International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 10, Issue 6, 2018, pp.-1283-1286.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

EPIDEMIOLOGY OF *ACINETOBACTER* SPECIES IN A TERTIARY CARE HOSPITAL WITH SPECIAL REFERENCE TO SEASONAL VARIATION

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Received: June 18, 2018; Revised: June 26, 2018; Accepted: June 27, 2018; Published: June 30, 2018

Abstract- Background: The members of the genus *Acinetobacter* are persistent nosocomial pathogens. Strains with increased resistance to most β-lactam antibiotics, fluoroquinolones, and aminoglycosides continue to be detected worldwide. They are responsible for various nosocomial infections. This study aimed to find the prevalence, epidemiology, and changing trend of this organism in a tertiary care hospital over a period of three years. Materials and methods: *Acinetobacter* species isolated from various clinical samples of a hospital were studied. Susceptibility test was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Multidrug-resistant (MDR) strains and extended-spectrum beta-lactamases (ESBLs) were detected. Results: Out of 8,905 isolates, 490 (5.5%) were *Acinetobacter* species. Maximum isolates 47.1% were obtained from intensive care units (ICUs). Off the MDR isolates, 87% were from the ICUs. All the ceftazidime-resistant isolates obtained from the outpatient department (OPD) were ESBL producers (100%). Amongst the indoor wards paediatric ward showed the highest percentage of ESBL producers (71.4%). The isolation rates throughout the three years were high in the late summer and monsoon, that is, may to oct. Conclusions: Isolation of MDR *Acinetobacter* species continues to be significant among ICUs. It is a major challenge to control infections caused by MDR *Acinetobacter* spp. Therefore, the continuous surveillance of the organism is needed to understand the changing trends in epidemiology and antibiotic resistance pattern.

Keywords- *Acinetobacter spp, ICUs, Epidemiology, Multidrug resistance*

Citation: Gupta Neetu, *et al.,* (2018) Epidemiology of *Acinetobacter* Species in a Tertiary Care Hospital with Special Reference to Seasonal Variation. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 10, Issue 6, pp.-1283-1286.

Introduction

The increasing role of *Acinetobacter* spp. in the establishment of nosocomial infections is a worldwide concern. The Progressive development of resistance to several antimicrobial agents used in the treatment poses a major challenge in health care [1]. In a study by the Global Burden of Diseases, mortality due to infections, in general, has decreased in the past few years but it still remains a major factor [2]. *Acinetobacter* spp. are primarily opportunistic pathogens that affect critical care units; however, it is gradually becoming a persistent nosocomial agent in other wards. Once established, remains endemic in the institutions and causes sequential outbreaks [3]. The ubiquitous presence of *Acinetobacter* poses a challenge in eradicating it from the hospital environment. According to recent studies, weather plays an additional epidemiological determinant in the incidence of these hospital associated infections [4]. However, in developing countries there is a lack in the published data on the seasonal trends of these pathogens. Therefore, this study aimed to find the prevalence of *Acinetobacter* in a tertiary care hospital over the past three years, to understand its distribution in the different areas of the hospital and different samples; to understand the changing trends in the epidemiology and antibiotic resistance patterns of antibiotics in various wards.

Material and Methods

The study period extended from January 2014 to December 2016 and involved the collection of continuous data of *Acinetobacter* spp. isolated from various samples received in the Department of Microbiology. The samples obtained from the patients admitted and treated in the various wards and ICUs of Dr D Y Patil Medical College, Hospital, and Research Centre, Dr D Y Patil Vidyapeeth, Pune, India, were included in the study. Various samples such as blood, urine, sputum, pus, and other body fluids received in the laboratory were processed. The growth of genus *Acinetobacter* on agar plates was identified by colony morphology, Gram stain, positive catalase test, negative oxidase test, the absence of motility, and standard biochemical reactions [1,5]. Antimicrobial susceptibility testing of the isolates was performed by Kirby-Bauer disc diffusion method for ciprofloxacin (5 µg), ceftazidime (30 µg), cefotaxime (30 µg), imipenem (10 µg), amikacin (30 µg), gentamicin (10 µg), ampicillin (10 µg), and cotrimoxazole (25 µg) discs (HiMedia, Mumbai, India). Zone size was interpreted according to CLSI guidelines [6,7]. Multidrug-resistant (MDR) strains (the isolates resistant to at least one agent in three or more categories of drugs) were detected using antibiotics from different categories [8,9]. Extended-spectrum beta-lactamase (ESBL) production in *Acinetobacter* spp. was detected in the isolates showing reduced susceptibility to ceftazidime by double disc approximation test performed on Mueller–Hinton agar plates using the discs of ceftazidime alone, ceftazidime-clavulanic, and ceftazidime-tazobactam [10-12]. To determine the seasonal trends in the isolation rate of *Acinetobacter* sps., we analyzed the monthly isolation rate from different samples and wards.

Results

During the study period, a total of 34,910 samples were processed and 8,905 isolates were obtained. Out of these isolates, 490 (5.5%) were *Acinetobacter* spp. In ward-wise distribution, the maximum number of isolates were obtained from ICUs 231 (47.1%), followed by surgery 94 (19.1%), medicine 89 (18.1%), pediatric 29 (5.91%) and gynecology wards 15 (3.06%),[Table-1].

Table-1 Isolation of *Acinetobacter* spp. from different wards and ICUs

Place of isolation	Three years (2014 to 2016)						
	Blood	Pus	Urine	Respiratory	C.S.F	Body Fluid	Total
ICUs	50	5	4	134	2	36	231
Surgery	3	76	1	0	0	14	94
Medicine	29	6	1	41	1	11	89
Pediatric	26	1	1	1	0	0	29
Gynecology	3	11	0	0	0	1	15
Others	1	14	0	0	0	13	28
OPD	1	3	0	0	0	0	4
Total	113	116	7	176	3	75	490

Table-2 Antibiotic resistance patterns in *Acinetobacter* isolates

Antibiotics	ICUs	Surgery	Med.	Gynec.	Pediatric	OPD	Other wards	Total
Ampicillin (10µg)	98.2%	93.5%	92%	100%	89.2%	100%	92.8%	95.4%
Ceftazidime (30µg)	95.2%	88.2%	91%	100%	72.4%	75%	75%	90.6%
Cefotaxime (30 µg)	93%	82.9%	67.4%	86.6%	65.5%	75%	78.5%	83.6%
Imipenem (10 µg)	17.7%	50%	16.8%	20%	3.4%	0%	17.8%	22.8%
Amikacin (30µg)	75.7%	70.21%	32.5%	66.6%	17.8%	0%	67.8%	62%
Gentamicin (10µg)	77%	63.8%	42.6%	73.3%	17.2%	0%	60.7%	63%
Ciprofloxacin (5µg)	84.4%	79.7%	47.1%	73.3%	24.1%	0%	67.8%	71.2%
Trimethoprim sulfamethoxazole (1.25/23.75 µg)	81.6%	79.7%	59.5%	80%	37.9%	25%	57.1%	72.7%

Table-3 Percentage of MDR (n=386/490) and ESBL (189/444) *Acinetobacter* spp. from different wards

Place of isolation	MDR isolates	ESBL isolates
ICUs	87%	43.6%
Surgery	85.1%	20.4%
Medicine	66.2%	50%
Pediatric	41.3%	71.4%
Gynecology	80%	53.3%
Others	75%	45.4%
OPD	25%	100%
Total	78.7%	42.5%

Table-4 Frequency of isolates in descending order in three years

2014 n=3242 (Percentage)	2015 n=2844 (Percentage)	2016 n=2819 (Percentage)
S.aureus 998 (30.7%)	*S. aureus* 888 (31.2%)	*S. aureus* 687 (24.3%)
Klebsiella spp.564 (17.3%)	*E. coli* 571 (20%)	*Klebsiella* spp. 528 (18.7%)
E. coli 484 (14.9%)	*Klebsiella* spp.444 (15.6%)	*E. coli* 499 (17.7%)
P.aeruginosa 272 (8.3%)	*P.aeruginosa* 275(9.6%)	*P. aeruginosa* 266 (9.4%)
Citrobacter spp.230 (7.09%)	*Citrobacter* spp. 195(6.8%)	*Acinetobacter* spp.181(6.4%)
Enterococcus spp.197 (6.07%)	*Acinetobacter* spp.169 (5.9%)	*Enterococcus* spp. 169 (5.9%)
Acinetobacter spp.140 (4.3%)	*Enterococcus* spp.120 (4.2%)	*Citrobacter* spp.132 (4.6%)
Remaining 357 (11%)	Remaining 182 (6.3%)	Remaining 357 (12.6%)

Table-5 Seasonal variation in isolation of *Acinetobacter* species from clinical samples

Clinical Samples	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Blood	7	10	6	0	2	4	29	16	20	6	7	6	113
Urine	1	1	2	1	0	0	1	0	0	0	1	0	7
Respiratory sample	5	20	10	16	19	8	19	17	19	21	8	14	176
Pus	5	3	3	12	13	16	16	12	10	9	12	5	116
Body fluids	2	5	3	7	4	11	8	9	8	14	2	5	78
Total	20	39	24	36	38	39	73	54	57	50	30	30	490

Table-6 Seasonal variation in isolation rates of *Acinetobacter* species in various wards and ICUs

Wards and ICUs	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
ICUs	10	23	13	16	13	10	30	28	26	30	12	20	231
Medicine wards	5	13	7	4	14	6	22	15	19	6	6	5	122
Surgery wards	4	3	4	16	11	23	20	11	11	14	11	5	133
OPD	1						1		1		1		4
Total	20	39	24	36	38	39	73	54	57	50	30	30	490

The sample-wise distribution of the isolates revealed that the maximum number of isolates were obtained from the respiratory samples 176 (35.9%) followed by pus 116 (23.6%), blood 113 (23%), body fluids including C.S.F 78(15.9%) and urine 7 (1.4%), [Table-1]. The maximum isolates from respiratory samples were obtained from ICUs (134/176), 76.1% followed by medicine ward (41/176), 23.2%. In addition, maximum isolates from blood samples were obtained from ICUs (50/113), 44.2%, followed by medicine (29/113), 25.6% and pediatric ward (26/113), 23%. Whereas, maximum pus isolates were obtained from surgery ward (76/116), 65.5%, followed by gynecology ward (11/116), 9.4%, [Table-1]. A gender-wise distribution of *Acinetobacter* isolates revealed male predominance in all wards except pediatric ward where the number of both sex was almost same (14-male,15-female). Overall distribution showed male: female ratio of 1.9:1. Isolates from the samples of gynecology ward showed 100% resistance to ampicillin and ceftazidime.

Strains isolated from ICUs samples showed high resistance to ampicillin, ceftazidime, cefotaxime, ciprofloxacin and cotrimoxazole (above 80%). Similarly, in other wards also maximum resistance to these antibiotics observed. In ICUs and wards imipenem is the most sensitive drug followed by amikacin and gentamicin [Table-2]. Out of the 490 *Acinetobacter* isolates, 386 (78.7%) were MDR organisms. Maximum MDR organisms were isolated from ICUs (201/231), 87%, followed by surgery (80/94), 85.1%, gynecology (12/15), 80%, medicine (59/89), 66.2%, pediatric (12/29), 41.3%, and OPD (1/4), 25%. The isolation rate from the remaining wards was (21/28) 75%, [Table-3]. Out of 490 *Acinetobacter* isolates, 444 (90.6%) showed reduced susceptibility to ceftazidime, which was further confirmed for ESBL production using the double disc approximation test. Out of 444 ceftazidime-resistant isolates, 189 (42.5%) were confirmed as ESBL producers. Ceftazidime-resistant *Acinetobacter* isolates from OPD were all ESBL producers (3/3), 100%. In the hospitalized patients, the maximum *Acinetobacter* isolates were obtained from pediatric ward (15/21), 71.4%, followed by gynecology (8/15), 53.3%, medicine (40/80), 50%, and surgery wards (17/23), 20.4%. Maximum ESBL producers were detected using β-lactamase inhibitor tazobactam (181/189), 95.7% compared with clavulanic acid (59/189), 31.2%, [Table-3]. Year-wise distribution revealed that maximum isolates were obtained from ICUs in the year 2015. However, in the surgery and medicine wards, the isolation was higher in 2014. The isolation rate of *Acinetobacter* spp. was the highest in 2016. Table-4 shows the occurrence of *Acinetobacter* spp. in the order of frequency during the three years. The isolation of *Acinetobacter* spp. was higher in the summer and rainy seasons (May to Oct) in these three years [Fig-1]. Sample-wise distribution revealed seasonal peak in two major type of infections blood stream infections (July-Sep) months and pyogenic infection (Apr-Nov). Three cases of meningitis were there in these three years which were in the months of June-July-august [Table-5]. While in the ward-wise isolation, seasonal trend was seen in ICUs with highest isolation rate during the months of July-oct, Medicine wards in July-Sep and in surgery ward extended from the month of Apr-Nov [Table 6]. Seasonal trend was not observed in the isolation of MDR and non-MDR isolates.

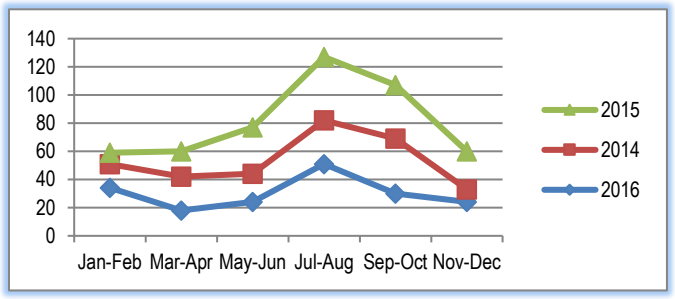

Fig-1 Trend of monthly and yearly isolation of *Acinetobacter*

Discussion

In this study, out of total 8,905 isolated organisms, 490 (5.5%) were *Acinetobacter*. Kaur, *et al*. found 10.2% prevalence of *Acinetobacter* in Bathinda, India, Dash, *et al*. found 3% prevalence in Odisha, India, and Uwingabiye, *et al*. found 6.94% prevalence of all bacterial isolates in Morocco [9,13,14]. The maximum isolates of *Acinetobacter* were obtained from ICUs (47.1%), followed by surgery (19.1%) and medicine wards (18.1%). In the study of Jaggi, *et al*., out of the total *Acinetobacter* isolates, 76.7% were obtained from ICUs [15]. The isolation rate is highest in ICUs due to the existing environment and risk factors for the persistence of this nosocomial pathogen. These consist of frequently admitted immunocompromised patients with multiple comorbidities, colonization followed by infection, prolonged stay of patients which leads to cross-infection among the patients, prolonged use of broad-spectrum antibiotics and indwelling devices. In this study, the maximum isolation of *Acinetobacter* spp. was from respiratory samples (35.9%), followed by pus (23.6%), blood (23%), body fluids (15.3%), and urine (1.4%). Similarly, in the study of Jaggi, *et al*., maximum isolates of *Acinetobacter* were obtained from respiratory samples (57.4%), followed by blood (23.8%) and pus (13.5%) [15]. In addition, Uwingabiye, *et al*. obtained maximum isolates of *Acinetobacter* spp. from bronchopulmonary samples (44.67%), followed by blood (14.51%) and deep pus (12.47%) [14]. Hospital-associated infections are most likely to involve the respiratory tract followed by urinary tract and may progress to septicemia [16,17]. In this study, urine isolates were less frequent compared with others. Among the isolates in respiratory samples received, maximum isolates were from ICUs (76.1%), followed by medicine (23.2%). Further among isolates in blood samples received, maximum was from ICUs (44.2%), followed by medicine (25.6%) and pediatric wards (23%). While among isolates from pus samples received, maximum isolates were from surgery ward (65.5%), followed by gynecology ward (9.4%). The factors responsible for a higher isolation rate in the respiratory and blood samples received from ICUs and pus samples received from surgery and gynecology wards are mechanical ventilation, nasogastric tubing, the disruption of anatomical barriers because of the use of invasive devices, surgeries, impaired immunological response, and ability of this organism to survive in high fluid content. *Acinetobacter* spp. were isolated more frequently from samples of male (59.1%) patients than that of female patients (40.8%), which is concords other studies [18]. The susceptibility testing results revealed that *Acinetobacter* spp. were resistant to most frequently used antibiotics. The isolates from ICUs and other wards showed the highest resistance to ampicillin (95.4%), followed by ceftazidime (90.6%) and cefotaxime (83.6%), similar to the results of other studies and lowest resistance to imipenem (22.8%), followed by amikacin (62%), and gentamicin (63%) [12,19]. Overall, a high resistance pattern was observed in ICUs, followed by surgery and gynecology wards. This study found a prevalence of 78.7% MDR strains; however, Begum Shahzeera, *et al*. in Islamabad reported 100% prevalence of MDR *A. baumannii* strains [20]. A meta-analysis conducted by Bialvaei, *et al*. revealed that the prevalence of MDR *A. baumannii* is 72% in Iranian population [21]. Maximum MDR strains were found in samples received from ICUs (87%), followed by surgery (85.1%) and gynecology wards (80%) and least in the samples received from pediatric ward (41.3%). *Acinetobacter* and MDR *Acinetobacter* spp. are persistent pathogens in ICUs. In the gynecology ward, although the number of isolated strains of *Acinetobacter* spp. was low, maximum strains obtained were MDR. The sample-wise distribution, revealed that maximum MDR *Acinetobacter* strains were obtained from respiratory samples (88.6%), followed by pus samples (85.3%) and minimum from blood samples (55.7%). In addition, Haung, *et al*. found maximum MDR strains from respiratory samples [22].

In this study, 42.5% of ceftazidime-resistant isolates were ESBL producers. In a study conducted by Banerjee, *et al*. in 2013, 50.7% of the *Acinetobacter* isolates were ESBL-producing, and Sinha, *et al*., in 2007, detected 28% isolates to be ESBL-producing [18,23]. Maximum ESBL producers were detected by screening positive isolates obtained from OPD (100%). During ward-wise and sample-wise distribution, maximum ESBL producers were detected from the pediatric ward and blood samples, respectively. This finding is not consistent with other studies that reported a trend of occurrence of ESBL producers similar to that of the detection of the MDR isolates [18,24]. In this study, the maximum MDR strains were detected in the respiratory sample received from ICUs, whereas maximum ESBL producers were detected in the blood samples, followed by respiratory samples, received from pediatric ward. The reason for this trend is not clear. It was observed that the isolation frequency of *Acinetobacter* spp. is increasing over the years. From being the seventh commonest isolate in 2014, it became the fifth commonest isolate in 2016. In addition, this upward trend of isolation has also been reported in other studies [3]. The reasons for this increasing trend are multifactorial such as the endemic presence of the organism in the environment, development of multidrug-resistant strains, and longer hospital stay leading to colonization [3,25,26]. Coming to the discussion of seasonal trends, there are two main studies on the isolation of *Acinetobacter* based on seasonality performed by CDC. These studies observed higher rate of infection during the months of July-October than November to June. In this study, we found seasonal variation in case of Blood stream infection, pyogenic infection, meningitis cases and not in other infections. This could be due to increased moisture and warmth in the environment, leading to an increase in their growth. Furthermore, skin colonization is higher in temperate climates than cold climates [27]. Therefore, measures to prevent skin colonization need to be more stringently followed during these months. While observing the seasonal trend in different hospital areas, trend seen in all ICUs, Medicine wards and surgery wards.

It is not surprising as the organism survive both in dry and moist conditions and hospital environment serve as reservoir where critical patients are lying but many studies including molecular need to be performed to understand the mechanism of seasonal variation.

Conclusion

In conclusion, *Acinetobacter* spp. can spread easily and remain endemic in hospitals. Therefore, the epidemiological profile of this organism should be monitored and any change in the trend must be highlighted. The infection control measures could include environmental surveillance and detection of colonization. The isolation of a patient (colonized or infected) with MDR is necessary.

Application of research: Continuous training of staff regarding the correct aseptic care of indwelling devices mainly vascular catheters and endotracheal tubes and extensive surveillance during summer and rainy seasons are the optimal approaches to eliminate *Acinetobacter* spp. from the hospital environment. As the isolation of MDR and ESBL *Acinetobacter* spp. is very high, the detection of MDR and ESBL producers is necessary to prevent therapeutic failures.

Research category: Epidemiology of *Acinetobacter*

Acknowledgement / Funding: Authors are thankful to Department of Microbiology Dr D Y Patil Medical College, Hospital and Research Centre, Dr D Y Patil Vidyapeeth, Pimpri, Pune, 411018

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Author Contributions: All authors contributed equally.

Author Statement: All authors read, reviewed, agreed and approved the final manuscript

Conflict of interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors

References

[1] Anitha M., Monisha D.M., Mohamed AS., Pandurangan S. (2016) *Sch. Acad. J. Biosci*, 4(4A), 335–41.
[2] Ghanshani R., Gupta R., Gupta B.S., Kalra S, Khedar R.S., Sood S. (2015) *Lung India*, 32(5), 441–8.
[3] Morfín-Otero R., Alcántar-Curiel M.D., Rocha M.J., C.M. Alpuche-Aranda C.M., Santos-Preciado J.I., Gayosso-Vázquez C *et al*., (2013) *Chemotherapy*,59, 57–65.
[4] Caldeiral S.M., Cunha A.R da., Akazawa R.T., Moreira R.G., Rosário de Souza L do R de., Fortaleza Carlos Magno Castelo C.B. (2015) *Rev. Saúde Pública* ,49, 19.
[5] Lone R., Shah A., Kadri S.M., Lone S., Shah F. (2009) *Bangladesh J Med Microbiol*, 03, 34–8.
[6] Clinical and Laboratory Standards Institute. M100-S23. Wayne, PA, USA: CLSI; 2013
[7] Clinical and Laboratory Standards Institute. M100-S25. Wayne, PA, USA: CLSI; 2015.
[8] Magiorakos A.P., Srinivasan A., Carey R.B., Carmeli Y, Falagas M.E., Giske C.G., *et al*., (2012) *Clin Microbiol Infect*, 18, 268–81.
[9] Kaur A., Gill A.k., Singh S., Kaur N., Mahajan A., Mittal V. (2016) *Int J Health Sci Res*, 6(6), 83–9.
[10] Singla P., Sikka R., Deeep Antariksh., Gagneja D., Chaudhary U. (2014) *J Clin Diag Res*, 8(4) ,16–9.
[11] Pandey A. (2009) *Indian J Pathol Microbiol*, 52(3), 456–7.
[12] Dhara M., Disha P., Sachin P., Manisha J., Seema B., Vega M.M. (2012) *National J Med Re*, 2(3), 348–53.
[13] Dash M., Padhi S., Patnaik S., Mohantyl, Misra P. (2013) *Avicenna J Med*, 3(4), 97–102.
[14] Uwingabiye J., Frikh M., Lemnouer A., Bssaibis F., Belefquih B., Maleb A., *et al*., (2016) *Pan Afr Med J*, 23, 191.
[15] Jaggi N., Sissodia P., Sharma L. (2012) *J Microbiol Infect Dis*, 2(2), 57–63.
[16] Bergogne-Berezin E. and Towner K.J. (1996) *Clin. Microbiol. Rev*, 9, 148–65.
[17] Cisneros J.M., Reyes M.J., Pachon J., Becerril B., Caballero F.J., Gracia-Garmendia., *et al*., (1996) *Clin Infect Dis*, 22,1026–1032.
[18] Banerjee M., Chaudhary B.L., Shukla S. (2015) *IJSR.NET*. 2015, 4(6), 1183–6.
[19] Gupta N., Gandham N., Jadhav S., Misra R.N. (2015) *J Nat Sc Biol Med*, 6(1), 159–62.
[20] Begum S., Hasan F., Hussain S., Shah A.A. (2013) *Pak J Med Sci*, 29(5), 1253–8.
[21] Bialvaei A.Z., Kouhsari E., Abargouei AS., Amirmozafari N., Ramazanzadeh R., Ghadimi-Dareajini A., *et al*., (2017) *J Chemother*, 1–9
[22] Gandham P. (2014) *Int. J. Microbiol. App. Sci*, 3(2), 9–13.
[23] Sinha M., Srinivase H., Macaden R. (2007) *Indian J Med Re*, 126, 63–7.
[24] Nath H., Barkataki D. (2016) *Int. J. Curr. Microbiol. App. Sci*, 5(11), 515–22.
[25] Gandham N.R., Gupta N., Jadhav S.V., Misra R.N. (2012) *Med. J DY Patil Univ*, 5(2), 151–3.
[26] Baang J.H., Axelrod P., Decker B.K., Hujer A.M., Dash G., Truant A.R., et a., (2012) *Am J Infect Control*, 40(2), 134–7.
[27] Alan M., Klompas M. (2010) *J Clin Outcomes Manag*, 17(8), 363–9.