Available online at **www.ijmrhs.com**

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2017, 6(4): 36-42

Comparative Evaluation of *In-Vitro* Doripenem Susceptibility with Other Carbapenem Antibiotics among Gram Negative Bacterial Isolates Obtained from VAP Patients in a Super-Speciality Hospital: A Pilot Study

Mohit Bhatia*, Poonam Sood Loomba, Bibhabati Mishra, and Vinita Dogra

Department of Microbiology, Govind Ballabh Pant Institute of Post Graduate Medical Education and Research, New Delhi, India

**Corresponding e-mail: docmb1984@gmail.com*

ABSTRACT

Context: *Few studies have been published about the in vitro Doripenem susceptibility profiles of Gram negative bacteria obtained from lower respiratory tract samples of patients suffering from Ventilator Associated Pneumonia (VAP).* ***Aims:*** *To generate preliminary data on in vitro Doripenem susceptibility profile of Gram negative bacteria isolated from mucus trap samples of patients suffering from VAP and also compare the organism wise in vitro susceptibility pattern of Doripenem, ertapenem, imipenem and meropenem.* ***Settings and Design:*** *A pilot study was conducted in a super speciality hospital from October 2015 to June 2016.* ***Material and Methods:*** *Patients on ventilator admitted in various intensive care units (ICUs) satisfying the defining criteria for VAP as per standard guidelines were included in the study. Seventy-Seven Gram negative bacterial isolates obtained from mucus trap samples of fifty-seven non-consecutive patients were identified and subjected to antibiotic susceptibility testing (AST) as per standard guidelines.* ***Statistical analysis used:*** *Descriptive statistics.* ***Results:*** *Klebsiella pneumoniae followed by Acinetobacter baumannii and Pseudomonas aeruginosa were the predominant bacterial isolates. The Doripenem resistance rates among K. pneumoniae, A. baumannii and P. aeruginosa were 55.10%, 96.43% and 52.94% respectively. There was 100% concordance between resistance to Doripenem, imipenem and meropenem respectively in A. baumannii. Fourteen (82.35%) out of seventeen Enterobacteriaceae spp. (Klebsiella pneumoniae and Escherichia coli) Doripenem resistant isolates were also resistant to ertapenem, imipenem and meropenem respectively. In case of P. aeruginosa, five (55.55%) Doripenem resistant isolates were also resistant to imipenem and meropenem respectively.* ***Conclusions:*** *This is probably the first report on high level of Doripenem resistance in K. pneumoniae from India. More number of studies should be conducted in order to substantiate our findings.*

Keywords: Doripenem, VAP, *K. pneumoniae, A. baumannii, P. aeruginosa*

INTRODUCTION

Carbapenems are gradually assuming a major role in the treatment of severe nosocomial bacterial infections. Doripenem is a new parenteral carbapenem antibiotic having significant *in vitro* activity against *Streptococci*, methicillin-susceptible *Staphylococci*, Enterobacteriaceae (including extended-spectrum beta-lactamase producing strains), *Pseudomonas aeruginosa*, *Acinetobacter* spp., and *Bacteroides fragilis* [1]. Doripenem was approved by United States Food and Drug Administration (US-FDA) in 2007 for the treatment of complicated intra-abdominal infections (cIAI), complicated urinary tract infections (cUTI) and pyelonephritis. In Europe and several Asia-Pacific countries, it has also been approved for treatment of nosocomial pneumonia (NP), including ventilator-associated pneumonia (VAP) [1,2]. Very few studies have been published about the *in vitro* doripenem susceptibility profiles of Gram negative bacterial isolates obtained from lower respiratory tract samples of patients suffering from VAP. Also, limited data is available about comparative evaluation of *in vitro* carbapenem susceptibility pattern of Gram negative bacteria.

This study was conducted with the aim of generating preliminary data on *in vitro* Doripenem susceptibility profile of

Gram negative bacteria isolated from mucus trap samples of patients suffering from VAP. Comparison of the organism wise *in vitro* susceptibility pattern of Doripenem, ertapenem, imipenem and meropenem was also attempted.

SUBJECTS AND METHODS

A pilot study was conducted in a super speciality hospital from October 2015 to June 2016. Patients on ventilator admitted in various intensive care units (ICUs) of this hospital satisfying the defining criteria for ventilator associated pneumonia (VAP) as per centres for disease control (CDC) guidelines 2015 were included in the study. Lower respiratory tract samples obtained using mucus extractors (mucus trap samples) from fifty-seven non-consecutive patients hospitalized during the study period were subjected to Gram stain and culture. Seventy-seven bacterial isolates, all of which were Gram negative, were obtained from these samples. All isolates were identified and subjected to antibiotic susceptibility testing (AST) in the form of minimum inhibitory concentration (MIC) using VITEK-2 (BioMérieux India Pvt. Ltd., New Delhi) automated system. MIC values of Amikacin, Gentamicin, Amoxicillin-Clavulanate, Piperacillin-Tazobactam, Ertapenem, Meropenem, Imipenem, Cefepime, Ceftriaxone, Cefuroxime, Cefuroxime axetil, Ciprofloxacin and Trimethoprim-Sulphamethoxazole respectively were determined as per Clinical Laboratory Standards Institute (CLSI) guidelines 2015. MIC values of tigecycline and colistin (for *Enterobacteriaceae* spp.) respectively were determined as per European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines 2015. The susceptibility to Doripenem (MIC) was determined using E-test strips (BioMérieux India Pvt. Ltd., New Delhi), the results of which were also interpreted as per CLSI guidelines 2015. *K. pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains for this purpose. Susceptibility to additional antibiotics namely Ampicillin-sulbactam, Cefotaxime, Ceftazidime, Levofloxacin, Ofloxacin, Netilmicin, Tobramycin and Ticarcillin-Clavulanate (as applicable for different Gram negative bacterial isolates) was determined using modified Kirby-Bauer disk diffusion method as per CLSI guidelines 2015.

RESULTS

The study population was constituted by thirty-two male and twenty-five female patients respectively. The mean age (± 2 SD) of the study participants was 51.36 ± 15.09 years. *Klebsiella pneumoniae* followed by *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were the predominant bacterial isolates as depicted in Figure 1. Figure 2 depicts the *in vitro* Doripenem susceptibility results of *K. pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 respectively. Figure 3 depicts the E-test results of two test isolates one of which was susceptible and the other resistant to Doripenem respectively.

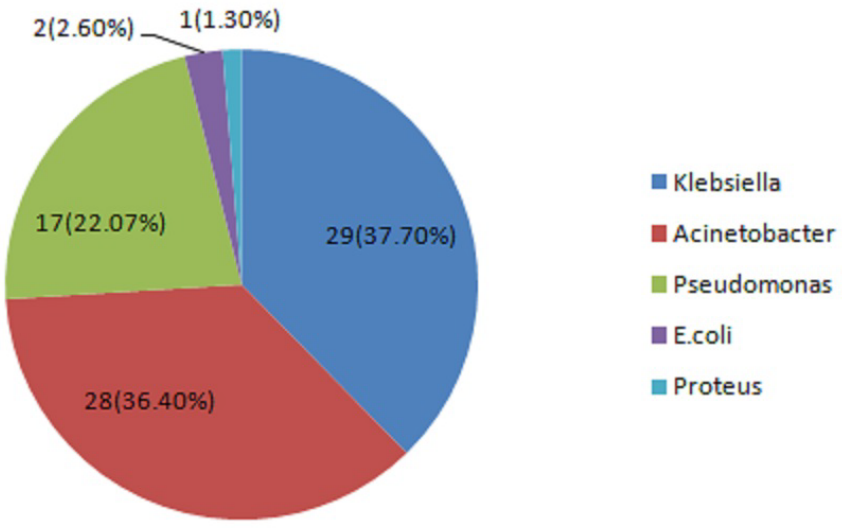

Figure 1 Percentage distribution of bacterial isolates obtained from patients suffering from VAP during the study period

Figure 2 *In vitro* Doripenem susceptibility results of *K. pneumoniae* ATCC 700603, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 respectively

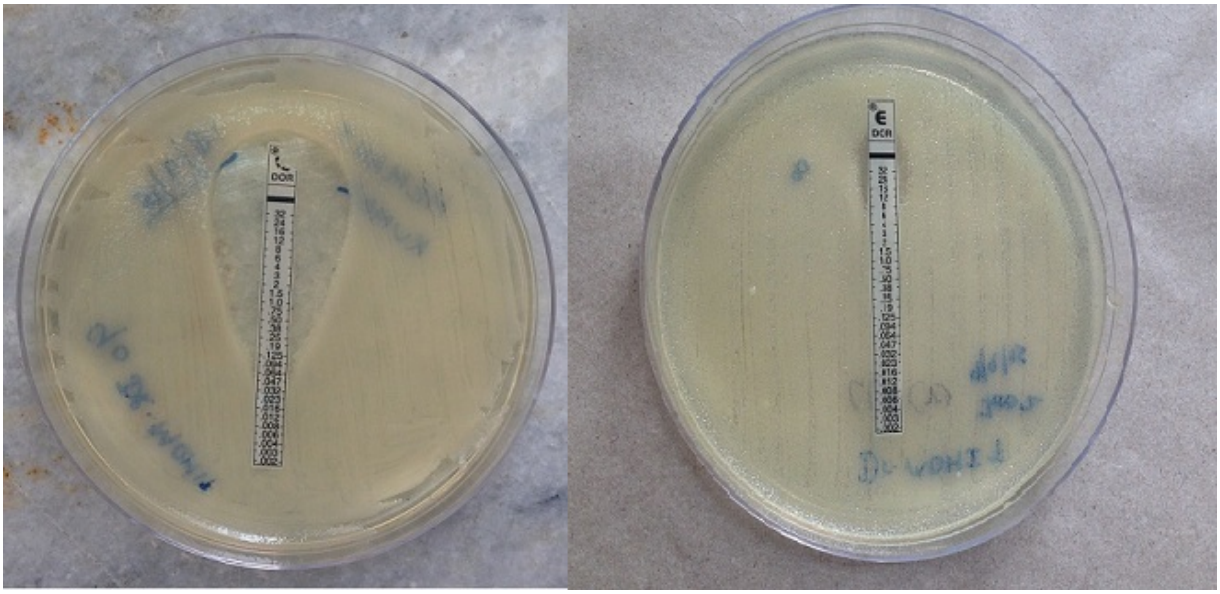

Figure 3 The E-test results of Doripenem sensitive and resistant isolates respectively

The *in vitro* antibiotic resistance profile of all 77 bacterial isolates is shown in Table 1. Majority of the bacterial isolates exhibited high resistance rates to all major antibiotic classes with the exception of glycyl cyclins and lipopeptides. While 27.60% of *K. pneumoniae* and 35.30% of *A. baumannii* isolates were respectively resistant to tigecycline, 34.48% of *K. pneumoniae*, 7.14% of *A. baumannii* and 23.53% of *P. aeruginosa* isolates were respectively resistant to colistin.

Table 1 The *in vitro* resistance profile of all bacterial isolates under study to different antibiotics

Antibiotic Group	*Acinetobacter baumannii* No. (%)	*Escherichia coli* No. (%)	*Klebsiella pneumoniae* No. (%)	*Proteus mirabilis* No. (%)	*Pseudomonas aeruginosa* No. (%)
Aminoglycosides					
Amikacin	28 (100)	2 (100)	27 (93.10)	1 (100)	14 (82.35)
Gentamicin	26 (92.80)	2 (100)	27 (93.10)	1 (100)	11 (64.70)
[@]Netilmicin	N.A.	2 (100)	28 (96.55)	1 (100)	10 (58.82)
Tobramycin	28 (100)	2 (100)	28 (96.55)	1 (100)	11 (64.70)
β-Lactam/β-Lactamase inhibitor combinations					
[@@]Amoxicillin-clavulanate	N.A.	2 (100)	29 (100)	1 (100)	N.A.
[#]Ampicillin-sulbactam	13 (46.40)	N.A.	N.A.	N.A.	N.A.

Piperacillin-tazobactam	27 (96.43)	2 (100)	27 (93.10)	1 (100)	10 (58.82)
Ticarcillin-clavulanate	27 (96.43)	2 (100)	29 (100)	0 (0)	15 (88.23)
Carbapenems					
Doripenem	27 (96.43)	1 (50)	16 (55.10)	0 (0)	9 (52.94)
[##]Ertapenem	N.A.	2 (100)	26 (89.60)	1 (100)	N.A.
Imipenem	28 (100)	2 (100)	24 (82.76)	1 (100)	6 (35.30)
Meropenem	28 (100)	2 (100)	27 (93.10)	1 (100)	12 (70.60)
Cephalosporins					
Cefepime	27 (96.43)	2 (100)	29 (100)	1 (100)	14 (82.35)
[*]Cefotaxime	28 (100)	2 (100)	29 (100)	0 (0)	N.A.
Ceftazidime	28 (100)	2 (100)	29 (100)	0 (0)	12(70.60)
[*]Ceftriaxone	28 (100)	2 (100)	29 (100)	1 (100)	N.A.
[*]Cefuroxime-axetil	N.A.	2 (100)	29 (100)	1 (100)	N.A.
[*]Cefuroxime	N.A.	2 (100)	29 (100)	1 (100)	N.A.
Fluoroquinolones					
Ciprofloxacin	27 (96.43)	2 (100)	28 (96.55)	1 (100)	11 (64.70)
Levofloxacin	27 (96.43)	2 (100)	29 (100)	0 (0)	12 (70.60)
[**]Ofloxacin	N.A.	2 (100)	29 (100)	1 (100)	N.A.
Folate pathway inhibitors					
[$]Trimethoprim-sulphamethoxazole	27 (96.43)	2 (100)	25 (86.20)	1 (100)	N.A.
Glycyl cyclins					
[$$]Tigecycline	6 (35.30)	0 (0)	8 (27.60)	1 (100)	N.A.
Lipopeptides					
[+]Colistin	2 (7.14)	0 (0)	10 (34.48)	1 (100)	4 (23.53)

[@]Susceptibility of *A. baumannii* to netilmicin could not be recorded as only MIC and not zone diameter of netilmicin has been defined for *A. baumannii* as per CLSI guidelines 2015. VITEK-2 automated system does not calculate MIC of netilmicin for Gram negative bacilli.
[@@]Amoxicillin-clavulanate is not recommended for use against *P. aeruginosa* and *Acinetoacter* spp. respectively as per CLSI guidelines 2015.
[#]Ampicillin-sulbactam is only used for *Acinetobacter* spp. as per CLSI guidelines 2015.
[##]Ertapenem is not recommended for use against *P. aeruginosa* and *A. baumannii* respectively as per CLSI guidelines 2015.
[*]Cefotaxime, Ceftriaxone, Cefuroxime Axetil and Cefuroxime are not recommended for use against *P. aeruginosa* as per CLSI guidelines 2015. Cefuroxime Axetil and Cefuroxime are also not recommended for use against *A. baumannii* as per CLSI guidelines 2015.
[**]Ofloxacin is not recommended for use against *A. baumannii* as per CLSI guidelines 2015.
[$]Trimethoprim-sulfamethoxazole is not recommended for use against *P. aeruginosa* as per CLSI guidelines 2015.
[$$]MIC for tigecycline was recorded as per EUCAST guidelines 2015. As per both CLSI and EUCAST guidelines 2015, tigecycline is not recommended for use against *P. aeruginosa.*
[+]MIC for colistin was recorded for *P. aeruginosa* and *A. baumannii* as per CLSI guidelines 2015. However, for members of the family Enterobacteriaceae, MIC for this antibiotic was recorded as per EUCAST guidelines 2015.

Table 2 depicts the summary of organism wise comparative susceptibility results of four different carbapenem antibiotics namely imipenem, meropenem, ertapenem and Doripenem. 15 (51.72%) out of 29 *K. pneumoniae* isolates were resistant to both Doripenem and ertapenem, Doripenem and imipenem and Doripenem and meropenem respectively. 27 (96.43%) out of 28 *A. baumannii* isolates were resistant to both Doripenem and imipenem and Doripenem and meropenem respectively. While 5 (29.41%) out of 17 *P. aeruginosa* isolates were resistant to both Doripenem and imipenem, 8 (47.06%) out of 17 *P. aeruginosa* isolates were resistant to both Doripenem and meropenem respectively.

Table 2 Summary of organism wise comparative susceptibility results of four different carbapenem antibiotics

Organisms	Doripenem/Ertapenem *Number				Doripenem/Imipenem *Number				Doripenem/Meropenem *Number			
	S/S	S/R	R/S	R/R	S/S	S/R	R/S	R/R	S/S	S/R	R/S	R/R
[**]*A. baumannii*	-	-	-	-	-	-	-	27	-	-	-	27
E. coli	-	1	-	1	-	1	-	1	-	1	-	1
[@]*K. pneumoniae*	1	9	2	15	2	7	3	15	1	9	1	15
P. mirabilis	-	1	-	-	-	1	-	-	-	1	-	-
[@@]*P. aeruginosa*	-	-	-	-	5	-	4	5	3	2	-	8

[*]S/S-Sensitive to both carbapenem antibiotics; S/R-Sensitive to first and resistant to second carbapenem antibiotic; R/S-Resistant to first and

sensitive to second carbapenem antibiotic; R/R-Resistant to both carbapenem antibiotics.
[**]One *A. baumannii* isolate was intermediate susceptible to Doripenem and resistant to both imipenem and meropenem respectively.
[@]While two isolates of *K. pneumoniae* were intermediate susceptible to Doripenem and resistant to imipenem, meropenem and ertapenem respectively, one isolate was resistant to Doripenem and intermediate susceptible to meropenem.
[@@]Two isolates of *P. aeruginosa* were intermediate susceptible to Doripenem and susceptible to imipenem, one isolate was intermediate susceptible to Doripenem and resistant to both imipenem and meropenem and another isolate was intermediate susceptible to Doripenem and resistant to meropenem.

Out of the sixteen Doripenem resistant isolates of *K. pneumoniae*, thirteen (81.25%) had MIC>32 µg/ml each, while other three (18.75%) had MIC=8 µg/ml each. Out of 13 Doripenem resistant *K. pneumoniae* isolates with MIC values >32 µg/ml, 1 isolate was susceptible to imipenem (MIC ≤ 1 µg/ml) only, 1 isolate was intermediate susceptible to ertapenem (MIC=1 µg/ml) but susceptible to both imipenem (MIC ≤ 1 µg/ml) and meropenem (MIC ≤ 1 µg/ml) and 1 isolate was susceptible to ertapenem (MIC ≤ 0.5 µg/ml), imipenem (MIC ≤ 1 µg/ml) and meropenem (MIC ≤ 1 µg/ml) respectively. All 3 Doripenem resistant *K. pneumoniae* isolates which had MIC=8 µg/ml were also resistant to ertapenem (MIC ≥ 2 µg/ml), imipenem (MIC ≥ 4 µg/ml) and meropenem (MIC ≥ 4 µg/ml) respectively. One isolate of Doripenem resistant *E. coli* also had MIC >32 µg/ml and was resistant to ertapenem (MIC ≥ 2 µg/ml), imipenem (MIC ≥ 4 µg/ml) and meropenem (MIC ≥ 4 µg/ml) respectively.

All twenty-seven (100%) and nine (100%) Doripenem resistant *A. baumannii* and *P. aeruginosa* isolates respectively had MIC >32 µg/ml each. All 27 Doripenem resistant *A. baumannii* isolates were also resistant to both imipenem (MIC ≥ 8 µg/ml) and meropenem (MIC ≥ 8 µg/ml) respectively. While 3 out of 9 Doripenem resistant *P. aeruginosa* isolates were susceptible to imipenem (MIC ≤ 2 µg/ml) only, 1 isolate was susceptible to imipenem (MIC ≤ 1 µg/ml) and intermediate susceptible to meropenem (MIC=4 µg/ml).

DISCUSSION

With the exception of colistin and tigecycline, high antibiotic resistance rates were observed in the present study. Antibiotic resistance rates are rising steeply among several Gram-negative bacteria like *Acinetobacter* spp., *P. aeruginosa* and members of the family Enterobacteriaceae, that often cause serious nosocomial infections [3]. The frequent usage of broad-spectrum antibiotics results in the selection of multi-drug resistant bacteria. Colonization and subsequent serious infections with these microorganisms results in increased morbidity and mortality among hospitalized patients [4-7].

Among the three major bacterial isolates obtained in this study, *P. aeruginosa* followed by *K. pneumoniae* showed the lowest Doripenem resistance rates of 52.94% and 55.10% respectively. Overall 53.13% of bacterial isolates belonging to the family Enterobacteriaceae (*K. pneumoniae, E. coli* and *P. mirabilis*) were resistant to Doripenem. 96.43% of *A. baumannii* isolates were found to be resistant to Doripenem.

Goyal, et al. had first reported high level of resistance against Doripenem in *A. baumannii* from a tertiary care referral hospital in India. In their study, *P. aeruginosa* showed sensitivity of 60.3% for Doripenem and 44.8% for meropenem. However, Doripenem and meropenem were effective against 6.4% and 6.3% of *A. baumannii* isolates, respectively [1]. In a multi-centric study conducted by Mendes, et al., the Doripenem resistance rate among *Enterobacteriaceae* spp. was found to be 1.3% [8]. In another multi-centric study conducted by Yun Li, et al. highest and lowest Doripenem resistance rates of 67.40% and 1.90% were observed among *A. baumannii* and *Enterobacteriaceae* spp. isolates respectively. In the same study, Doripenem resistance rate of *P. aeruginosa* was found to be 16.2% [9]. To the best of our knowledge, ours is the first report on high level of Doripenem resistance in *K. pneumoniae* and only the second report on high level of Doripenem resistance in *A. baumannii* from India.

Another highlight of this study was that more number of *P. aeruginosa* isolates were susceptible to imipenem than both Doripenem and meropenem respectively. Doripenem is generally considered to be more active than both meropenem and imipenem versus *P. aeruginosa* and Acinetobacter spp. due to its strong affinity for penicillin binding protein (PBP) targets that are species specific [10]. The reason for our aberrant findings could be due to the fact that only 17 isolates of *P. aeruginosa* were subjected to AST in the present study.

There was 100% concordance between resistance to Doripenem (MIC ≥ 8 µg/ml), imipenem (MIC ≥ 8 µg/ml) and meropenem (MIC ≥ 8 µg/ml) respectively in *A. baumannii*. Fourteen (82.35%) out of seventeen Enterobacteriaceae

spp. (*K. pneumoniae* and *E. coli*) Doripenem resistant isolates (MIC ≥ 8 µg/ml) were also resistant to ertapenem (MIC ≥ 2 µg/ml), imipenem (MIC ≥ 4 µg/ml) and meropenem (MIC ≥ 4 µg/ml) respectively. In case of *P. aeruginosa*, five (55.55%) Doripenem resistant isolates were also resistant to imipenem (MIC ≥ 8 µg/ml) and meropenem (MIC ≥ 8 µg/ml) respectively. In a multi-centric study conducted by Jean, et al. similar kind of analysis was done with the aim of providing an insight about choosing appropriate carbapenem agents to treat infections in critically ill hospitalized patients. In this study, *E. coli*, *K. pneumoniae* and *Enterobacter cloacae* with ertapenem MICs ≥ 4 mg/l were synchronously not susceptible to imipenem, meropenem and Doripenem. Additionally, *P. aeruginosa* and *A. baumannii* isolates with imipenem MICs ≥ 8 mg/l were also not susceptible to meropenem and Doripenem [11].

A major drawback of our study was small sample size owing to which no statistical evaluation of our findings could be done. Also, due to the same reason the MIC50 and MIC90 values could not be obtained for different bacterial isolates.

The results obtained in our study point towards the possibility of existence of high level of Doripenem resistance among members of the family Enterobacteriaceae and non-fermenter Gram negative bacilli. More number of multi-centric studies should be conducted in order to substantiate our findings. Also, more number of randomized control trials should be conducted in order to evaluate the efficacy of Doripenem and other carbapenems in clinical settings.

REFERENCES

[1] Goyal, K., Gautam, V., and Ray, P. "Doripenem vs meropenem against Pseudomonas and Acinetobacter." *Indian journal of medical microbiology* 30.3 (2012): 350.
[2] Qu, Xiao-Yu, Ting-Ting Hu, and Wei Zhou. "A meta-analysis of efficacy and safety of Doripenem for treating bacterial infections." *Brazilian Journal of Infectious Diseases* 19.2 (2015): 156-162.
[3] Slama, Thomas G. "Gram-negative antibiotic resistance: there is a price to pay." *Critical Care* 12.4 (2008): S4.
[4] Günseren, Filiz, et al. "A surveillance study of antimicrobial resistance of gram-negative bacteria isolated from intensive care units in eight hospitals in Turkey." *Journal of Antimicrobial Chemotherapy* 43.3 (1999): 373-378.
[5] Kucukates, Emine. "Antimicrobial resistance among Gram-negative bacteria isolated from intensive care units in a Cardiology Institute in Istanbul, Turkey." *Japanese journal of infectious diseases* 58.4 (2005): 228.
[6] Kollef, Marin H., et al. "The impact of nosocomial infections on patient outcomes following cardiac surgery." *Chest* 112.3 (1997): 666-675.
[7] Trouillet, Jean-Louis, et al. "Ventilator-associated pneumonia caused by potentially drug-resistant bacteria." *American journal of respiratory and critical care medicine* 157.2 (1998): 531-539.
[8] Mendes, Rodrigo E., et al. "Doripenem activity tested against a global collection of Enterobacteriaceae, including isolates resistant to other extended-spectrum agents." *Diagnostic microbiology and infectious disease* 63.4 (2009): 415-425.
[9] Li, Yun, et al. "Antimicrobial resistance surveillance of Doripenem in China." *The Journal of antibiotics* 68.8 (2015): 496-500.
[10] Canadian Antimicrobial Resistance Alliance (CARA). "Doripenem." Available from: http://www.can-r.com/mediaResources/About%20Doripenem.pdf
[11] Jean, Shio-Shin, et al. "Carbapenem susceptibilities and non-susceptibility concordance to different carbapenems amongst clinically important Gram-negative bacteria isolated from intensive care units in Taiwan: results from the Surveillance of Multicentre Antimicrobial Resistance in Taiwan (SMART) in 2009." *International journal of antimicrobial agents* 41.5 (2013): 457-462.