ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 8, Issue 6, 2015

ISSN - 0974-2441

Research Article

AN EPIDEMIOLOGICAL STUDY ON DEADLY DRUG-DEFYING UNTREATABLE CARBAPENEM-RESISTANT GRAM-NEGATIVE BACTERIA ENCOUNTERED IN SOUTH INDIA

SHARMILA DEVI NATARAJAN[1], VISWADEVAN VISWAJITH[2], MAHENDRAN KANDHASAMY[2], MYTHILI RAVICHANDRAN[1], PRATHABAN MUNISAMY[1], VARADHARAJU CHANDRASEKAR[1]*

[1]Department of Microbiology, K.S. Rangasamy College of Arts and Science, Tiruchengode - 637 215, Tamil Nadu, India. [2]Department of Microbiology, Bioline Laboratory, Coimbatore - 641 002, Tamil Nadu, India. Email: vchandraasekar@yahoo.com

Received: 04 August 2015, Revised and Accepted: 29 September 2015

ABSTRACT

Objective: The rise of deadly "superbugs" among the pathogenic bacterial community is becoming a serious threat and the most centered concern in the field of clinical microbiology. The aim of this study was to investigate the epidemiological report on the current scenario of the multidrug-resistance (MDR) and extensive drug-resistance (XDR) characteristics of the frequent clinical isolates in South India.

Methods: In this study, isolates were analyzed using standard microbiological techniques for a 3-month period (January 2013-March 2013). Antimicrobial susceptibility test in accordance with Clinical Laboratory Standards Institute guidelines (MICRO S23) 2013 were conducted. Resistant pattern analysis was done using penicillin, aminoglycosides, monobactam, cephalosporins, quinolones, carbapenems, tetracyclines, amphenicols, sulfonamides, and nitrofurantoin classes.

Results: The highest frequency of MDR characters was owned equally by both the *Escherichia coli* and *Klebsiella* sp. with 29% resistance. However, *Acinetobacter* sp., among the MDRs, though it contributed to only 6% prevalence, it exhibited 100% resistance toward carbapenems compared to all other organisms. Our study revealed that the prevalence of carbapenem-resistant Gram-negative bacteria (GNB) in South India is 21.42%.

Conclusion: This study mainly underlines the increasing resistance in GNB to carbapenem with the data analysis over a 3-month period. This epidemiological report also insists on the dissemination of MDR among GNB in the South India. Hence, our team is working on the data for the similar period in the coming years also for having a strong hold on the status of MDR and XDR each year.

Keywords: Antibiotic resistance, Epidemiology, Extreme drug-resistant gram-negative bacteria, Multidrug-resistant gram-negative bacteria, Resistance pattern.

INTRODUCTION

Gram-negative bacilli, which include Enterobacteriaceae family and the non-fermentors including *Pseudomonas* sp., *Acinetobacter* sp., show multidrug-resistance (MDR) as they are fast in acquiring the ability to resist the effects of more than one or two classes of antibiotics to which they were previously sensitive. Recent studies on antibiotic resistance have focused on extensive drug-resistance (XDR), that is, resistance to all except one or two antibiotics [1]. Gram-negative bacteria (GNB) are the potent organisms responsible for bloodstream infections, intra-abdominal infections, urinary tract infections, hospital-acquired pneumonia, respiratory tract infections, and also infections in the wound and central venous catheters [2-4]. Now-a-day, both the fermentative and non-fermentative GNB are more rapidly acquiring MDR than Gram-positive bacteria. A recent study has reported that multidrug-resistant GNB have become serious threat with the mortality rate ranging from 30% to 70%, particularly in hospitalized patients. In Enterobacteriaceae, extended-spectrum β-lactamases enzymes of carbapenem-hydrolyzing carbapenemases constitute an important growing public threat due to the widespread usage of carbapenem drugs [5-7]. In 2011, a survey report of the Indian Situation Analysis by the Global Antibiotic Resistance Partnership (GARP) clearly stated that resistance against certain antibiotics is already at higher levels in India but the problem is hidden due to limited surveillance, but the issue was highlighted after the New Delhi metallo-β-lactamase-1 was reported, which conferred broad resistance to all major antibiotics including highly stable carbapenem [8]. A very recent review reported that the sporadic or endemic outbreaks of carbapenemases in Enterobacteriaceae can be categorized under three types of classes: A, B, or D [9,10]. Although MDR is the growing issue for both the gram-positive and GNB, the greater attention is on GNB. They are mainly associated with complicated, invasive medical devices and surgical things and serve as a major source of nosocomial infection. This scenario drew our attention toward the highly pathogenic gram-negative bacilli during our clinical survey on the MDR and XDR characteristics against carbapenem antibiotics in South India.

This is a strong epidemiological report based on large data gathered from South India to explore the worsening scenario of carbapenem-resistant GNB (CR-GNB) prevalence in routine clinical samples along with the overview of emerging XDR organisms beyond the MDR organisms.

The objective of this study was to perform an epidemiological work on the current scenario of clinical infections in South India by focusing on two major aspects. In the first phase, the steadily increasing bacterial infections were observed by screening the routine clinical samples including blood, urine, pus, and all kinds of swabs for microbiological analysis from different places of south India. In the second phase, the prevalence of MDR, XDR, and CR-GNB pattern was analyzed using percentage analysis. This is the first moralistically done epidemiological report from South India having vast sample study within a short period of investigation since a global level attention is required from rural and urban regions all over.

METHODS

This study was conducted in the Department of Clinical Microbiology, Bioline Laboratory, Coimbatore, Tamil Nadu, India, during the period from January 2013 to March 2013.

Clinical sample collection

The prospective samples (blood, urine, pus, and all kinds of swabs) were received from different places of the South India and were subjected to microbiological testing [11].

Isolation and identification

Isolation and identification of pathogenic bacterial strains were carried out as described by standard microbiological techniques [12]. Blood agar and MacConkey agar plates were used for the isolation and differentiation of bacterial isolates. Furthermore, identification and confirmation of strains were carried out using Vitek2 Compact (Software version 6.01, bioMerieux, France).

Antibiotic sensitivity test

Antibiotic susceptibility test was performed on Muller–Hinton agar plates by disk diffusion method according to National Committee for Clinical Laboratory Standards guidelines, 2013 [12]. The diameter of the zone of inhibition around the antibiotic disk was recorded and interpreted as sensitive or intermediate resistant or resistant based on the analysis of their inhibition zone, and thus the susceptibility was determined according to an Automated Osiris system (Version 9, Bio-Rad, USA) as per Clinical Laboratory Standards Institute guidelines, 2013. Criteria for resistance were evaluated in accordance with the calculation of resistance in percentage for every antibiotic in a class. The MDR was confirmed in bacteria if they were found to be resistant to more than three classes of antibiotic agents among the total antibiotics subjected [13], which included the following classes of antibiotics: penicillin, amino glycosides, monobactam, quinolones, tetracyclines, sulfonamides, nitrofurantoin, amphenicols, and carbapenem. The antibiotic-resistant pattern was analyzed with their resistance characters toward the above-mentioned antibiotics and the average percentage for the resistance was calculated for the confirmation of MDR-GNB. Organisms with intermediate levels of resistance toward the antibiotics were excluded from the percentage analysis of antibiotic resistance pattern. Finally, the most concerned CR MDR organisms were targeted for the better understanding of resistance characters.

RESULTS

Current status of bacterial infection in the South India

The first phase of the epidemiological study on the bacterial infections in the South India during January 2013 to March 2013 and distribution of samples are clearly summarized in Table 1. Maximum numbers of clinical samples were received from the highly populated geographical regions such as Coimbatore and Madurai. It was observed that majority of the route of infection is evidenced in urine samples of women aged above 25, which coincides with the recent reports of urinary tract infections [14].

Epidemiology and prevalence of MDR organisms and XDR organisms in the South India

Antimicrobial susceptibility test

Table 2a and b show the antibiogram and the basic epidemiological characteristics of the MDR and XDR bacterial isolates tested. The second phase of this epidemiological study was the surveillance of MDR from the processed samples. Of 9152 processed samples, 15 Gram-negative MDR showed extreme resistance, of which 2, MDR were from the same sample (Table 1). Among them, 10 isolates were of Gram-negative MDR from urine samples, and 5 isolates were confirmed Gram-negative MDR from other samples (swab and pus). Our study reveals that the majority of the MDR were isolated from patients aged 40 years and above, whereas 3 samples were from patients in the age group 25-30 years.

Our study shows that 100% of the *Escherichia coli* and *Klebsiella* species are resistant to second, third, and fourth generations of cephalosporins. The resistance toward the penicillin class of antibiotics such as ureidopenicillins and aminopenicillins with the combination of tazobactam and sulfactam varies with *E. coli* (93.3%), *Klebsiella* sp. (86.66%), *Pseudomonas* sp. (85.41%), and finally *Acinetobacter* sp. (75%) [15]. The monobactam, one of the beta-lactam drugs that are structurally related to the above-mentioned class of antibiotics penicillin and cephalosporin, shows 100% resistance against *E. coli*, *Klebsiellla* sp., and *Acinetobacter* sp., except *Pseudomonas* sp. (75%). These organisms also showed MDR against protein synthesis inhibitors such as aminoglycosides and tetracycline, which is as follows: *E. coli*, 100%; *Klebsiella* sp., 88.83%; *Pseudomonas* sp., 81.21%; and *Acinetobacter* sp., 75% and *E. coli,* 75%; *Klebsiella* sp. 60%; *Pseudomonas* sp., 62.5%; and *Acinetobacter* sp., 100%. *Acinetobacter* sp., which causes DNA damage and cell death, showed 100% resistance against the major generations of quinolones, whereas the others showed resistance against *E. coli* (82%) [4,16], *Klebsiella* sp. (96%), and *Pseudomonas* sp. (82.5%). Toward the member of the folate pathway inhibitors sulfonamides, all the MDR showed 100% resistance except *E. coli* (80%). In contrast, the related drug nitrofurantoin was subjected to all the three MDR except *Acinetobacter* sp., which showed 0% resistance by *E. coli,* whereas *Klebsiella* sp. and *Pseudomonas* sp. showed 100% and 75% resistance,

Table 1: Distribution of bacterial isolates in clinical samples

Samples (January 2013 to March 2013)	Number of samples
Urine samples	6708
Pus samples	868
Sputum samples	416
Swab samples	330
Blood samples	830
Total number of samples	9152

Table 2a: Antibiotic resistant attributes of occurred clinical isolates

Sample no.	Age	Sex	Sample	Antibiotic resistance attributes (%±SD)					
				Isolates	P	A	M	C	Q
01-101322	*	F	Pus	*E. coli*	100±0.5	100±0.7	100±0.5	100±0.4	100±0.5
01-101322	*	F	Pus	*Pseudomonas* sp.	75±0.6	25±0.3	100±0.5	100±0.6	50±0.8
15-008937	44	M	Swab	*Acinetobacter* sp.	75±0.9	75±0.7	100±0.5	100±0.5	100±0.3
15-9421	44	M	Sputum	*Klebsiella* sp.	100±0.6	75±0.3	100±0.9	100±0.4	100±0.5
01-116632	*	M	Pus	*E. coli*	100±0.5	100±0.3	100±0.8	100±0.5	50±0.3
05-21119	27	M	Urine	*Klebsiella* sp.	66.66±0.9	100±0.5	100±0.3	100±0.8	80±0.9
06-037257	30	M	Urine	*E. coli*	66.66±0.5	100±0.8	100±0.3	100±0.5	80±0.8
15-8630	65	F	Urine	*Klebsiella* sp.	100±0.5	66.66±0.9	100±0.5	100±0.8	100±0.5
01-104301	62	M	Urine	*Pseudomonas* sp.	66.66±0.9	100±0.5	0	100±0.8	100±0.3
01-107632	79	M	Urine	*Pseudomonas* sp.	100±0.5	100±0.3	100±0.5	100±0.5	100±0.2
01-109560	59	M	Urine	*Klebsiella* sp.	100±0.2	100±0.8	100±0.1	100±0.8	100±0.5
01-110423	46	F	Urine	*E. coli*	100±0.2	100±0.5	100±0.8	100±0.7	80±0.2
06-040256	49	M	Urine	*Pseudomonas* sp.	100±0.5	100±0.8	100±0.9	100±0.6	80±0.9
01-115018	70	F	Urine	*E. coli*	100±0.8	100±0.6	100±0.7	100±0.8	100±0.2
15-63	52	M	Urine	*Klebsiella* sp.	66.66±0.7	100±0.8	100±0.5	100±0.5	100±0.3

*100% carbapenem-resitant organisms. *Age missing SID: 01-101322, 01-116632. M: Male, F: Female, P: Penicillin, A: Aminoglycosides, M: Monobactam, C: Cephalosporins, Q: Quinolones, *E. coli: Escherichia coli*, SD: Standard deviation

Table 2b: Antibiotic resistant attributes of occurred clinical isolates

Sample no.	Age	Sex	Sample	Antibiotic resistance attributes (%±SD)					
				Isolates	CA	T	AM	S	N
01-101322	*	F	Pus	*E. coli*	66.66±0.9	50±0.9	0	100±0.4	–
01-101322	*	F	Pus	*Pseudomonas* sp.	0	50±0.9	0	100±0.5	–
15-008937	44	M	Swab	*Acinetobacter* sp.	100*±0.5	100±0.4	0	100±0.3	–
15-9421	44	M	Sputum	*Klebsiella* sp.	66.66±0.8	0	100±0.8	100±0.4	–
01-116632	*	M	Pus	*E. coli*	66.66±0.3	0	0	0	–
05-21119	27	M	Urine	*Klebsiella* sp.	33.33±0.9	100±0.5	–	100±0.7	100±0.3
06-037257	30	M	Urine	*E. coli*	66.66±0.8	100±0.3	–	100±0.5	0
15-8630	65	F	Urine	*Klebsiella* sp.	66.66±0.2	100±0.4	–	100±0.4	100±0.7
01-104301	62	M	Urine	*Pseudomonas* sp.	33.33±0.8	0	–	100±0.4	100±0.5
01-107632	79	M	Urine	*Pseudomonas* sp.	66.66±0.8	100±0.5	–	100±0.1	100±0.3
01-109560	59	M	Urine	*Klebsiella* sp.	66.66±0.8	0	–	100±0.1	100±0.4
01-110423	46	F	Urine	*E. coli*	66.66±0.8	100±0.5	–	100±0.4	0
06-040256	49	M	Urine	*Pseudomonas* sp.	100*±0.4	100±0.2	–	100±0.4	0
01-115018	70	F	Urine	*E. coli*	100*±0.4	100±0.4	–	100±0.5	0
15-63	52	M	Urine	*Klebsiella* sp.	33.33±0.9	100±0.2	–	100±0.5	100±0.5

*100% carbapenem-resistant organisms. *Age missing SID: 01-101322, 01-116632. M: Male, F: Female, CA: Carbapenems, T: Tetracyclines, AM: Amphenicols, S: Sulfonamides, N: Nitrofurantoin, *E. coli: Escherichia coli*, SD: Standard deviation

respectively. One of the significant protein synthesis inhibitors, amphenicols (chloramphenicol), showed 0% resistance by all the three MDR except *Klebsiella* sp. (100%) [13,16]. Significantly, toward the most concerned carbapenem antibiotics, the *Acinetobacter* sp. showed 100%, and *E. coli*, *Klebsiella* sp. and *Pseudomonas* sp. showed 73.32%, 53.33%, and 49.99% resistance, respectively [15]. Tables 2 and 3 summarize the detailed distribution of MDR and XDR in the South India with patient characteristics and antibiotic resistance pattern.

The frequency rate of 15 gram-negative MDR isolates is shown in Fig. 1. Of these 15 isolates, 5 isolates are non-fermentative gram-negative MDR. The percentage of the isolated MDRs prevalence is as follows: E. coli (33.33%), Klebsiella sp. (33.3%), Pseudomonas sp. (26.66%), and Acinetobacter sp. (6.66%). Our study shows that the frequent multiple drug-resistant character was predominantly incurred with both E. coli and Klebsiella sp. [11,14]. Similarly, 60% of the most predominant MDR of E. coli were isolated from urine samples [17].

Fig. 2 depicts that of the 15 MDR-GNB observed, 11 were confirmed to be XDR [1]. The percentage of emerging XDR among the MDR in South India includes *E. coli* (80%), *Klebsiella* sp. (80%), *Pseudomonas* sp. (50%), and finally *Acinetobacter* sp. (100%).

DISCUSSION

The first phase of our study clearly shows the epidemiology of bacterial infections influenced by multiple factors. The samples of blood, urine, pus, and swabs were the only targeted clinical samples in this study, because bloodstream, urinary tract, and wound infections are the significant route for many bacterial infections. In addition, we selected the most frequently received samples for this study. With the overall analysis of clinical samples from many places, the maximum numbers of samples received were from the highly populated cities such as Coimbatore and Madurai. This study shows that the number of people affected with severe bacterial infections and taking treatment to the level of microbiological analysis remains high in the Coimbatore and Madurai region compared to other places included in this study. The reasons for inclusion of less number of samples from other regions were a lack of hospital and diagnostic facilities. In addition, due to the inadequate number of health care personnel, unexpected nosocomial infections are also emerging as a broader way of MDR transmission among patient community [18]. This epidemiological report also highlights the steadily increasing rate of urinary infections among women aged 20 and above. This was indicated by the highest number of urine samples (6708) evaluated (73.29%), maximum from females in premenopausal and menopausal periods, of a total of 9152 samples [4].

Table 3: Incidence of MDR and extensive drug-resistance in clinical isolates

MDR isolates (January 2013 to March 2013)	Number of isolates
E. coli	5
Klebsiella sp.	5
Pseudomonas sp.	4
Acinetobacter sp.	1
Total number of Gram-negative MDROs	15

Of these 15, MDR 11 organisms were found to be XDR. MDR: Multidrug-resistance, XDR: Extensive drug-resistance, *E. coli: Escherichia coli*

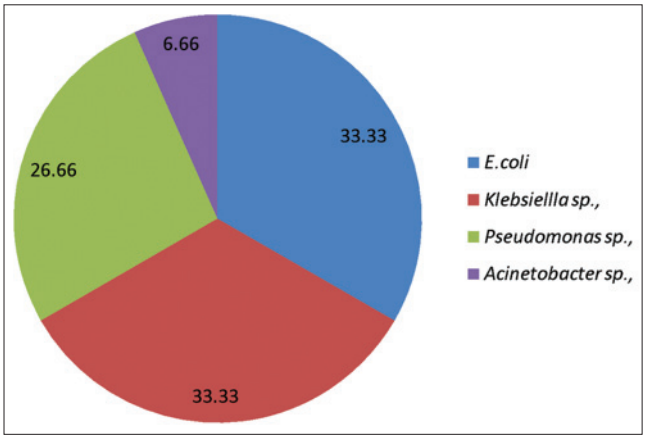

Fig. 1: Prevalence of multidrug-resistance using percentage analysis

The second phase of this epidemiological study was the investigation of MDR prevalence in South India to find out the most threatening CR Gram-negative MDR and XDR. The well-known reasons behind this antibiotic pressure in bacteria are the improper use of antibiotics and marketing strategies adopted by pharmaceutical companies for promotion of their product. In our study, for every 538 samples, 1 sample was confirmed to be the case of MDR, with a ratio of 1:500. On the basis of the 3-month MDR surveillance study, the percentage of MDR in South India was found to be 0.2%. It was also estimated that the nature of MDR attributes is likely to be more in GNB than in Gram-positive bacteria. In our study, 15 MDR-GNB included *E. coli*, *Klebsiella* sp., *Pseudomonas* sp., and *Acinetobacter* sp., as the major resistant organisms. Importantly, the high frequency of MDR was found in *E. coli* and *Klebsiella* sp. consistently with a similar ranking

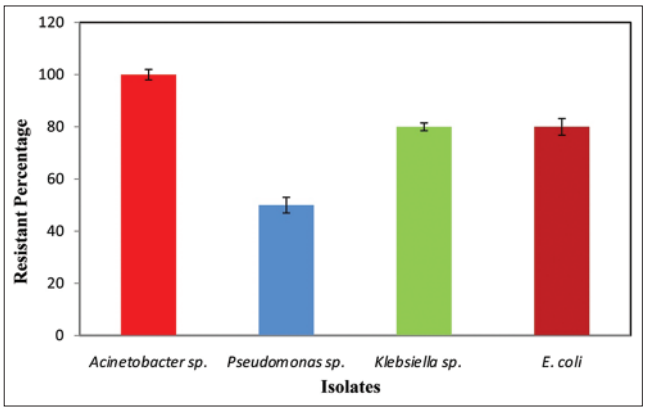

Fig. 2: Frequency of extensive drug-resistance organisms from total multidrug-resistance organisms

percentage (33.3%). While comparing the percentage obtained for these two nosocomial pathogens, *Pseudomonas* sp. ranked lower in frequency (26.6%). At last, the frequency of *Acinetobacter* sp. was very less, that is, 6.6%. However, this observation is more often with intensive care unit (ICU) patients with severe health problems leading to complications during treatment and this similar with previous studies in last few decades [17]. In another way, this study also clearly shows that maximum number of MDR prevalence (41.17%) was observed at Coimbatore, a highly populated geographical region. This may be because Coimbatore like other highly populated city provides all kinds of advanced diagnostic and hospital facilities by accompanying health care services to all nearby districts and states. In this study, we restricted the antibiotic resistance pattern analysis only to MDR GNB based on two aspects. Second, GNB infections cause more complications in treatment with higher mortality rate in early reports with the note on *Acinetobacter* sp. and *Pseudomonas* sp. in particular [14,19-21]. This antibiotic-resistant pattern analysis includes all the first-line and the second-line antibiotics, mainly following three antibiotics in carbapenem class: Meropenem, imipenem, and ertapenem. It clearly identifies that the antibiotic-resistant pattern is succeeded with 100% resistance in some of the major antibiotics in the first line except the second-line carbapenems. It highlights the default 100% resistances against cephalosporins, which was followed by monobactam, sulfonamides, nitrofurantoin (excluding *Acinetobacter* sp.), aminoglycosides, and quinolones with a slight variation. Finally, it was found that penicillin and tetracycline groups still show minimum sensitivity. Today's worldwide concern of CR in our study was observed with 21.42%. This study also reveals the total number of XDR organisms among the total MDR organisms observed, and it was found to be 11 XDR. It is notable that *Acinetobacter* sp. shows 100% resistance to all the carbapenem groups (meropenem, imipenem, and ertapenem) [4]. In contrast, two strains (*E. coli* [115018] and *Pseudomonas* sp. [040256]) were found to be resistant to all the antibiotics in the carbapenem class except imipenem. Previous investigations reported that in the United States, 60-70% *E. coli* strains showed resistance to fluoroquinolones, whereas in our study resistance was seen in more than 80% strains of *E. coli* in South India, which is increasing steadily [4,16,22]. In a previous study, it was reported that lactose non-fermenting Gram-negative pathogens such as *Acinetobacter* sp. and *Pseudomonas* sp. differ from other MDR GNB by their impermeability of the outer membrane structures, which made them to attain such defense mechanism against multiple antibiotics [23,24]. These are the two significant pathogens that frequently cause nosocomial infections among patients admitted to ICU [4,16,20]. It is clear that GARP has recommended two complementary types of surveillance including surveillance for antibiotic resistance and surveillance for antibiotic use for facilitating better prophylaxis [8,24].

CONCLUSION

We conclude that CR-GNB is very often found in clinical settings. In addition, XDR is also slowly stepping in along with the commonly existing issue and ticking horror from all the populated ends. Antibiotic susceptibility for the clinical pathogens may enhance the awareness about the prevalence of MDR and XDR worldwide to uplift the treatment (carbapenem) with better choices. Hence, we are working at molecular level to understand the genetic features of deadly MDR and XDR for the future development of novel drugs and preventive approaches.

ACKNOWLEDGMENT

We acknowledge the project funding by the UGC. We also acknowledge the laboratory support and team support provided by Department of Microbiology, Bioline Laboratory HO, Coimbatore, and all other leading branches of South India of Bioline. And I thank Department of Biotechnology, K.S. Rangasamy College of Arts and Science, Tiruchengode, and my team members Suriya Prabha Prabhu, and Karunakaran G for their support.

REFERENCES

1. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, *et al*. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18(3):268-81.
2. Paterson DL. Resistance in gram-negative bacteria: Enterobacteriaceae. Am J Infect Control 2006;34 5 Suppl 1:S20-8.
3. Gaynes R, Edwards JR. The National Infections Surveillance System. Overview of nosocomial infections caused by gram-negative bacilli. Clin Infect Dis 2005;41:848-54.
4. da Costa PM, Loureiro L, Matos AJ. Transfer of multidrug-resistant bacteria between intermingled ecological niches: The interface between humans, animals, and the environment. Int J Environ Res Public Health 2013;10:278-94.
5. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Bala Krishnan R, *et al*. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: A molecular, biological, and epidemiological study. Lancet 2010;10(9):597-602.
6. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: Here is the storm! Trends Mol Med 2012;18:263-72.
7. Hawkey PM. Prevalence and clonality of extended-spectrum beta-lactamases in Asia. Clin Microbiol Infect 2008;14 Suppl 1:159-65.
8. Global Antibiotic Resistance Partnership (GARP) — India Working Group. Rationalizing antibiotic use to limit antibiotic resistance in India. Indian J Med Res 2011;134:281-94.
9. Engel LS. Multidrug-resistant gram-negative bacteria: Trends, risk factors, and treatments. J Emerg Med 2009;18-27.
10. Maltezou HC. Metallo-beta-lactamases in Gram-negative bacteria: Introducing the era of pan-resistance? Int J Antimicrob Agents 2009;33(5):405.e1-7.
11. Blekher L, Siegman-Igra Y, Schwartz D, Berger SA, Carmeli Y. Clinical significance and antibiotic resistance patterns of *Leminorella* spp. an emerging nosocomial pathogen. J Clin Microbiol 2000;38(8):3036-8.
12. Gupta E, Mohanty S, Sood S, Dhawan B, Das BK, Kapil A. Emerging resistance to Carbapenems in a tertiary care hospital in north India. Indian J Med Res 2006;124(1):95-8.
13. Huang XZ, Frye JG, Chahine MA, Glenn LM, Ake JA, Su W, *et al*. Characteristics of plasmids in multi-drug-resistant Enterobacteriaceae isolated during prospective surveillance of a newly opened hospital in Iraq. PLoS One 2012;7(7):e40360.
14. Linhares I, Raposo T, Rodrigues A, Almeida A. Frequency and antimicrobial resistance patterns of bacteria implicated in community urinary tract infections: A 10-years surveillance study (2000-2009). BMC Infect Dis 2013;13:19.
15. Tripathi KD. Essentials of Medical Pharmacology. 6th ed. India: JP Brothers; 2008.
16. Levy SB, Marshall B. Antibacterial resistance worldwide: Causes, challenges and responses. Nat Med 2004;10 Suppl 2:S122-9.
17. Peleg AY, Hooper DC. Hospital-acquired infections due to gram-negative bacteria. N Engl J Med 2010;362:1804-13.
18. Siegel SG, Rhinehart E, Jackson M, Chiarello L. The Healthcare Infection Control Practices Advisory Committee. Management of Multidrug-Resistant Organisms in Healthcare Settings. Healthcare

Infection Control Practices Advisory Committee (HICPAC); 2006. Available from: http://www.cdc.gov/hicpac/pdf/guidelines/MDROGuideline2006.pdf.
19. Kollef MH, Golan Y, Micek ST, Shorr AF, Restrepo MI. Appraising contemporary strategies to combat multidrug-resistant gram-negative bacterial infections-proceedings and data from the gram-negative resistance summit. Clin Infect Dis 2011;53 Suppl 2:S33-55.
20. Bonomo RA, Szabo D. Mechanisms of multidrug-resistance in *Acinetobacter* species and *Pseudomonas aeruginosa*. Clin Infect Dis 2006;43 Suppl 2:S49-56.
21. Panta K, Ghimire P, Rai SK, Mukhiya RK, Singh RN, Rai G. Antibiogram typing of gram-negative isolates in different clinical samples of a tertiary hospital. Asian J Pharm Clin Res 2013;6(1):153-6.
22. Tamma PD, Cosgrove SE, Maragakis LL. Combination therapy for treatment of infections with gram-negative bacteria. Clin Microbiol Rev 2012;25(3):450-70.
23. Giske CG, Monnet DL, Cars O, Carmeli Y. Clinical and economic impact of common multidrug-resistant gram-negative bacilli. Antimicrob Agents Chemother 2008;52(3):813-21.
24. Bisht R, Katiyar A, Singh R, Mittal P. Antibiotic resistance: A global issue of concern. Asian J Pharm Clin Res 2009;2(2):34-9.