from the ventilator circuit, suction apparatus, beds, air-conditioning vents, floor, medicine trolley, window sills, door handles, and wash basins. Humidifier fluid used for humidifying the oxygen supplied to the patients was also sampled. Ten *Pseudomonas aeruginosa* and ten *Acinetobacter baumannii* isolated from 36 VAP patients during the study period were used for comparison. The details of these VAP patients have been described in our previously published study [7].

Microbiological processing

Throat and nasal samples from HCWs were collected with swabs pre-moistened with sterile distilled water, while their hand impressions were taken directly on blood agar. Environmental samples from dry surfaces were taken with absorbent cotton-wool swabs, which were moistened with peptone water. Air cultures were made on blood agar settle plates [15]. The swabs were inoculated within one hour in enriched brain heart infusion broth and incubated for 24 hours at 37°C. After incubation the broth was subcultured on 5% sheep blood agar and MacConkey agar. The bacteria isolated from all these cultures were identified based on standard bacteriological techniques [16]. The susceptibility of the clinical isolates to amikacin, ceftazidime, ciprofloxacin, meropenem, piperacillin-tazobactam and colistin was determined by the Kirby-Bauer disc diffusion method [17].

Typing of the bacterial isolates

I. Quantitative antibiogram typing: Quantitative antibiogram typing was done based on the Euclidean distance (ED) as a similarity coefficient [18]. The Euclidean distance is the square root of the sum of the squared differences between inhibition zones for various antibiotics. For example, if 4, 3, and 1 are the differences in the inhibition zones of two isolates to antibiotic A, B and C respectively, the Euclidean distance is calculated as follows:

$$ED = \sqrt{(4)^2 + (3)^2 + (1)^2} = \sqrt{16 + 9 + 1} = \sqrt{26} = 5.1.$$

The smaller the distance between two isolates, the greater is the resemblance between them. Some amount of variation in the inhibition zones are known to occur even when the same isolate is tested repeatedly [18]. Therefore, the antibiograms of several isolates obtained from various sources were determined twice on different days and similarities between them were analyzed to define the cut-off distance below which the differences are due to such permissible variations. The cut-off is taken as the distance below which the difference in the inhibition zones of 95% of isolates tested twice would be smaller than the cut-off. Accordingly, two isolates are considered similar if their Euclidean distance is lesser than the cut-off.

II. Molecular typing of *Pseudomonas aeruginosa*: PCR-RFLP was performed for *Pseudomonas*

aeruginosa from the VAP patients, the ICU environment, and the HCWs to determine how the isolates are related to each other.

1. DNA preparation

DNA was prepared from bacteria as described by Liu *et al.* [19]. Lysis buffer containing 0.25% (vol/vol) sodium dodecyl sulfate and 0.05 N NaOH was prepared. A few colonies of the test strain were suspended in 20 µl of lysis buffer and heated for 15 minutes at 95°C. After heating, 180 µl of high-performance liquid chromatography-grade H₂O was added to it. The lysis suspension was stored at -20 °C.

2. Polymerase chain reaction (PCR)

PCR was done as described by Spilker *et al.* [20]. In brief, the amplification of targeted DNA was carried out in 25-µl reaction volumes, each containing 2 mM MgCl₂, 50 mM Tris-HCl, 250 µM (each) deoxynucleoside triphosphates, 0.4 µM (each) primer, 1 U of Taq polymerase, and 2 µl of whole-cell bacterial lysate, and adjusted to 25 µl by the addition of high-performance liquid chromatography-grade H₂O. The primer sequences used were GACGGGTGAGTAATGCCTA (Forward) and CACTGGTGTTCCTTCCTATA (Reverse). After an initial denaturization for 2 minutes at 95°C, 30 cycles were completed, each consisting of 20 seconds at 94°C, 20 seconds at 54°C and 40 seconds at 72°C. A final extension of 1 minute at 72°C was applied. The length of the amplicon was about 618 base pairs.

3. Restriction fragment length polymorphism (RFLP)

The amplified PCR products were digested with 3 U of restriction enzyme, *HinfI* according to the recommendations of the manufacturer (Bangalore Genei, Bangalore, India) and electrophoresed in 2% agarose gel in the presence of ethidium bromide at 75 V for 2 hours [21].

Ethical consideration

This study was approved by the institute research and ethical committees and informed consent was obtained.

Statistical Analysis

In quantitative antibiogram typing, the similarity between two strains was analyzed based on the Euclidean distance between them and a dendrogram was obtained according to the unweighted pair group

method of analysis using statistics software (SPSS 16.0, SPSS Inc, Chicago, Illinois).

Results

A total of 352 samples, 192 from the HCWs and another 160 from the ICU environment, were collected. Sixty-four bacteria were recovered on different occasions from these surveillance samples. The details of those bacterial isolates are summarized in Table 1.

Surveillance for potential VAP pathogens

Of the 64 bacterial isolates from the surveillance cultures, only 37 were pathogenic organisms, while the remaining 27 were either normal skin flora (coagulase-negative staphylococci) or environmental contaminants (*Bacillus* spp). Members of Enterobacteriaceae such as *Klebsiella pneumoniae*, *Enterobacter* spp. and *Escherichia coli* were predominantly recovered from the ICU personnel, while non-fermentative Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were more often isolated from the environment (Table 1).

Typing of *Pseudomonas aeruginosa*

Quantitative antibiogram typing and PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) was performed on 22 *Pseudomonas aeruginosa*, which includes 12 organisms from the surveillance samples and another 10 from VAP patients. The *Pseudomonas aeruginosa* isolates used for typing are summarized in Table 2.

Quantitative antibiogram typing

A total of 53 *Pseudomonas aeruginosa* isolated from various samples such as pus, tracheal aspirate, sputum, ear discharge, urine, etc. were tested on two different days to assess the reproducibility of zone diameter measurement. Fifty-one of the 53 isolates (96.2%) tested twice had a Euclidean distance less than 7.0. So, a distance of 7.0 was defined as cut-off below which the differences were considered to be due to casual variation. Therefore, isolates with a Euclidean distance less than 7.0 were considered as similar strains.

The dendrogram obtained by antibiogram cluster analysis (quantitative antibiogram typing) of the 22 *Pseudomonas aeruginosa* isolates from surveillance samples and VAP patients is depicted in Figure 1.

Table 1. Bacteria isolated from the surveillance cultures

Surveillance site	No. of swabs	Organism isolated	No. of isolates
Health care worker			
Throat	64	<i>Klebsiella pneumoniae</i>	4
		<i>Enterobacter</i> spp.	3
		<i>Escherichia coli</i>	2
Anterior nares	64	CONS	6
		<i>Staphylococcus aureus</i>	2
Hands	64	CONS	16
		<i>Staphylococcus aureus</i>	4
		<i>Klebsiella pneumoniae</i>	2
		<i>Pseudomonas aeruginosa</i>	1
Environment			
Ventilator circuit	16	<i>Pseudomonas aeruginosa</i>	1
Suction apparatus	16	<i>Pseudomonas aeruginosa</i>	3
		<i>Acinetobacter baumannii</i>	2
		<i>Escherichia coli</i>	1
Bed	16	-	-
Air-condition vents	16	-	-
Floor	16	<i>Pseudomonas aeruginosa</i>	2
		<i>Acinetobacter baumannii</i>	1
		<i>Bacillus</i> spp.	1
		<i>Pseudomonas aeruginosa</i>	2
Medicine/dressing trolleys	16	<i>Acinetobacter baumannii</i>	1
		<i>Bacillus</i> spp.	1
Window sills	16	<i>Pseudomonas</i> spp.	1
		<i>Pseudomonas aeruginosa</i>	1
		<i>Acinetobacter baumannii</i>	1
Door handles	16	<i>Pseudomonas aeruginosa</i>	1
		<i>Acinetobacter baumannii</i>	1
Wash basins	16	<i>Escherichia coli</i>	1
		<i>Pseudomonas aeruginosa</i>	1
		-	-
Humidifier fluid	8	-	-
Air (Settle plate)	8	<i>Pseudomonas aeruginosa</i>	1
		<i>Bacillus</i> spp.	3

CONS – Coagulase-negative Staphylococci

The dendrogram shows two broad clusters of *P. aeruginosa*, of which one is susceptible to most of the antibiotics, while the other cluster includes multi-drug resistant (MDR) strains. The MDR cluster is further divided into two small clusters comprised of four different strains (Strain 1, 2, 3 and 4). Similarly, the drug-susceptible cluster consists of four dissimilar strains (Strain 5, 6, 7 and 8). Strain 2, the most predominant strain, was isolated from the VAP patients as well as the surveillance samples of both the ICUs. But the extremely drug-resistant strain 4 was exclusively recovered from the CCU, while strains 1 and 8 were present only in the MICU (Table 3).

RFLP typing

Restriction fragment length polymorphism of the *Pseudomonas aeruginosa* isolates showed two different patterns, *i.e.*, Type A and B (Figure 2). Among the isolates recovered from the VAP patients in CCU and MICU, 67% and 50% respectively

belonged to type A. Similarly, 57% and 60% of the *Pseudomonas aeruginosa* isolated from the surveillance samples obtained from the CCU and the MICU respectively were type A.

Quantitative antibiogram typing of *Acinetobacter baumannii*

The reproducibility of zone diameter measurement was assessed by repeated testing of 48 *Acinetobacter baumannii* isolated from a variety of samples such as wound discharge, tracheal aspirate, sputum, blood, etc. Forty-six of the 48 isolates (95.8%) tested twice on different occasions had a Euclidean distance of less than 6.0. So, a distance of 6.0 was considered as the cut-off to evaluate the similarity of the 15 *Acinetobacter baumannii* isolates (5 from the surveillance samples and 10 from VAP patients). The *Acinetobacter baumannii* isolates used for typing are summarized in Table 2. The dendrogram obtained by antibiogram cluster analysis (quantitative antibiogram typing) of those 15

Table 2. Bacterial isolates used for typing.

Isolate	Source of the isolate	Isolate code(s)*	No. of isolates
<i>Pseudomonas aeruginosa</i>	Hand of HCW	P/M/S/05	1
	Ventilator circuit	P/C/S/07	1
	Suction apparatus	P/C/S/11, P/M/S/04, P/C/S/10	3
	Floor	P/M/S/02, P/C/S/06	2
	Medicine/ dressing trolleys	P/C/S/08, P/M/S/03	2
	Door handle	P/M/S/01	1
	Wash basins	P/C/S/09	1
	Air (Settle plate)	P/C/S/12	1
	VAP patients	P/M/V/13 to P/M/V/16, P/C/V/17 to P/C/V/22	10
<i>Acinetobacter baumannii</i>	Suction apparatus	A/M/S/02, A/C/S/05	2
	Floor	A/C/S/03	1
	Dressing trolley	A/C/S/04	1
	Door handle	A/M/S/01	1
	VAP patients	A/M/V/06 to A/M/V/10, A/C/V/11 to A/C/V/15	10

* - Isolate code includes the following information: Isolate's name/ place of isolation/ source/ isolate number. E.g. *Pseudomonas aeruginosa*/ Medicine intensive care unit/ Surveillance sample/ 05 or P/M/S/05 *Pseudomonas aeruginosa*, A - *Acinetobacter baumannii*, M - Medicine intensive care unit, C - Critical care unit, S - Surveillance sample, V - VAP patient, HCW - Health-care worker
VAP - Ventilator-associated pneumonia

Acinetobacter baumannii isolates is depicted in Figure 3. The dendrogram shows a large MDR cluster consisting of four different strains and a small cluster comprised of two susceptible strains. Strain 2 and strain 4 were isolated only from patients in the MICU, while strain 6 was isolated from a patient suffering from VAP in the CCU. Strains 1, 3 and 5 were recovered evenly from both the MICU and the CCU (Table 3).

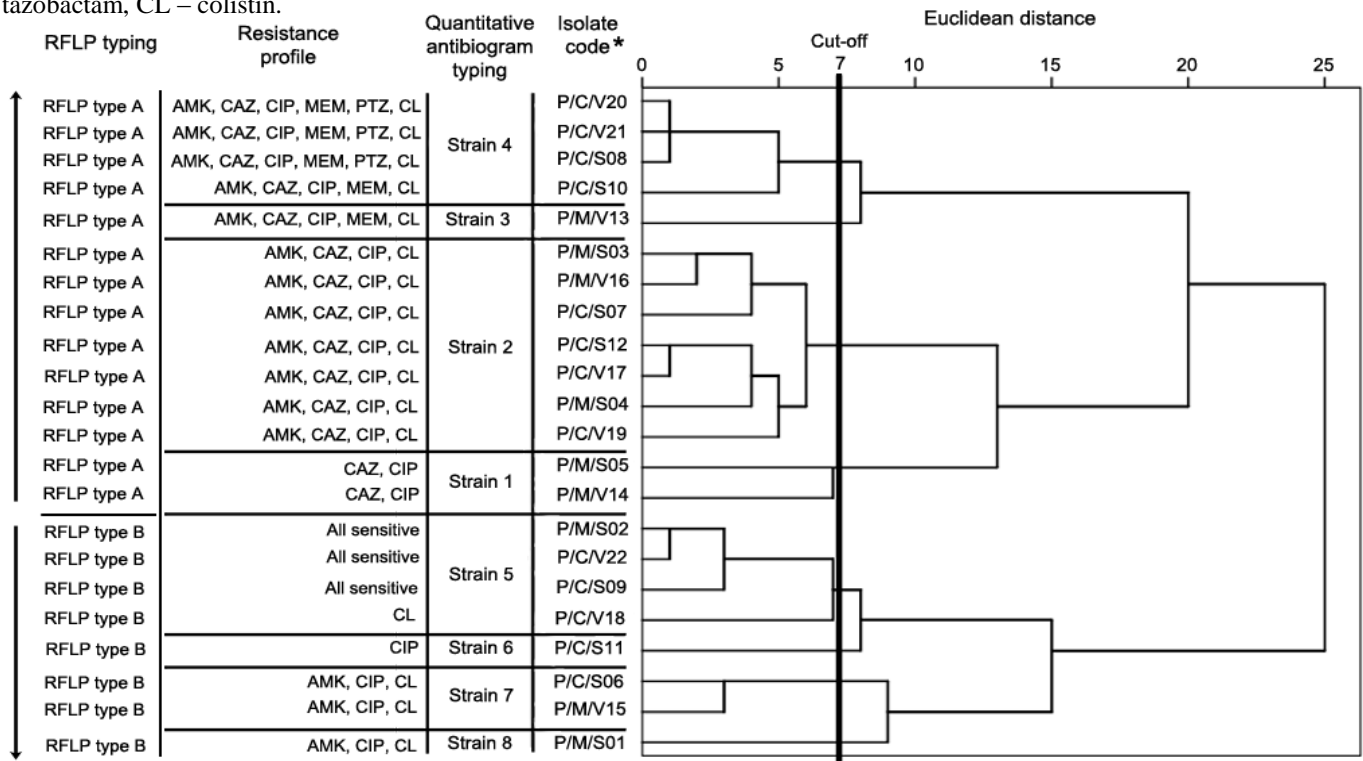
Discussion

VAP causes significant mortality and morbidity among ICU patients receiving MV. There are various sources from which the microorganisms can gain access to the respiratory tract and eventually cause VAP. The source of infection can be endogenous or exogenous [22,23]. The oropharyngeal colonization and gastric colonization can act as the endogenous source of microorganisms [8,22,24,25]. Contaminated respiratory instruments (bronchoscopes, ventilator circuits, humidifiers, and suction catheters), infective aerosols from the ICU environment, and contaminated hands and apparel of the HCWs (due to contact with other patients, contaminated taps, medicine trolleys and other fomites) are the major exogenous sources of infection [22,23,25]. Multidrug resistant VAP pathogens such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are known to survive in health care environments and are very effective human colonizers [9,26]. In our study these organisms were isolated on different occasions from our ICU environment including the ventilator circuit, floor,

medicine/dressing trolleys, and wash basins. The ICU environment and the equipment can get contaminated directly with the secretions/ discharges from patients during various patient care activities or indirectly through the contaminated hands of the HCWs. But in our study, only one HCW was found to be colonized by *P. aeruginosa*. Nevertheless, members of Enterobacteriaceae were recovered from many of the ICU personnel in our study. Methicillin resistant *Staphylococcus aureus* (MRSA) is usually the most common Gram-positive bacteria found as colonizers on the hands of HCWs [27]. In the present study, however, we did not isolate MRSA from any of the ICU personnel. A few months previous to our study, some HCWs were identified as MRSA carriers by routine surveillance and they have been treated successfully, which could be the reason for our failure to detect any MRSA carriers during the present study.

RFLP of the *Pseudomonas aeruginosa* strains revealed the presence of two clusters, of which type A was most prevalent. Comparison of the resistance profile of the isolates with their RFLP pattern reveals that the MDR strains show type A RFLP pattern, while the susceptible strains have type B pattern. The comparison also reveals the likely evolution of the strains. Strain 1, which shows resistance to only ceftazidime and ciprofloxacin, appears to be the parent strain of the type A cluster. It seems to have gradually acquired the other resistance genes, sequentially becoming strain 2, strain 3 and finally the most resistant strain 4. Strain 4 has established

Figure 1. Dendrogram of the 22 *Pseudomonas aeruginosa* isolates from the surveillance cultures and the VAP patients, based on antibiogram cluster analysis (quantitative antibiogram typing). The resistance profiles and the RFLP typing of the isolates are mentioned for comparison. AMK – amikacin, CAZ – ceftazidime, CIP – ciprofloxacin, MEM – meropenem, PTZ – piperacillin-tazobactam, CL – colistin.



* For details refer to Table 2.

Figure 2. RFLP using *HinfI*. Lanes M, 100 bp DNA ladder; lane 1 to 3, isolates from patients admitted in MICU; lane 4, isolate from medicine trolley in MICU; lane 5, isolate from a HCW in MICU; lane 6 to 8, isolates from patients admitted in CCU; lane 9, isolate from suction apparatus in CCU. Lane 1, 2, 4, 7, 8 and 9 are showing type A pattern, while lane 3, 5 and 6 are showing type B pattern.

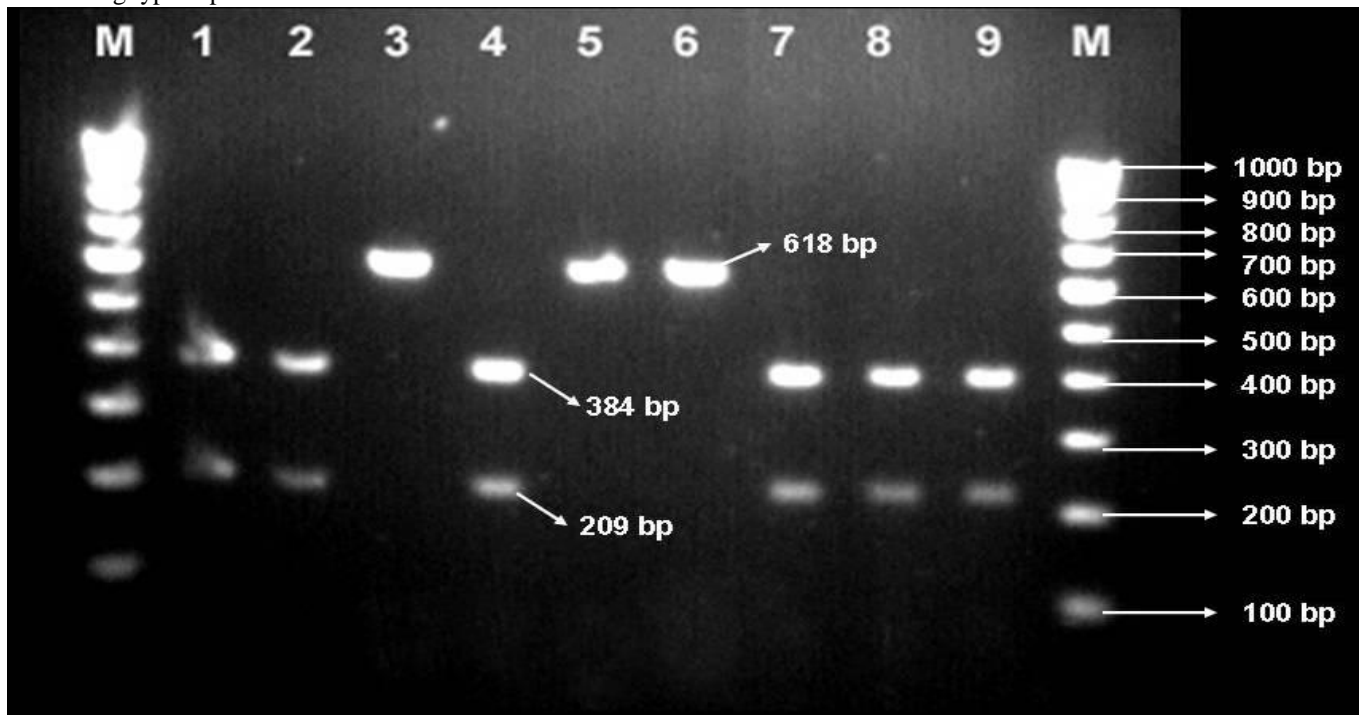


Table 3. Details of the strains prevalent in different ICUs

Bacteria	Strain	ICU	Source
<i>Pseudomonas aeruginosa</i>	Strain 4	CCU	VAP patients, trolley, suction apparatus
	Strain 6	CCU	Suction apparatus
	Strain 3	MICU	VAP patient
	Strain 1	MICU	Hands of HCW and VAP patient
	Strain 8	MICU	Door handle
	Strain 2	MICU and CCU	Trolley, suction apparatus and VAP patient in MICU and ventilator circuit, air and VAP patients in CCU
	Strain 5	MICU and CCU	MICU floor and VAP patients and wash basin in CCU
<i>Acinetobacter baumannii</i>	Strain 7	MICU and CCU	CCU floor and VAP patient in MICU
	Strain 2	CCU	VAP patient
	Strain 4	CCU	VAP patient
	Strain 5	MICU	VAP patients
	Strain 6	MICU	VAP patient
	Strain 1	MICU and CCU	Door handle and VAP patient in MICU and dressing trolley and VAP patient in CCU
	Strain 3	MICU and CCU	VAP patient and suction apparatus in MICU and VAP patients, suction apparatus and floor in CCU

M ICU – Medicine intensive care unit

CCU – Critical care unit

VAP – Ventilator-associated pneumonia

itself as a major pathogen and has become a cause for concern in the CCU. In the type B cluster, strain 5, the most susceptible strain, appears to be the parent strain which has evolved into strains 6, 7 and 8 in a similar fashion.

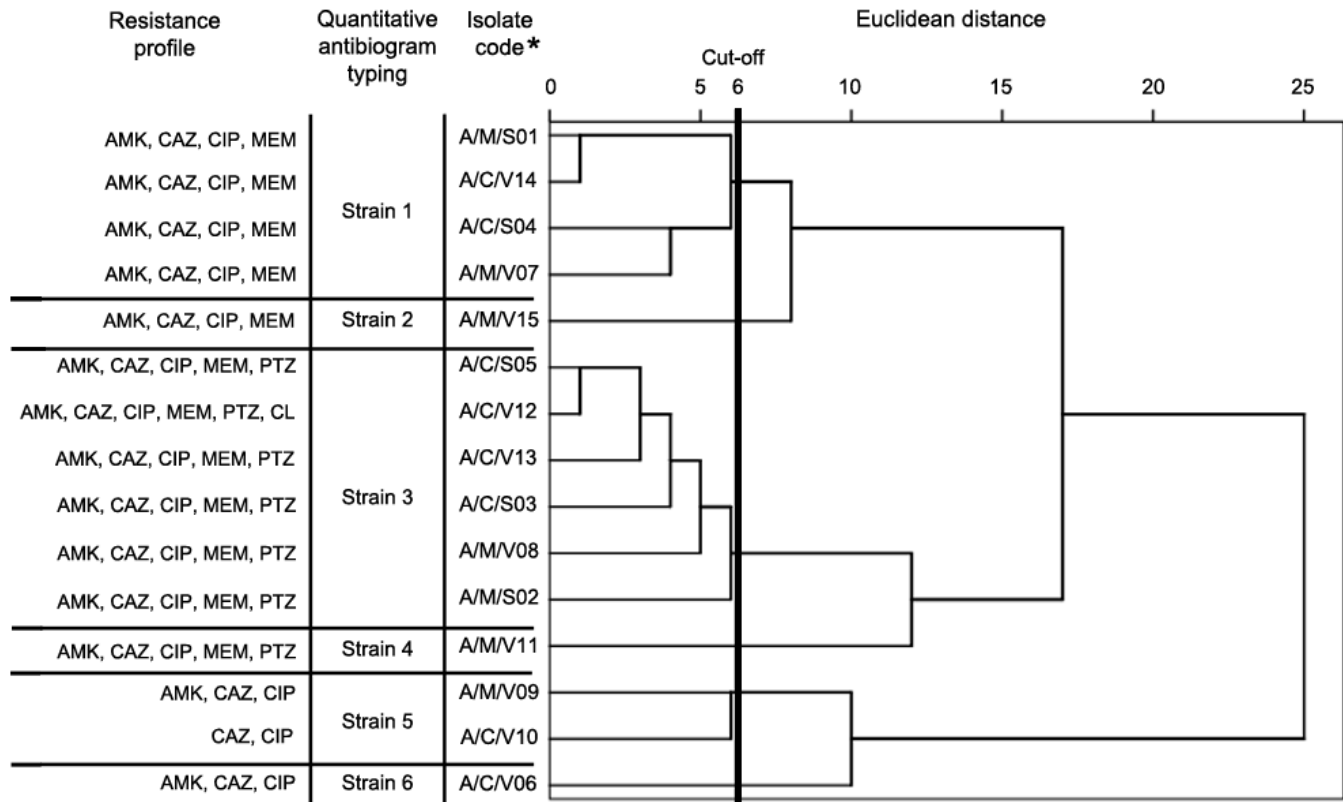
We observed that though *Acinetobacter baumannii* is quite rampant among our ICU patients, only a few isolates were recovered from the ICU environment compared to *P. aeruginosa*. This suggests that *Acinetobacter baumannii* is probably not as efficient as *Pseudomonas aeruginosa* in surviving in the environment. The quantitative antibiogram typing revealed that there were two clusters of *Acinetobacter baumannii*, of which the MDR cluster is larger, while only very few isolates belonged to the susceptible cluster. In the MDR cluster, strain 3 was the most resistant and prevalent strain in both the ICUs. The dendrogram of *Acinetobacter baumannii* also suggests that strain 1 could have been the parent strain which has subsequently evolved into strain 2, strain 3 and strain 4.

Although certain strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were indigenous to a particular ICU, others were found to be circulating between both the ICUs, as quite often the patients from one of these ICUs are transferred to the other ICU carrying the new strain with them. During our study, we could not document an apparent outbreak, as there was no clustering of VAP cases caused by a single strain from a single point source.

But in our study, based on the quantitative antibiogram typing, we noted that similar strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were present in the VAP patients as well as the ICU milieu. For instance, *Pseudomonas aeruginosa* strain 4 isolated from VAP patients in the CCU was also recovered from the suction apparatus and trolley in the CCU. This confirms that the same strains are being transmitted from the environment to the patients and/or vice versa. HCWs are generally considered as the primary mediators involved in such transmissions [28]. However, in our study, only one strain of *Pseudomonas aeruginosa* isolated from a VAP patient in the MICU was found on the hands of a HCW. We failed to recover other strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from the HCWs because most of them washed their hands with disinfectants just before we collected their samples, as they did not want to be identified as carriers of potential VAP pathogens. Consequently, we could not elucidate the exact mode of transmission of the pathogens between the environment and the patients. Nevertheless, we have isolated many VAP pathogens from various sites and instruments in the ICUs. So, although the ICU environmental surfaces cannot be considered as the *de facto* sources of exposure, they are potential reservoirs for the VAP pathogens.

As the health-care environment often contains a diverse population of microorganisms, the Centre for

Figure 3. Dendrogram of the 15 *Acinetobacter baumannii* isolates from the surveillance cultures and the VAP patients, based on antibiogram cluster analysis (quantitative antibiogram typing). The resistance profiles of the isolates are mentioned for comparison. AMK – amikacin, CAZ – ceftazidime, CIP – ciprofloxacin, MEM – meropenem, PTZ – piperacillin-tazobactam, CL – colistin.



* For details refer to Table 2.

Disease Control and Prevention (CDC) recommends strict adherence to hand hygiene to prevent health-care-associated infections [28]. In addition, the current CDC guidelines also recommend disinfection of medical equipment surfaces, bedside equipment, and environmental surfaces (e.g., bedrails, bedside tables, carts, commodes, doorknobs, and faucet handles) with a low- or intermediate-level disinfectant to prevent the spread of health-care-associated infections [29]. However, the routine use of germicidal chemicals to disinfect hospital floors is not recommended as within a few hours after floor disinfection, the bacterial count returns to the pre-treatment level [29]. If these infection control measures are not practiced, there is a potential risk of future outbreaks.

The major limitation of our study is the relatively low discriminatory power of PCR-RFLP as we had used only one restriction enzyme (*HinfI*), instead of multiple restriction enzymes. Therefore, we could only broadly classify the clusters of *Pseudomonas*

aeruginosa without being able to genetically distinguish the different strains within the cluster. The other limitation is that we have not studied hand hygiene and other infection control measures practiced in our ICUs; therefore, future studies are needed to examine the various infection control measures and evaluate the usefulness of such measures.

To conclude, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were the most common potential VAP pathogens isolated from the surveillance cultures. Eight different strains of *Pseudomonas aeruginosa* were present in our ICUs, but MDR strain 2 and strain 4 were the most prevalent strains. Six strains of *Acinetobacter baumannii* were found in our ICUs, of which MDR strain 1 and strain 3 were the most common. There was no evidence of an outbreak, but similar strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were observed in the VAP patients as well as in the ICU milieu. Only one HCW was found to be the carrier of a *Pseudomonas aeruginosa* strain

present in a VAP patient. However, the ICU environment was observed to be the potential reservoir for VAP pathogens. Therefore, strict adherence to environmental infection control measures is essential to prevent health-care-associated infections.

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References

- Davis KA (2006) Ventilator-associated pneumonia: a review. *J Intensive Care Med* 21: 211-226.
- Koenig SM and Truitt JD (2006) Ventilator-associated pneumonia: diagnosis, treatment, and prevention. *Clin Microbiol Rev* 19: 637-657.
- Lizan-Garcia M, Peyro R, Cortina M, Crespo MD, Tobias A (2006) Nosocomial infection surveillance in a surgical intensive care unit in Spain, 1996-2000: a time-trend analysis. *Infect Control Hosp Epidemiol* 27: 54-59.
- Nicastro E, Petrosillo N, Martini L, Larosa M, Gesu GP, Ippolito G (2003) Prevalence of nosocomial infections in 15 Italian hospitals: first point prevalence study for the INF-NOS project. *Infection* 31: 10-15.
- Erbay RH, Yalcin AN, Zencir M, Serin S, Atalay H (2004) Costs and risk factors for ventilator-associated pneumonia in a Turkish university hospital's intensive care unit: a case-control study. *BMC Pulm Med* 4: 3.
- Rello J, Ollendorf DA, Oster G, Vera-Llonch M, Bellm L, Redman R, Kollef MH (2002) Epidemiology and outcomes of ventilator-associated pneumonia in a large US database. *Chest* 122: 2115-2121.
- Joseph NM, Sistla S, Dutta TK, Badhe AS, Parija SC (2009) Ventilator-associated pneumonia in a tertiary care hospital in India: incidence and risk factors. *J Infect Dev Ctries* 3: 771-777.
- Chastre J and Fagon JY (2002) Ventilator-associated pneumonia. *Am J Respir Crit Care Med* 165: 867-903.
- Mayank D, Anshuman M, Singh RK, Afzal A, Baronia AK, Prasad KN (2009) Nosocomial cross-transmission of *Pseudomonas aeruginosa* between patients in a tertiary intensive care unit. *Indian J Pathol Microbiol* 52: 509-513.
- Baraibar J, Correa H, Mariscal D, Gallego M, Valles J, Rello J (1997) Risk factors for infection by *Acinetobacter baumannii* in intubated patients with nosocomial pneumonia. *Chest* 112: 1050-1054.
- Longtin Y, Troillet N, Touveneau S, Boillat N, Rimensberger P, Dharan S, Gervaix A, Pittet D, Harbarth S (2010) *Pseudomonas aeruginosa* outbreak in a pediatric intensive care unit linked to a humanitarian organization residential center. *Pediatr Infect Dis J* 29: 233-237.
- Habibi S, Wig N, Agarwal S, Sharma SK, Lodha R, Pandey RM, Kapil A (2008) Epidemiology of nosocomial infections in medicine intensive care unit at a tertiary care hospital in northern India. *Trop Doct* 38: 233-235.
- Mehta A, Rosenthal VD, Mehta Y, Chakravarthy M, Todi SK, Sen N, Sahu S, Gopinath R, Rodrigues C, Kapoor P, Jawali V, Chakraborty P, Raj JP, Bindhani D, Ravindra N, Hegde A, Pawar M, Venkatachalam N, Chatterjee S, Trehan N, Singhal T, Damani N (2007) Device-associated nosocomial infection rates in intensive care units of seven Indian cities. Findings of the International Nosocomial Infection Control Consortium (INICC). *J Hosp Infect* 67: 168-174.
- Mukhopadhyay C, Bhargava A, Ayyagari A (2003) Novel nosocomial infections by *Stenotrophomonas maltophilia*: first reported case from Lucknow, North India. *J Clin Microbiol* 41: 3 989-3990.
- Senior BW (1996) Examination of water, milk, food and air. In Collee JG, Marmion BP, Fraser AG, Simmons A, editors. *Mackie and McCartney's Practical Medical Microbiology*. New York: Churchill Livingstone. 883-921.
- Collee JG, Marmion BP, Fraser AG, Simmons A (1996) *Mackie and McCartney's Practical Medical Microbiology*, 14th edition. New York: Churchill Livingstone, 978 p.
- Clinical Laboratory Standards Institute (2006) Performance standards for antimicrobial disk susceptibility tests. In: CLSI document M2-A9. CLSI: Wayne, PA.
- Blanc DS, Lugeon C, Wenger A, Siegrist HH, Francioli P (1994) Quantitative antibiogram typing using inhibition zone diameters compared with ribotyping for epidemiological typing of methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 32: 2505-2509.
- Liu L, Coenye T, Burns JL, Whitby PW, Stull TL, LiPuma JJ (2002) Ribosomal DNA-directed PCR for identification of *Achromobacter (Alcaligenes) xylosoxidans* recovered from sputum samples from cystic fibrosis patients. *J Clin Microbiol* 40: 1210-1213.
- Spilker T, Coenye T, Vandamme P, LiPuma JJ (2004) PCR-based assay for differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* species recovered from cystic fibrosis patients. *J Clin Microbiol* 42: 2074-2079.
- bd-El-Haleem D, Kheiralla ZH, Zaki S, Rushdy AA, bd-El-Rahiem W (2003) Multiplex-PCR and PCR-RFLP assays to monitor water quality against pathogenic bacteria. *J Environ Monit* 5: 865-870.
- Safdar N, Crnich CJ, Maki DG (2005) The pathogenesis of ventilator-associated pneumonia: its relevance to developing effective strategies for prevention. *Respir Care* 50: 725-739.
- Bergmans DC, Bonten MJ, van Tiel FH, Gaillard CA, Van der GS, Wilting RM, de Leeuw PW, Stobberingh EE (1998) Cross-colonisation with *Pseudomonas aeruginosa* of patients in an intensive care unit. *Thorax* 53: 1053-1058.
- Torres A, El-Ebiary M, Soler N, Monton C, Fabregas N, Hernandez C (1996) Stomach as a source of colonization of the respiratory tract during mechanical ventilation: association with ventilator-associated pneumonia. *Eur Respir J* 9: 1729-1735.
- Niederman MS, Craven DE (2005) Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *Am J Respir Crit Care Med* 171: 388-416.
- Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA (2007) Global challenge of multidrug-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 51:3471-3484.
- Pawun V, Jiraphongsa C, Puttamasute S, Putta R, Wongnai A, Jaima T, Tithsayatikom P, Wattanasri S (2009) An outbreak of hospital-acquired *Staphylococcus aureus* skin infection among newborns, Nan Province, Thailand, January 2008. *Euro Surveill* 14.

28. Sehulster LM, Chinn RY, Arduino MJ, Carpenter J, Donlan R, Ashford D, Besser R, Fields B, McNeil MM, Whitney C, Wong S, Juranek D, Cleveland J (2003) Guidelines for Environmental Infection Control in Health-Care Facilities. Recommendations from CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC). Available: http://www.cdc.gov/ncidod/dhqp/pdf/guidelines/eic_in_HCF_03.pdf . Accessed 26 February 2010.
29. Rutala WA, Weber DJ (2008) Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008. Available: http://www.cdc.gov/ncidod/dhqp/pdf/guidelines/Disinfection_Nov_2008.pdf . Accessed 26 February 2010.

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