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Detection of Extended Spectrum B-Lactamase (ESBL) and Metallo B-Lactamase (MBL) In *Pseudomonas aeruginosa* and *Acinetobacter* Species

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A B S T R A C T

Infections caused by multidrug-resistant bacteria expressing *β*-lactamases pose serious challenges to clinicians worldwide because these bacteria are resistant to a broad range of *β*-lactams. This study was undertaken to detect extended spectrum β-lactamases (ESBL) and metallo-beta-lactamase (MBL) producing *Pseudomonas aeruginosa* and *Acinetobacter* species from wound pus and sputum samples. A prospective study was performed over a period of 8 months in a tertiary care hospital. A total of 17 clinical isolates of *P. aeruginosa* and 18 of *Acinetobacter* species were tested for the presence of ESBL, and metallo-beta-lactamase enzyme. Detection of ESBL was done by the Cephalosporins/Clavulanate Combination Disk method as per Clinical and Laboratory Standards Institute (CLSI) guidelines, and MBL was detected by Imipenem - EDTA Combined disk synergy test. Among 17 isolates of *P. aeruginosa*, 64.7% were MDR, 41.17 % of were positive for ESBL while 35.2% were MBL producers. Among 18 isolates of *Acinetobacter* spp, 44.4% were MDR, 16.6% were ESBL producers while 44.4 % were MBL-producing species. A high prevalence of MDR, ESBL, MBL positive isolates among these non-fermentating bacteria is increasing at an alarming rate. Thus, proper antibiotic policy and measures to restrict the indiscriminative use of cephalosporins and carbapenems should be taken to minimize the emergence of this multiple beta-lactamase-producing pathogens.

Keywords

Pseudomonas aeruginosa, *Acinetobacter* spp, Multidrug Resistance, ESBL, MBL

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Introduction

β-Lactamases are the commonest cause of bacterial resistance to *β*-lactam antimicrobial agents, which are used in the treatment of various serious infections. With the increased use of antimicrobial agents, bacteria responded with a variety of new *β*-lactamases including extended-spectrum *β*-lactamases, metallo-*β*-lactamases. Infections caused by multidrug-resistant bacteria expressing *β*-lactamases pose serious challenges to clinicians worldwide because these bacteria are resistant to a broad range of *β*-lactams, including third-generation cephalosporins, and nosocomial infections caused by these organisms complicate therapy and limit treatment options (1).

Acinetobacter species and *Pseudomonas aeruginosa* are widely distributed in nature, including the domestic and hospital environment and are noted for their intrinsic resistance to antibiotics and for their ability to acquire genes encoding resistance determinants. Foremost among the mechanisms of resistance in both of these pathogens is the production of beta‑lactamases and aminoglycoside ‑ modifying enzymes (2,3).

Clinical infections with organisms harboring carbapenemases pose serious therapeutic challenges, with increasing reports of poor patient outcomes and death. So, early detection of resistance strains is crucial which helps in timely implementation of strict infection control practices as well as formulation of clinical guidelines regarding the potential risks for therapeutic failure (4). Hence, this study was carried out to determine the occurrence of ESBL and MBL among imipenem resistant strains of *P. aeruginosa* and *Acinetobacter* species from various clinical samples of patients admitted and visiting a Tertiary Hospital in Nepal.

Materials and Methods

The study was conducted at microbiology laboratory of KIST Medical College and Teaching Hospital, Gwarko, Lalitpur , Nepal from July 2015 to February 2016 to detect ESBL and MBL producers in *P. aeruginosa* and *Acinetobacter* spp isolated from various clinical samples. A total of 540 samples were collected in which 170 wound pus and 370 sputum samples were included under study, which were sent to laboratory for routine culture and antibiotic susceptibility testing.

Isolation and identification of isolates

The clinical samples were inoculated on Blood Agar(BA) and MacConkey agar (MA) and was incubated at 37^{o}C for 24 hours. Plates were observed for colony morphology and characteristic smell. Gram staining was performed for the presumptive identification of the bacteria according to standard technique using acid alcohol as decolourizer. Typical colonies of bacterial isolates were sub-cultured on nutrient broth and incubated at 37ºC for 4 hours. After incubation, fresh culture of test organism was inoculated into different biochemical media.

Identification of *Acinetobacter* spp

Acinetobacter spp was identified on the basis of typical non fermenting colonies on MA, various characteristics such as positive catalase test, negative oxidase test, non-motile, citrate positive, urease variable, Alk/Alk H_2S^- G^- in triple sugar iron agar (TSIA) medium, oxidative in Hugh and Leifson's medium and its ability to grow at 37° C and 41° C (5).

Identification of *P. aeruginosa*

P. aeruginosa were identified on the basis of various characteristics such as non-lactose fermenting colonies on MA, positive catalase and oxidase test, pigment production, growth on cetrimide agar and growth at 42° C (5).

Antibiotic Susceptibility test

AST was performed by Kirby-Bauer disc diffusion method as per the Clinical and Laboratory Standards Institute guidelines (6). The diameter of the zone of inhibition produced by each antibiotic disc was measured, recorded and the isolate were classified as resistant and sensitive on the basis of guidelines published by the CLSI. *Pseudomonas aeruginosa* and *Acinetobacter* spp isolates that showed resistance to at least one agent in three or more classes of antibiotics was titled as MDR (7) and further preserved for other analysis. Control strains of

E. coli (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were tested primarily.

Detection of ESBL production

The strains resistant to Ceftazidime and/or Cefotaxime were further tested for ESBL production by the Cephalosporins/Clavulanate Combination Disk method using ceftazidime (30μg) and cefotaxime discs (30 μg) with and without clavulanic acid (10 μg) as recommended by the CLSI. An increase of ≥ 5 mm in the inhibition zones of either cephalosporin in combination with clavulanic acid compared to the cephalosporin alone was interpreted as ESBL producer (8).

Detection of MBL production

The strains which showed resistance to imipenem were further tested and interpreted for MBL production by Imipenem - EDTA Combined disk synergy test (CDST-IPM) in which two imipenem (10ug) discs were placed on the surface of an agar plate and 10 μl 0.5 M EDTA solution was added to one of them to obtain a desired concentration of 750 ug. Plates were incubated for 16 to 18 hours at 35˚C. If zone of inhibition of imipenem-EDTA disc were ≥ 7 mm more than that of imipenem disc alone, it was considered MBL positive (8).

Data management and analysis

All the results obtained were entered into the worksheet of statistical package for social science (SPSS) software (Verson 21.0), then analyzed. Frequency and percentages were calculated and Chi-square test was done whenever applicable with $p<0.05$ regarded as significant.

Results and Discussion

Out of total 170 wound pus and 370 sputum samples, 18 isolates of *Acinetobacter* spp and 17 isolates of *Pseudomonas aerugionosa* were isolated. The culture positive samples obtained from total sample were 3.1 % for *P. aeruginosa* and 3.33% of *Acinetobacter* spp.

Out of 17 *P. aeruginosa* isolates, 8 (47.05%) were from General ward, 6 (35.2%) were from ICU and 3 (17.6%) were from OPD. Similarly, out of total 18 *Acinetobacter* spp., 13 (72.22%) were from General ward patients, 3 (16.66%) were from ICU and only 2 (11.11%) from OPD patients. There was no significant association (p-value > 0.05) between growth of organisms and different wards.

In this study, Imipenem showed the highest susceptibility of 58.8%, followed by Meropenem, Amikacin of 52.9% and 47.1% respectively against *Pseudomonas aeruginosa*. Likewise, for *Acinetobacter* spp; Imipenem, Gentamycin and Tobramycin showed the highest susceptibility of 55.6% each (Table 1).

In this study done at KIST hospital, Gwarko, Lalitpur, the incidence rate of MDR *Acinetobacter* spp and *P. aeruginosa* were 64.7% and 44.4%. Similarly, the rate of ESBL *Acinetobacter* spp and *P. aeruginosa* were 16.6% and 41.17% and MBL rates 44.4% and 35.2% respectively (Table 2).

Majority of MDR strains of *P. aeruginosa*: 5 (29.41%) each from general wards and ICU were isolated respectively. Similarly, highest MDR *Acinetobacter* spp were isolated from general ward 5 (27.77%), followed by ICU accounting 3 (16.66%). There was no significant association (p-value > 0.05) between multidrug resistant organisms and different wards.

Out of 17 isolates of *P. aeruginosa* 6 (35.2%) were found to be MBL producers. These were isolated from general ward 4 (23.52%) and 2 (11.76%) from ICU. Similarly, out of 18 isolates of *Acinetobacter* spp 8 (44.4%) MBL producers which were isolated from general

ward 5 (27.77%) and ICU 3 (16.66%) respectively. There was no significant association (p-value > 0.05) between MBL producing organisms and different wards..

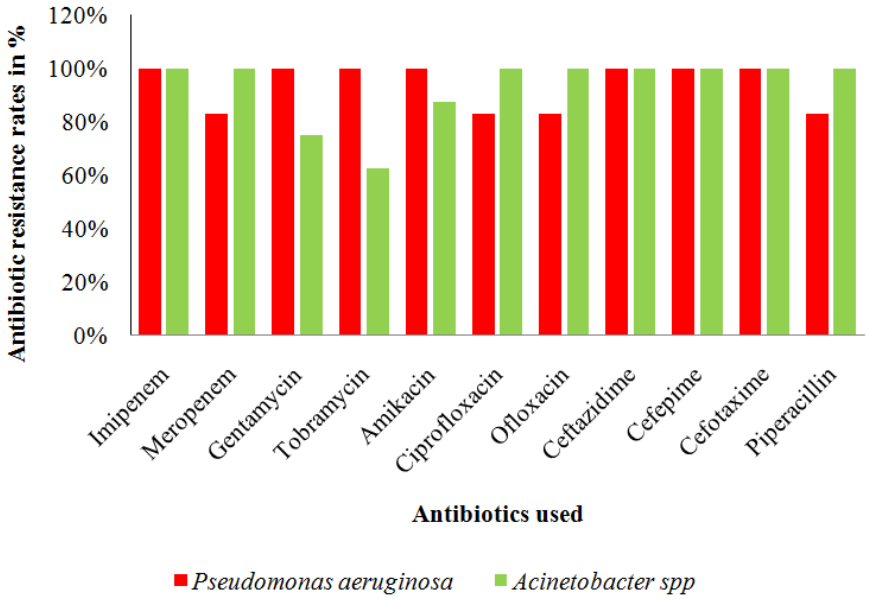
Table.1 Antibiotic Susceptibility Pattern of *Pseudomonas aeruginosa* and *Acinetobacter* spp

Antibiotics used	*Pseudomonas aeruginosa*				*Acinetobacter* spp			
	Resistant		Sensitive		Resistant		Sensitive	
	No.	%	No.	%	No.	%	No.	%
Imipenem	7	41.2%	10	58.8%	8	44.4%	10	55.6%
Meropenem	8	47%	9	52.9%	9	50.0%	9	50.0%
Gentamycin	11	64.7%	7	41.2%	8	44.4%	10	55.6%
Tobramycin	11	64.7%	7	41.2%	8	44.4%	10	55.6%
Amikacin	9	52.9%	8	47.1%	9	50.0%	9	50.0%
Ciprofloxacin	12	70.6%	5	29.4%	11	61.1%	7	38.9%
Ofloxacin	14	82.4%	3	17.6%	11	61.1%	7	38.9%
Ceftazidime	15	88.2%	2	11.8%	18	100.0%	0	0.0%
Cefepime	15	88.2%	2	11.8%	16	88.9%	2	11.1%
Cefotaxime	16	94.1%	1	5.9%	18	100.0%	0	0.0%
Piperacillin	15	88.2%	2	11.7%	18	100.0%	0	0.0%

Table 2 Distribution of MDR, ESBL and MBL producers

Organism	Total	MDR	ESBL	MBL
Pseudomonas aeruginosa	17	11 (64.7%)	7 (41.17%)	6 (35.2%)
***Acinetobacter* spp**	18	8 (44.4%)	3 (16.6%)	8 (44.4%)

Figure.1 Antibiotic Resistance pattern among MBL producers



All MBL producers *P. aeruginosa* were resistant to Imipenem, Gentamycin, Tobramycin, Amikacin, Ceftazidime, Cefepime, Cefotaxime. Likewise, all MBL producers *Acinetobacter* spp were resistant to Imipenem, Meropenem, Ciprofloxacin, Ofloxacin, Ceftazidime, Cefepime, Cefotaxime and Piperacillin as illustrated in Figure 1.

The emergence of antibiotic resistant bacteria is threatening the effectiveness of many antimicrobial agents. It has increased the hospital stay of the patients, thus leading to an increased economic burden on them. In the present study, the rate of isolation of *P. aeruginosa* was 47.05% from General ward and 35.2% from ICU. Similarly, 72.22% and 16.66% *Acinetobacter* spp were from General ward patients and ICU. Very less percentage of both bacteria were isolated from OPD. Other studies reported the highest incidence of infections with these bacterias from ICUs (9,10).

In the present study, we observed an increased resistance of *P. aeruginosa* to carbapenems. Similar result of imipenem resistance in this organism was also recorded in China (30.5%) (11) and Thailand (33.3%) (12). High antibiotic pressure due to greater empirical or in-discriminate use of broad-spectrum antibiotics is probably the main reason for an increase carbapenem resistance in the hospital setting (13).

Data of *P. aeruginosa* from other researcher who reported susceptibility figure of 65.9% for Gentamicin, 78% for Amikacin (14) was much higher with value of susceptibility of 41.3% for Gentamicin and 48% for Amikacin of present study. In a previous study of *P. aeruginosa* from Nepal 82.3% isolates were resistant to ceftazidime, 77.4% to piperacillin, 88.7 % to cefotaxime and 64.5% isolates were resistant to ciprofloxacin (15), which is much similar to the resistance rates of *P. aeruginosa* of our finding.

In the same study, they reported lower (35.5%) imipenem resistance but higher (50%) rate of meropenem resistance in *Acinetobacter* clinical isolates and its susceptibility to the Aminoglycosides-amikacin (68.4.% vs 58.4%) was much higher and gentamicin (37.1% vs 55.6%) much lower than our study (15). Decreased susceptibility to aminoglycosides is most commonly caused by aminoglycoside modifying enzymes, including acetyltransferases, phosphotransferases and nucleotidyltransferases and more recently, 16S rRNA methylases (11). Low sensitivity pattern of *Acinetobacter* spp to antibiotics was also reported in another study from Nepal where 82.75% isolates of *Acinetobacter* spp were resistant to cefotaxime, 86.2% to cefepime, 82.75% to ciprofloxacin (16).

MDR is pervasive and growing clinical problem, which is recognized as a threat to public health in causing significance effect on morbidity and mortality and increased economic burden, which stems from the misuse of antibiotics, particularly, excessive use. The rise of multidrug-resistant (MDR) *P. aeruginosa* is being reported by many researchers. In our study, 64.7% *P. aeruginosa* were found to be MDR, which is comparable with the study done by Mishra *et al.*, (2012) at Institute of Medicine (IOM), TUTH (14). The number of multidrug resistant *P. aeruginosa* ranges from 20% to 85.4% in various studies throughout the world (17, 18, 19). In our study the number of MDR was equally found on General wards and ICU each accounting for 29.41%. MDR *P. aeruginosa* was significantly higher for ICU patients (37%) than for non-ICU patients (30.3%) in another studies (20), where ICU *P. aeruginosa* isolates were highly MDR compared to other wards.

In our study 44.4% of *Acinetobacter* species were found to be MDR which is much lower than the other research findings of India 89.71% and 78% (21,22). Other studies from Nepal have records of 85.4%, 79.31% of MDR in *Acinetobacter* spp (1,16).

ESBLs occur rarely in non-fermenters. Our investigation accords with the study done in Shimla region of Himachal Pradesh for assessing the prevalence of ESBL producing *P. aeruginosa*, 32.75% isolates were confirmed as ESBL producers by DDST method (23). In a study conducted at a tertiary care hospital, Aligarh, India; 23.1% ESBL *P. aeruginosa* were reported (24). ESBL *Acinetobacte*r spp were less compared to ESBL *P. aeruginosa* in our study. While our data deviates from the report of the study done in Mayo Institute of Medical Sciences, Barabanki where higher rate of ESBL Acinetobacter spp accounting for 50.70% was issued (25). ESBL tests were not developed for *Acinetobacter* spp., *Pseudomonas aeruginosa*. False positive results with *Acinetobacter* spp. are common owing to inherent susceptibility to clavulanate

MBL-producing non-fermenters especially *Acinetobacter* spp and *P. aeruginosa* have been reported with increasing frequency from several countries worldwide as the main mechanism of resistance to imipenem (26). However, the prevalence rate may vary greatly in different geographical areas and from institute to institute. The rate of MBL production in *Acinetobacter* spp in present study was relatively low (44.4%) than that reported from India, where 80.3% imipenem-resistant *Acinetobacter* isolates was MBL producers (27). Similarly, in the study conducted by Bhandari (2015), Pokhrel *et al.*, (2010) in Nepal reported a variable per cents of MBL *Acinetobacter* spp with records of 63.8% and 40% (16,28).

35.2% of *P. aeruginosa* were MBL producers in present study. Previous studies from Nepal reported MBL-producing *P. aeruginosa* in 33.3% of imipenem-resistant isolates (1), according to which MBL may not play a major role in imipenem resistance. In another study from tertiary level hospital,(14) reported MBL production in 3.3% of all *P. aeruginosa*. In a tertiary level hospital from Egypt, 68.7% of carbapenem resistant *P. aeruginosa* were MBL producers (29). High (69.8%) prevalence of MBL in IRPA (Imipenem resistant *P. aeruginosa*) was observed in an Indian hospital (30). These great differences in MBL prevalence between different countries are probably due to different antibiotherapy policies.

All of metallo β- lactamase producers in our study were MDR and resistant to most of the antibiotic used. MBL *P. aeruginosa* were resistant to Imipenem, Gentamycin, Tobramycin, Amikacin, Ceftazidime, Cefepime, Cefotaxime. MBL *Acinetobacter* spp were resistant to Imipenem, Meropenem, Ciprofloxacin, Ofloxacin, Ceftazidime, Cefepime, Cefotaxime and Piperacillin. Similar result was obtained by Mishra *et al.*, (2012) (14). This may be due to the fact that the MBL genes are often carried along with other resistance genes resulting in multi-drug resistance limiting treatment options (31).

In conclusion all MBL *P. aeruginosa* and *Acinetobacter* spp were MDR, leaving very few antibiotics for treatment options.. MDR, ESBL and MBL producers were predominantly isolated from ward patients than ICU.

Thus, detection of such isolates is of paramount importance both in hospital and community setting. Immediate infection control, and antibiotic stewardship programs should be implemented in order to limit the spread of β-lactamase producing organisms.

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References

1. Khanal S, Joshi DR, Bhatta DR, Devkota U and Pokhrel BM (2013). β-lactamase-producing multidrug-resistant bacterial pathogens from tracheal aspirates of intensive care unit patients at national institute of neurological and allied sciences Nepal. ISRN Microbiol 2013: 847569.
2. Vila J, Marco F. (2010). Interpretive reading of the non‐fermenting gram‐negative bacilli antibiogram. Enferm Infecc Microbiol Clin; 28: 726‐36.
3. Goel V, Sumati AH, Karadesai SG (2013). Prevalence of extended-spectrum beta-lactamases, AmpC beta‐lactamase, and metallo‐beta‐lactamase producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in an intensive care unit in A tertiary Care Hospital. Journal of Scientific Society 40: 1.
4. Chaudhary AK, Bhandari D, Amatya J, Chaudhary P and Acharya B. (2016). Metallo-Beta-Lactamase Producing Gram-Negative Bacteria among Patients Visiting Shahid Gangalal National Heart Centre. Austin J Microbiol.; 2(1): 1010.
5. Patrica MT (2014). Bailey and Scott's Diagnostic Microbiology, Thirteenth Edition, Elsevier Inc, pp 329-345.
6. Clinical Laboratory and Standard Institute (2015). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement 35: 3.
7. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, *et al.,* (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect.; 18: 268-281.
8. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM and Chong Y (2002). Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp. J Clin Microbiol 40:3798-3801.
9. Rahbar M, Mehragan H, Akbari NH *et al.,* (2010). Prevalence of antibiotic-resistant *Acinetobacter baumannii* in a 1000-bed tertiary care hospital in Tehran, Iran. Indian J Pathol & Microbiol; 53:290-3.
10. Sharma D, Vyas N, Sinha P, Mathur A (2014). Non fermentative gram negative bacilli as nosocomial pathogens: Identification and antibiotic sensitivity in clinical samples of indoor patients. NJMS; 03: 02.
11. Xiao YH, Giske CH, Wei ZQ, Shen P, Heddini A and Li LJ (2011). Epidemiology and characteristics of antimicrobial resistance in China. Drug Resist Updat 14:236-250.
12. Piyakul C, Tiyawisutsri R and Boonbumrung K (2012). Emergence of metallo-β-lactamase IMP-14 and VIM-2 in *Pseudomonas aeruginosa* clinical isolates from a tertiary-level hospital in Thailand. Epidemiol Infect 140:539–541.

13. Xu J, Duan X Wu H and Zhou Q (2013). Surveillance and Correlation of Antimicrobial Usage and Resistance of *Pseudomonas aeruginosa*: A Hospital Population-Based Study. PLoS one 8:78604.
14. Mishra SK, Acharya J, Kattel HP, Koirala J, Rijal BP and Pokhrel BM (2012). Metallo-beta-lactamase producing Gram-negative bacterial isolates. J Nepal Health Res Counc 10: 208-213.
15. Mishra SK, Rijal BP and Pokhrel BM (2013).Emerging threat of multidrug resistant bugs *Acinetobacter calcoaceticus baumannii* complex and Methicillin resistant *Staphylococcus aureus*. BMC Res Notes 6:98-104.
16. Bhandari P, Thapa G, Pokhrel BM, Bhatta DR and Devkota U (2015). Nosocomial Isolates and Their Drug Resistant Pattern in ICU Patients at National Institute of Neurological and Allied Sciences, Nepal. Int J Microbiol., 6.
17. Jayakumar S and Appalaraju B (2007). Prevalence of multi and pan drug resistant *Pseudomonas aeruginosa* with respect to ESBL and MBL in a tertiary care hospital. Indian Journal of pathology and Microbiology.50:922-925.
18. Kohanteb J, Dayaghi M and Motazedian M (2007). Comparsion of biotyping and antibiotyping of *Pseudomonas aeruginosa* isolated from patients with burn wound infection and nosocomial pneumonia in Shiraz, Iran. Pak J Biol Sci. 10:1817-1822.
19. Shankar EM, Mohan V and Premalatha G (2005). Bacterial etiology of diabetic foot infections in South India. Eur J Intern Med. 16:567-570.
20. Slama KB, Gharbi S, Jouini A, Maarouf M, Fendri C, Boudabous A and Gtari M (2011). Epidemiology of *Pseudomonas aeruginosa* in Intensive Care Unit and Otolaryngology Department of a Tunisian Hospital. Afr. J. Microbiol. *Res.* 5:3005-3011.
21. Sukumaran J, Sriram L, Sumathi G (2014). Nonfermentative gram negative bacilli- characterisation and antibiotic resistant pattern study from a tertiary care hospital. Indian Journal of Basic and Applied Medical Research. 3 (4), P. 227-232.
22. Tripathi P, Gajbhiye S (2013). Prevalence of Multidrug Resistance, ESBL and MBL production in *Acinetobacter* spp. International Journal of Recent Trends in Science And Technology 6 (3), pp 139-143.
23. Bharti and Sharma PC (2014). Prevalence of Extended spectrum beta lactamase (ESBL) producing *Pseudomonas aeruginosa* strains recovered from human patients in Himachal Pradesh. Indian Journal of Basic and Applied Medical Research 4: (1), P. 430-440
24. Gupta R, Malik, A, Rizvi M and Ahmed M (2015). Biofilm Producing Multidrug and Extensive Drug Resistant Bacterial Pathogens from Tracheal Aspirates of Intensive Care Unit Patients; a Threat to Combat. Int.J.Curr.Microbiol.App.Sci (1): 1-9
25. Banerjee M, Chaudhary BL, Shukla S (2015). Prevalence of ESBL and MBL in *Acinetobacter* Species Isolated from Clinical Samples in Tertiary Care Hospital. International Journal of Science and Research (IJSR) 4: (6).
26. Walsh TR (2010). Emerging carbapenemases: a global perspective. Int J Antimicrob Agents 36:8-14.
27. Kaur A, Gupta Vand Chhina D (2014). Prevalence of metalo-β-lactamase-producing (MBL)

*Acinetobacter*species in a tertiary care hospital. Iran J Microbiol 6: 22–25.

28. Pokhrel BM, Shrestha S, Chaudhari R, Karmacharya S, Kattel HP, Mishra SK Dahal RK, Bam N, Banjade N, Rijal BP, Sherchand JB, Ohara H, Koirala J (2010). Prevalence of Nosocomial Lower Respiratory tract infection caused by MDR pathologens, Department of Microbiology, Tribhuvan University Teaching Hospital, Nepal.
29. Zafer MM, Al-Agamy MH,El-Mahallawy HA, Amin MA and Ashour SED (2015). Dissemination of VIM-2 producing *Pseudomonas aeruginosa* ST233 at tertiary care hospitals in Egypt. BMC Infect Dis 15:122-129.
30. Gupta V, Sidhu S and Chander J (2012). Metallo-β- lactamase producing nonfermentative gram-negative bacteria: An increasing clinical threat among hospitalized patients. Asian Pac J Trop Med 2012:718-721.

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