5.0 DISCUSSION

*P. aeruginosa* has gained significance as one of the important nosocomial bacterial pathogen. This organism is ubiquitous and may be present in intensive care units (ICU’s) and critical care units (CCU’s), ventilators, intubation pipes, on surgical devices, catheters, liquid soaps and everything else we could think of. It mainly causes infections in hospitalized hosts, immuno-compromised hosts and patients with cystic fibrosis. Besides, other disease conditions such as pneumonias, urinary tract infections (UTI’s), bloodstream infections, surgical site infections and skin infections in the setting of burn injuries may be caused by this bacterium. Strains of *P. aeruginosa* have been found to be resistant to most classes of clinically relevant antibiotics. Aminoglycosides, cephalosporins and fluoroquinolones are some of the examples. Treatment of *P. aeruginosa* infections is very difficult because of the emergence of multi-drug resistant strains. Carbapenems are generally preferred as the last resort drugs for treating multidrug resistant strains. Since the introduction of the first carbapenem, imipenem in the 1980’s, others meropenem, panipenem, biapenem, ertapenem and doripenem have also been developed but the emergence of carbapenemases have limited their use in the clinical settings. However, there is a tremendous increase in the frequency of carbapenem resistance among *P. aeruginosa* strains. An increasing prevalence of infections due to multidrug-resistant (MDR) isolates has been reported from many countries of the world including India. There is thus, an urgent need, to characterize the resistant strains so as to develop suitable therapeutic and other control strategies.

The present study has therefore been designed with the objective of identifying multidrug resistant and metallo-β-lactamase (MBL) producing strains in the hospital setting of Himachal Pradesh since such strains pose a threat due to their inter and intra species spread. MBLs are emerging enzymes in Gram-negative bacilli that hydrolyze virtually all β-lactam drugs. Twelve families of MBLs have been identified so far. These are imipenemases (IMPs), Verona imipenemases (VIMs), New Delhi metallo-β-lactamases (NDM), Sao Paulo metallo-β-lactamases (SPM-1), Seoul imipenemases (SIM-1), KHM-1, German imipenemase (GIM-1), Dutch imipenemase (DIM-1), Tripolimetallo-β-lactamase (TMB-1), Serratiametallo-β-lactamase (SMB-1), Florence imipenemase (FIM-1), Australian imipenemase (AIM-1) (Zhao and Hu, 2011, Lee et al, 2010, Yong et al,
Out of over 800 β-lactamases identified from Gram-negative bacilli, at least 120 β-lactamases have been detected in *P. aeruginosa* (Zhao and Hu, 2010). The integrons which are genetic elements composed of a gene encoding an integrase, an integration site for the gene cassettes (att) and a promoter play a major role in the spread of MBL producing strains. Gene cassettes are small mobile elements that carry antibiotic resistance genes e.g. MBLs and a specific recombination site. Gene cassettes are captured by the integrin via an int1-mediated site-specific recombination between the 59-base elements (50-be) and att1 sites. 59 be is a cassette-associated recombination site which consists of diverse sequence families all of which share a common structure as shown in Fig 2.2.

For achieving the objective of the study, the isolates recovered from clinical specimens (pus, blood and urine) of patients suffering from different clinical conditions such as wound and burn cases, urinary tract infection and septicemia at Indira Gandhi Medical College (IGMC) Shimla in the state of Himachal Pradesh have been characterized for multidrug resistance, occurrence of metallo-β-lactamases (*bla*VIM-2 and *bla*IMP-1) and integron classes, integron class 1 (*int*1), class 2 (*int*2) and class 3 (*int*3). A total of 141 isolates out of 153 examined were confirmed as *P. aeruginosa* on the basis of typical colony morphology (mucoid, cream coloured, elevated colonies), phenotypic characteristics such as pigment production (pyocyanin and pyoverdine), microscopic examination of Gram stained preparations (Gram negative rods), motility, growth at 42°C, β-hemolysis on blood agar, (Woo et al, 2000). All these characteristics confirmed *P. aeruginosa* isolates. The preliminary identification of *P. aeruginosa* can be made from its grape-like odour due to aminoacetophenone. The mucoid colonies are presumed to play a role in colonization and virulence. Biochemical examination further supported the morphological features. Biochemically, all the isolates were oxidase positive and catalase positive which further helped in confirmation of *P. aeruginosa* (Bergey’s Manual of Systematic Bacteriology, 2001). Additionally, the 16SrRNAs of 27 isolates which were MBL producers (by phenotypic tests as discussed below) were partially amplified, there nucleotide sequences compared with standard strains. Nucleotide sequence homology of 100% was observed. The complete 16S rRNA amplicon (1396bp) of one of the isolate
Pa99 revealed 100% homology to the published sequences of *P. aeruginosa* strains which further authenticated the identity of *P. aeruginosa* isolates.

The antibiotics used in the antibiotic cultural sensitivity assay belonged to nine different antibiotic groups/classes namely, aminoglycosides, carbapenems, cephalosporins, macrolides, monobactams, penicillins, glycopeptides, quinolones and tigecycline. The criterion for considering an isolate as multidrug resistant was as follows: any isolate which was resistant to four or more classes of antimicrobials was regarded as MDR in the present study. Such criterion has also been used by Goyal et al (2010). Following this criterion, 69.50% (98/141) *P. aeruginosa* isolates were found MDR which is quite alarming. However, different workers define MDR isolates differently. Irfan et al (2008) and Saderi et al (2010) defined MDR isolate as the one which was resistant to two or more drugs or drug classes of therapeutic relevance. Souli et al (2008) regarded those strains as MDR which were resistant to three or more classes of antimicrobial agents.

We recorded highest percentage (100%) of strains resistant to cefazolin followed by ofloxacin resistant strains (48.94%), gatifloxacin (42.55%), tigecycline (41.13%), ticarcillin/clavulanic acid (39.71%), netillin and cefotaxime (38.29%) each, tobramycin (33.33%), gentamicin (26.95%) and imipenem (23.4%). The reason for such high prevalence of resistant strains to multiple antibiotics may be attributable to indiscriminate use of these antibiotics for treating *P. aeruginosa* infections in this geographical region. The resistance was also observed against other antibiotics but to a lesser extent. The proportion of strains resistant to other antibiotics ranged from 2.12%- 17.02% with cefoperazone (17.02%) at the top followed by meropenem (16.31%), ertapenem (14.89%), doripenem (10.64%), colistin (8.51%), cefepime and ciprofloxacin (6.38%) each. 5.61% isolates were resistant to each of the following antibiotics: ceftazidime, aztreonam and piperacillin and 4.96% each to carbenicillin and azithromycin, levofloxacin (3.55%) and 2.12% each to amikacin and piperacillin/tazobactum. We found 39.0% (55/141) isolates resistant to imipenem and 21.27 % (30/141) isolates resistant to meropenem (including intermediate resistance). 15.60% (22/141) and 4.96% (7/141) isolates showed intermediate resistance for imipenem and meropenem respectively. The emergence of intermediate resistance is of particular interest in that such strains can spread to the community causing difficulty in treating *P. aeruginosa* infections. It is for
this reason that such isolates were included among resistant strains in the present study. All the isolates of *P. aeruginosa* were susceptible to polymixin B. Similar observation has been made by Jovcic et al (2011). All the strains tested by us were resistant to cefazolin which is consistent with the observation of Deshmukh et al (2011). Our observation regarding low resistance (8.51%) against colistin is substantiated by Deshmukh et al (2011) and Rit et al (2013) who reported 100% susceptibility to colistin. Some workers have reported variability in the susceptibility of *P. aeruginosa* strains to colistin. However, Varaiya et al (2008) has reported higher frequency (51.7%) of strains resistant to colistin. Low percentage of strains resistant to amikacin seen in the present study (5.17%) is in tune with the report of Gad et al (2007) who reported 8% strains resistant to this antibiotic. Variability in the percentage of amikacin resistant strains up to 79% have been reported by various workers. Although very less resistance was found against amikacin. The sensitivity of the isolates to polymixin B, colistin, amikacin and aztreonam may have therapeutic application in this geographic region. High proportion of resistant strains has however, been observed against other three aminoglycosides; netillin (75.86%), tobramycin (60.34%), gentamicin (37.93%). The latter observation is consistent with others (Ahir et al, 2012, Strateva et al, 2007, Lee et al, 2001, Lauretti et al, 1999). High resistance was also observed to ofloxacin and gatifloxacin as compared to other quinolones (ciprofloxacin and levofloxacin) used in this study. Gad et al (2007) reported *P. aeruginosa* strains resistant to ofloxacin (23%) and 29% and 31% to Ciprofloxacin and levofloxacin whereas Khan et al (2008) reported 68.4% strains resistant to ofloxacin which is similar to what we observed. Higher percentage of strains resistant to cefotaxime (79.31%) and cefoperazone (43.10%) were observed by us while Gad et al (2007) reported 68% and 36 % strains resistant to cefotaxime and cefoperazone, whereas strains were reported resistant to these antibiotics by Deshmukh et al (2011). Similar percentages of high resistance of 94% and 93.4% respectively for cefotaxime and cefoperazone have been observed by Vahdani *et al* (2012) and Varaiya *et al* (2008). The results of the sensitivity assay would be helpful to the clinicians in this region in selecting the appropriate antibiotic for treating *P. aeruginosa* infections. At the same time they would not use the antibiotics to which most strains are resistant.
Besides *in vitro* antibiotic cultural sensitivity assay, the determination of minimum inhibitory concentrations of different carbapenems was also undertaken using Ezy MIC test. This test is an accurate and sensitive one, easy to perform and less time consuming. This test can be considered as an alternative to the agar and broth microdilution methods for quantitative estimation of antimicrobial substance. In this assay, the susceptibility criterion followed for the four carbapenems for *P. aeruginosa* has been as follows: The strains which were inhibited by a conc of ≤2µg/ml were considered as sensitive while those inhibited by a concentration of 4µg/ml as intermediate resistant and those inhibited by ≥8µg/ml were considered as resistant in the cases of all the four carbapenems. Based on this criterion 37 isolate were sensitive, three intermediate sensitive and 60 resistant to imipenem whereas 42, 27 and 31 respectively were sensitive, intermediate resistant and resistant to meropenem. In the same manner 77, 2 and 21 isolates respectively were sensitive, intermediate resistant and resistant to doripenem. In case of ertapenem 59, 9 and 35 isolates respectively were sensitive, intermediate resistant and resistant to this drug.

In various studies across the world, varying resistance has been seen towards imipenem and meropenem. All MBL producers were found resistant to multiple antibiotics and 58/141 (41.13%) were found resistant (isolates resistant to one or more carbapenems: resistance to imipenem was exhibited by maximum number 55/58 (94.83%) of isolates followed by ertapenem 34/58 (58.62%), meropenem 30/58 (51.72%) and doripenem 23/58 (39.65%) (Table 4.1). The carbapenem resistant isolates were also screened for the presence of metallo-β-lactamases. Intrestingly, all the carbapenem resistant isolates were not MBL producers by phenotypic methods but some isolates with intermediate zones were found to produce metallo-β-lactamases. The frequency of isolation of MBL-producing *P. aeruginosa* strains from clinical cases has been on the increase throughout the world during past few years. Cornaglia et al (2000) observed that MBL producing *P. aeruginosa* could spread as hospital infections as these workers observed an outbreak of carbapenem resistant *P. aeruginosa* in the hospital where carbapenems were not used for treatment and only 15% patients had received therapy with this group of antibiotics.
For the lack of uniform and clearly defined guidelines for detecting MBL producers, different groups have employed different methods such as modified Hodge test (MHT) and spectrophotometric determination (Lee et al, 2001, Lauretti et al, 1999). Some workers have also used screening methods such as double disk synergy test (DDST), combined disk test (CDT) and E-test for detecting MBL producing strains (Yong et al, 2002, Sharma et al, 2010 and Angadi et al, 2012). These tests utilize metal chelators such as EDTA. Jesudasen et al (2005) suggested that EDTA disc synergy could detect more carbapenemases and metallo beta lactamase producers as compared to modified Hodge test. We detected MBL production in 27 isolates (19.15%) of the entire collection of 141 isolates tested. Among the 58 carbapenem resistant strains, 27/58 (46.55%) were MBL producers. It is interesting to note that all P. aeruginosa isolates recovered from pus were MBL producers. However, the strains which originated from urine and blood were resistant to carbapenems but did not produce MBLs. This observation is of particular interest in that MBL production might be related to pathogenesis of P. aeruginosa infections and might have implications in treating wound infections. The resistance in non-MBL producing strains could be due to mechanisms other than MBL production such as efflux systems, decreased outer membrane permeability, or production of AmpC enzymes. The MBL producing P. aeruginosa strain was first isolated in Japan in 1991 and reported from India in 2002. We employed two methods Ezy MIC strip test and combined disc test for screening MBL producing strains among MDR isolates of P. aeruginosa and found dis-concordance of results of the two methods as only 8/27 (29.6%) of the carbapenem resistant isolates were MBL producers by both tests while 10 (37.04%) were positive by combined disc test but negative by Ezy MIC strip test. Similarly, 9 (33.33%) isolates positive by Ezy MIC Strip Test were negative by CDT (Table 4.3).

The comparative analysis of 27 MBL positive and 31 MBL negative P. aeruginosa isolates reflected more or less similar or comparable patterns of resistance to different antibiotics used in the study except in case of MBL negative strains where more resistance was recorded as compared to MBL positive strains against cefepime, ciprofloxacin, piperacillin and piperacillin/tazobactam. Patwardhan et al (2013) recorded 28.89 and 22.22% isolates as MBL producers by combined disc test and Ezy MBL strip
respectively and thus, did not observe full concordance between the results of the two methods. The MBL producing *P. aeruginosa* strain was first isolated in Japan in 1991. In India it was first reported in 2002. In a study from Tamil Nadu, out of 67 MDR *P. aeruginosa* clinical isolates 62.7 % and 70.1% were resistant to imipenem and meropenem respectively and 70.1% were found to be MBL producers. Other workers reported MBL production in 69.5, 72, 83.33 and 85.7 percent of imipenem resistant isolates (Sharma et al, 2010, Shahcheraghi et al, 2010, Rit et al, 2013, Pandya and Agravat, 2013). 8.6% isolates of *P. aeruginosa* showed presence of MBL by the DDST in a study from Sevagram, Maharashtra in a rural hospital of India, using ceftazidime and imipenem as substrates and EDTA and 2 mercaptoethanol as chelators of the enzyme (Mendiratta et al, 2005). Several studies report combined disc test as more sensitive test for MBL detection but several workers have employed E test strip test also for the detection of MBL producing *P. aeruginosa* strains.

Ours is perhaps the first report regarding the prevalence of multidrug resistant MBL producing *P. aeruginosa* strains in the state of Himachal Pradesh. The higher frequency of multidrug resistant strains in the state which could be due to several factors such as indiscriminate use of antibiotics, lack of awareness among healthcare workers, increased frequency and interaction with tourists and visitors from other states carrying *P. aeruginosa* infections and lack of appropriate strategies to effectively control infections. The present study thus, provides the basic information regarding high proportion of multidrug resistant *P. aeruginosa* (69.50%) isolates and prevalence of metallo-β-lactamase producing MDR strains in Himachal Pradesh.

Since the phenotypic expressions of metallo-β-lactamases rely on several factors including the environmental factors under which detection is being made. The phenotypic methods also donot give a clue about the type of β-lactamase produced by the organism. The genotypic detection is definitely authentic and reliable method for detecting MBL producing strains. In order to establish the genotypic basis of MBLs, we designed primers to amplify selective regions of the *blaIMP-1* and *blaVIM-2* genes of the *P. aeruginosa* strains that were MBL positive by phenotypic tests since these two genes are of predominant worldwide occurrence (Zhao and Hu, 2011). These genes have been reported from Iran (Doosti et al, 2013 and Yousefi et al, 2010), Brazil (Mendes et al, 2007), France (Pitout et
In the PCR assays, the reference sequences of all the genes encoding IMP-1 and VIM-2 were downloaded from Gene Bank, and primers were designed with the help of Primer3 tool. The primer sequences matched with the \textit{bla_{IMP-1}} and \textit{bla_{VIM-2}} gene precursors of reported \textit{P. aeruginosa} strains by blast-2 analysis. The forward primer of \textit{bla_{IMP-1}} gene of PHB 62 (Accession no.GU831553.1) strain was derived from nucleotide no. 43 to 61 while reverse primer was derived from to nucleotide no. 655-675 of PHB 62 strain. The forward primer of \textit{bla_{VIM-2}} gene of P40 strain (Accession no. KJ679408.1) matched to nucleotides 797 to 817 whereas reverse primer to nucleotides 1541 to1560.

The amplification of \textit{bla_{VIM-2}} gene segments was achieved in 6/27 (22.22\%) phenotypically MBL positive isolates. We, however, did not achieve amplification of IMP-1 gene in any isolate which suggests that another MBL gene(s) may be present in these strains might be responsible for resistance. \textit{bla_{IMP-1}} gene was not detected in any isolate. It is possible that other \(\beta\)-lactamase genes might be responsible for the carbapenem resistance. It is equally possible that mechanisms other than the action of \(\beta\)-lactamase IMP-1 might operate in the development of resistance. For example, it is a well-established fact that EDTA used to detect MBL in phenotypic test may increase bacterial cell-wall permeability and that zinc (chelated by EDTA) accelerates imipenem decomposition and decreases OprD expression of \textit{P. aeruginosa} (Conejo et al, 2003). Another alternative would be the presence of OXA-10 and/or OXA-14 stabilized in the dimeric form by metal ions (Zn\(^{++}\)) as dimeric forms are more active than monomeric form, increasing its enzymatic activity and turning the enzyme more efficient to inactivate carbapenems and other \(\beta\)-lactam antibiotics (Danel et al, 2001). Wirth et al (2009) from Brazil and Shahcheraghi et al (2010) from Iran have produced experimental evidence to these observations. In India, other workers have detected \textit{bla_{IMP}} and \textit{bla_{VIM}} genes in 59.02\% of \textit{P. aeruginosa} isolates in Chennai (Amudhan et al, 2012) and 61.1\% strains carried \textit{bla_{VIM}} and 3\% carried \textit{bla_{IMP}} in Tamil Nadu (Arunagiri et al, 2012). Out of these 6 isolates having \textit{bla_{VIM-2}} gene, five were MBL producers by Ezy MBL strip test and four by combined disk test and among them two were MBL producers by both these tests. This suggests that the VIM-2 encoded enzymes were the most prevalent MBLs among clinical isolates of \textit{P. aeruginosa} in this region which is consistent with the
findings of others who reported types of \( \text{bla}_{\text{VIM}} \) gene only (Buchunde et al, 2012 and Manoharan et al, 2010). Castanheira et al (2009) detected VIM-2 gene in 57 out of 301 (19\%) \textit{Pseudomonas} isolates with elevated MICs of imipenem or meropenem (MIC\(\geq8\mu g/ml\)), VIM (subtype 2) was found. Bogiel et al (2010) from Poland reported a low prevalence rate of 1.4\% of \( \text{bla}_{\text{VIM}} \) gene and these workers did not find the occurrence of \( \text{bla}_{\text{IMP}} \) gene in the isolates. This observation is similar to ours as we detected \( \text{bla}_{\text{VIM}} \) gene in 6/141 (4.25\%) isolates. Similarly, Cavallo et al, 2007 reported VIM-2 gene in 2/76 (2.63\%) isolates in France. All the MBL producing isolates in our study were resistant to imipenem. However, resistance to other carbapenems was also observed but not against all the isolates. Intermediate resistance was also observed against all the carbapenems but not by all the isolates.

In the PCR assay for amplifying \( \text{bla}_{\text{VIM-2}} \) gene segment, we observed a band of expected size of 764bp. The nucleotide sequence homology of the amplicons of all the six isolates on analysis by multiple-sequence alignments by use of the CLUSTAL W program revealed 100\% homology amongst them. In order to determine the genetic relatedness based on the amplicon, the nucleotide sequence of the \( \text{bla}_{\text{VIM-2}} \) gene amplicon of one of the isolates, Pa16 of pus origin with the corresponding published sequences of this gene with other bacterial species revealed 99-100\% homology to \textit{Klebsiella oxytoca} (FJ627181.1), \textit{Acinetobacter berezinae} (EF125009.1), \textit{Citrobacter freundii} (JX486753.1), \textit{Pseudomonas sp.} (EF645359.1), \textit{Stenotrophomonas maltophilia} (KF471098.1), \textit{P. putida} (FJ715942.1), \textit{P. aeruginosa} (AM087407.1) and \textit{Acinetobacter baumanii} (AM749812.1). This observation reflects that the \( \text{bla}_{\text{VIM-2}} \) sequences are quite conserved among bacterial species and this gene can be transferred by way of intra-species and inter-species. Besides, the nucleotide sequences of the VIM-2 gene amplicon of one of the isolate, Pa16 were compared to the published sequences of other genes in the VIM subgroup 2 i.e. VIM-24 (HM855205.1), VIM-23 (GQ242167.1), VIM-11 (DQ022682.1), VIM-31 (JN566054.1) and VIM-6 (AY165025.1) which had 99-100\% homology with our sequence. Such sub-grouping has been done by Zhao and Hu (2010) who utilized the amino acid sequences for sub-grouping of VIM gene.
The MBL producing isolates of *P. aeruginosa* 27 in number were further analysed for the presence of integron classes, *int1*, *int2* and *int3* integrase genes by PCR found that 11 (40.7%) contained class 1 integrons. The primers utilized in the assay in case of *int1* corresponded to the nucleotide sequence (66 to 86-forward primer and 937-956 reverse primer) of strain B5 (Accession No. KM384735.1) of *P. aeruginosa* by blast-2 analysis. For *int2*, the forward primer sequence corresponded to nucleotide number 23-44 and reverse primer 650-672of strain 17 (Accession no. KF358999.1) of *P. aeruginosa*. Similarly, the primers for class 3 were derived from the nucleotide sequence of *Serratia marcescens* (Accession No.AF416297.1). The forward primer corresponded to the nucleotide no. 738-758 and reverse primer to nucleotide no. 1697 to 1717. The amplification was achieved in case of integron class 1 only in 11 (40.7%) isolates and no amplification was observed for *int2* and *int3* in any of the isolate. The frequency of occurrence of integrase gene is comparable to that reported by others (Chen et al, 2009, Gu et al, 2007 and Fonseca et al, 2005). Odumosu et al (2013) identified class 1 integron in 31 (57%) multidrug resistant clinical isolates of *P. aeruginosa* but none of them carried class 2 and class 3 integrons. Cicek et al (2013) screened for Class1 and 2 integrons but amplified class 1 integrons only in 10/205 (4.87%) isolates. Class 1 integrons are the most widely disseminated type in pathogens of human and animal origins (Gu et al, 2007, White et al, 2001, Machado et al, 2008, Vo et al 2007, Kang et al, 2005) and have also been found in aquatic ecosystems (Srinivasan et al, 2008 and Wright et al, 2008). We observed the presence of *bla*VIM-2 gene and *int1* gene in one isolate, Pa138 only. Several studies across the world describe the prevalence of class 1 integrons among metallo-β-lactamase positive isolates of *P. aeruginosa*. In a study from china, 45.3% of the *bla*VIM positive isolates carried integrons (Cheng et al, 2008). Lepšanović et al (2012) found class 1 integrons in all the *bla*VIM positive isolates of *P. aeruginosa* in Belgrade (Serbia). Kouda et al from Japan (2009) reported the prevalence of class1 integrons in all the *bla*IMP-1 and *bla*VIM-2 positive isolates.

The integrons carry genes resistant to antibiotics such as sulphonamide (*sul1*), quaternary ammonium compounds (*qacEΔ1*). Integron class 1 is characterized by two conserved regions, the 5' conserved segment (5'-CS), which includes the integrase gene (*int1*), the...
adjacent recombination site (attI1) and the promoter (Pc), and the 3' conserved segment (3'-CS), which contains the qacEΔ1 gene and sul1 and the orf5 (Levesque et al, 1995 and Gillings et al, 2008).

Sul1 codes for the enzyme dihydropteroate synthetases which is responsible for resistance against sulphonamides. This gene is mostly found linked to other resistance genes in class 1 integrons including carbapenemases (Skold, 2001). In order to see the association of sul1 gene, we amplified sul1 gene of all the MBL positive isolates in the PCR assay and achieved amplification in 14 isolates. The sul 1 gene was found linked to integron class 1 in case of nine isolates. This observation suggests that either class 1 integrons in our isolates have lost the sul1 gene region or that this gene is carried on another genetic context. Similar findings have been reported by other workers (Gundogdu et al, 2011, Grape et al, 2005). Primer sequences used in the PCR assay corresponded to Forward primer- nucleotide no. 9095 to 9115 and reverse primer to 9858 to 9878 of P. aeruginosa strain M140A (Accession No. KJ510410.1). The blaVIM-2 gene was associated with int1 in one isolates. The studies carried out with respect to the presence of int1 and sul1 reflect that they co-exist in a larger proportion. High prevalence of sul1 (95%) was observed among Gram negative bacilli in Thailand (Suvattanadeja et al, 1993). Similarly Gundogdu et al (2011) observed a high prevalence of 72% of sul1 gene among E. coli isolates recovered from patients having UTI. Of these 55.1% were positive for class 1 integrase. Antunes et al (2005) reported a high prevalence of sul1 (76%) and also had class 1 integrons (77%) in Salmonella enterica strains in Portugal. These workers reported 96.7% isolates carrying int1 and sul1 gene. In another study from Mangalore, all the 18 cotrimoxazole (sulphonamide) resistant strains of Salmonella spp. isolated from human, poultry and seafood sources tested positive for sul1 (Adesiji et al, 2014). Shahid et al (2014) observed 40.74% and 58.39% of environmental and clinical isolates of Gram negative bacteria respectively, carrying sul1. Ho et al (2009) and Kerrn et al (2002) also found a co-relation of integron int1 and sul1 as a conserved segment of an integron class 1 component.

It may be concluded from the present study that multidrug resistant MBL and integron positive strains are prevalent in the state of Himachal Pradesh. We report the occurrence
of int1 and blaVIM-2 in this region. The nucleotide sequence analysis of the amplicons of these genes revealed 99-100% homology among isolates of P. aeruginosa as well as other Gram-negative bacteria. Further studies are however required in order to understand the epidemiology of P. aeruginosa strains in the state which would ultimately be helpful in the proper management of infections due to P. aeruginosa in this region.