ORIGINAL ARTICLE

In vitro Efficacy of Meropenem, Colistin and Tigecycline Against the Extended Spectrum Beta-Lactamase Producing Gram Negative Bacilli

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ABSTRACT

Objective: To compare the *in vitro* efficacy of meropenem, colistin and tigecycline against extended spectrum Beta-lactamase producing Gram negative bacilli by minimal inhibitory concentration.

Study Design: Cross-sectional descriptive study.

Place and Duration of Study: Department of Microbiology, Army Medical College, National University of Sciences and Technology, Rawalpindi, from June to December 2010.

Methodology: Routine clinical specimens were subjected to standard microbiological procedures and the isolates were identified to species level. Extended spectrum β-lactamase producing Gram negative bacilli were detected by Jarlier disc synergy method and confirmed by ceftazidime and ceftazidime-clavulanate Etest. Minimum Inhibitory Concentration (MIC_{90}) of meropenem, colistin and tigecycline was determined by Etest (AB BIOMERIUX) and the results were interpreted according to the manufacturer's instructions and Clinical and Laboratory Standards Institute guidelines and Food and Drug Authority recommendations. Results were analyzed by using Statistical Package for the Social Sciences version 20.

Results: A total of 52 non-duplicate extended spectrum Beta-lactamase-producing Gram negative bacilli were included in the study. The MIC_{90} of tigecycline (0.75 µg/ml) was lowest as compared to the meropenem (2 µg/ml) and colistin (3 µg/ml).

Conclusion: Tigecycline is superior in efficacy against the extended spectrum Beta-lactamase producing Gram negative bacilli as compared to colistin and meropenem.

Key Words: *Colistin. Extended spectrum β-lactamase. Meropenem. Minimum inhibitory concentration. Tigecycline.*

INTRODUCTION

The rapid developments in the field of antibiotic research have been largely offset by emerging problem of antimicrobial resistance.[1] This has been due to various mechanisms including but not limited to gene mutations, resistance enzymes, efflux pumps and biofilm formation.[2] Non-judicious use of antimicrobial agents and improper antiseptic measures by the healthcare providers have further complicated the issue leading to the emergence of Multi-Drug Resistant (MDR) bacteria.[3] MDR bacteria like Extended Spectrum Beta-Lactamase (ESBL) producing Gram Negative Rods (GNRs) make the infection management difficult and also increase the mortality and morbidity.[4] ESBLs are enzymes which confer resistance in bacteria against all penicillins, most cephalosporins and the monobactams.[5] The need to evaluate the efficacy of newer antimicrobial groups in ESBL infections is of paramount importance.[6]

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Received: March 07, 2012; Accepted: March 03, 2015.

Carbapenem is a group of β-lactam antibiotics which act by inhibiting cell wall synthesis.[7] They have a diverse spectrum of activity against anaerobes, Gram positive and Gram negative bacteria.[7] Colistin is a polymyxin, first discovered in 1960s. It acts by disrupting active transport function of the cell membrane, and is active against infections caused by Gram negative bacilli.[7,8] The neurotoxic and nephrotoxic effects of the agent are dose-related and reversible so it can be a useful option for the management of infections by multi-drug resistant and pan-drug resistant bacteria.[8] Tigecycline is a newer semi-synthetic broad spectrum antibiotic which is active not only against Gram positive, Gram negative bacteria, anaerobes but also against atypical bacteria.[3,9] However, it is less effective against *Pseudomonas* spp. and *Proteus* spp.[3] It can counter the bacterial resistance by by-passing the efflux pump mechanism and also by binding avidly to the ribosomal receptors.[3]

Timely detection of infections by ESBL producing GNRs and proper management in light of the culture and susceptibility report is of prime importance to prevent the spread of these strains. This study was aimed to compare the *in vitro* efficacy of meropenem, colistin and tigecycline against extended spectrum β-lactamase producing Gram negative bacilli.

METHODOLOGY

This comparative, cross-sectional study was conducted in the Department of Microbiology, Army Medical College, National University of Sciences and Technology, Islamabad, from June to December 2010. Routine clinical specimens including urine, pus, blood, sputum, High Vaginal Swabs (HVS), fluids and tissues were cultured on blood, MacConkey and chocolate agars (Oxoid). The isolates were identified to species level by standard microbiological procedures like Gram staining, colony morphology, biochemical tests and Analytical Profile Index (API)- 20 E, if required.[10,11] Antimicrobial susceptibility was determined by Kirby Bauer disc diffusion method and ESBL detection was done by disc synergy method.[12,13] The total number of ESBLs isolated during the study period was quite high (n=348) however, due to financial constraints, 65 isolates (chosen by random selection) were subjected for confirmation of ESBL production to Etest using ceftazidime plus ceftazidime-clavulanate Etest strip (AB Biodisk, Solna, Sweden).[14] A total of 52 ESBL Etest positive specimens were subjected to minimum inhibitory concentration determination of meropenem, colistin and tigecycline by Etest method. All samples from the same patient during the same episode of illness were excluded.

A 0.5 McFarland's suspension of each isolate was prepared and applied on three Mueller Hinton Agar plates. Etest strips (AB Biodisk, Solna, Sweden) for meropenem, colistin and tigecycline were applied on the separate inoculated plates which were then incubated at 37°C for 16 - 24 hours. The results were read according to the manufacturer's instructions and interpreted by Clinical and Laboratory Standards Institute (CLSI) guidelines for meropenem, Galani *et al*. for colistin and Food and Drug Authority (FDA) recommendations for tigecycline.[5,15-17] MIC_{50} and MIC_{90} values of the three antimicrobial agents were calculated by cumulative percentage by calculating the percentage isolates exhibiting a particular MIC value and then arranging these MICs in ascending order (Table I). MIC_{50} was defined as that value of MIC which corresponded to the 50% of the isolates and MIC_{90} was defined as that value of MIC which corresponded to 90% of the isolates (Table I). The *in vitro* efficacy of the three antibiotics were compared descriptively on the basis of the lowest MIC_{90} value as a marker of better *in vitro* efficacy.

Results were analyzed on the Statistical Package for the Social Sciences (SPSS) version 20. Descriptive statistics were used to describe the results i.e. frequency and percentage were calculated for qualitative variables like isolation of ESBL producers in various clinical specimens and among different GNRs. Kruskal-Wallis test was applied to compare MIC of the three groups. A p-value < 0.05 was considered as significant.

RESULTS

Out of a total of 52 ESBL producing Gram negative bacilli, 40.4% were isolated from urine (n=21), followed by 19.2% from pus (n=10), 13.5% from Catheter tips (n=07), 7.7% from high vaginal swab (n=04), 5.8% (n=03) each from sputum and blood and only 3.8% (n=02) were isolated from body fluids and tissues. *Escherichia coli* (*E. coli*) was found to be the commonest (51.9%, n= 27) of the ESBL producing organisms during the period of this study, followed by *Klebsiella pneumoniae* (*K. pneumoniae*) (30.7%, n=16), *Enterobacter* spp. (9.6%, n=05) and only 7.7% (n=04) were found to be *Klebsiella oxytoca* (*K. oxytoca*). Taking ≤ 1.0 µg/ml as susceptible, 2.0 µg/ml as intermediate and ≥ 4.0 µg/ml as resistant for meropenem, 86.5% of the isolates were found to be susceptible, 11.5% intermediate and 1.9% resistant. In case of colistin, taking ≤ 2.0 µg/ml as susceptible and ≥ 4.0 µg/ml as resistant, 95.9% were found to be susceptible and 3.8% in intermediate range.[14] For tigecycline, ≤ 2.0 µg/ml was taken as susceptible and ≥8.0 µg/ml as resistant. Accordingly, 96% isolates were found to be susceptible and 3.8% intermediate and none was found to be resistant.[7,16]

The MIC values for meropenem ranged from 0.032 - 4.0 µg/ml with a median value (Q_2) 0.064 µg/ml and Q_1 and Q_3 (25th and 75th percentile) values 0.047 µg/ml and 0.094 µg/ml respectively (Figure 1). For colistin, the range was 0.094 - 3 µg/ml, median (Q_2) 0.38 µg/ml and Q_1 and Q_3 (25th and 75th percentile) values 0.38 µg/ml and 0.56 µg/ml respectively (Figure 1). For tigecycline, the range was 0.064-3.0 µg/ml, median value (Q_2) 0.38 µg/ml and Q_1 and Q_3 (25th and 75th percentile) values were 0.25 µg/ml and 0.5 µg/ml respectively (Figure 1). The interquartile range values of the three antimicrobial agents are elaborated in Table II. The difference in MICs of meropenem, colistin and tigecycline was evaluated by Kruskal-Wallis test which revealed a chi-square value 56.5, degree of freedom 2 and a p-value of 0.00

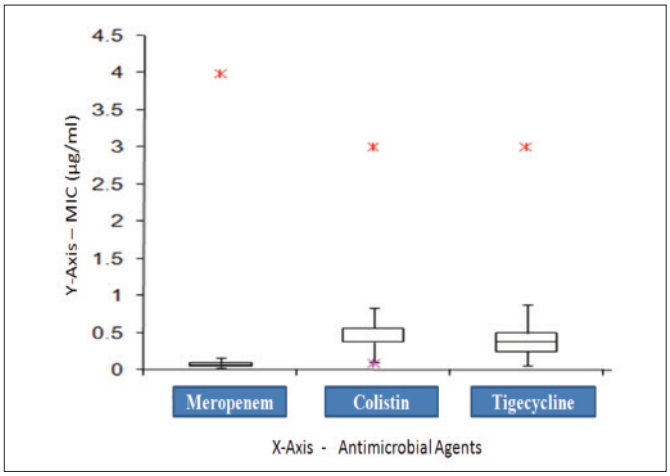

Figure 1: Box-plot of the MIC values of Meropenem, Colistin and Tigecycline.

Table I: Minimum Inhibitory Concentrations (MIC) of Meropenem, Colistin and Tigecycline against ESBL producers.

Meropenem		Colistin		Tigecycline	
MIC (µg/ml)	Percentage	MIC (µg/ml)	Percentage	MIC (µg/ml)	Percentage
0.032	21.1	0.094	3.8	0.064	1.9
0.047	25	0.125	1.9	0.125	3.8
0.064	25	0.19	1.9	0.19	11.5
0.094	15.4	0.25	9.6	0.25	19.2
1.5	3.8	0.38	44	0.38	15.4
3.0	7.7	0.5	13.5	0.5	25
4.0	1.9	0.75	7.7	0.75	15.4
		1.5	5.8	1.0	3.8
		2.0	7.7	3.0	3.8
		3.0	3.8		

MIC_{50} = 0.064 µg/ml *MIC_{50} = 0.38 µg/ml* *MIC_{50} = 0.38 µg/ml*
MIC_{90} = 3.0 µg/ml *MIC_{90} = 2.0 µg/ml* *MIC_{90} = 0.75 µg/ml*

Table II: Interquartile range values of MICs of Meropenem, Colistin and Tigecycline.

Interquartile range	Meropenem MIC (µg/ml)	Colistin MIC (µg/ml)	Tigecycline MIC (µg/ml)
Q_4-Q_3	3.91	2.44	2.5
Q_3-Q_2	0.03	0.182	0.12
Q_2-Q_1	0.017	0.0	0.13

indicating a statistically significant difference. The MIC_{50} values of tigecycline, colistin and meropenem were found to be 0.064 µg/ml, 0.38 µg/ml and 0.38 µg/ml respectively while MIC_{90} values of tigecycline, colistin and meropenem were 0.75 µg/ml, 2 µg/ml and 3 µg/ml respectively (Table I). Since a lower concentration of tigecycline was able to inhibit the growth of 90% of the isolates (MIC_{90}), therefore, it is more potent as compared to the other two antimicrobials. This suggests that tigecycline is the most reliable option for the treatment of infections caused by ESBL producing GNRs as compared to colistin and meropenem.

DISCUSSION

Keeping in view the limited treatment options in GNRs producing ESBLs, this study was planned to evaluate the efficacy of the drugs as old as colistin and the newer ones like meropenem and tigecycline. Though the use of colistin remained obsolete for many years, presently it is recommended that it can be used with proper dose adjustments.[8] The dose of colistin for patients with normal renal function is 80 - 160 mg (1 - 2 million IU)/8 hours.[18] The dose of colistin in patients with estimated Creatinine Clearance (CrCl in ml/minute) of 50 - 90, 10 - 50 and < 10 should be reduced to 160 mg (2 million IU)/12 hours, 160 mg (2 million IU)/24 hours and 160 mg (2 million IU)/36 hours respectively.[18] Unlike colistin, no renal dose-adjustment is required for tigecycline, however, hepatic dose-adjustment is required for it.[18] Tigecycline is given as 100 mg intravenous *stat* followed by 50 mg twice a day. In case of severe hepatic impairment, the dose is reduced to 25 mg twice a day.[18] Regarding suitability in pregnancy, meropenem belongs to class-B, colistin to class-C and tigecycline to class-D which means that tigecycline has a definite human risk but the benefit may outweigh the risk.[18]

In this study, tigecycline was found superior in the *in-vitro* efficacy against ESBL producing Gram negative bacilli as compared to meropenem and colistin, as demonstrated by its lower MIC_{90}. Taking a look on studies conducted in various parts of the world, it has revealed diverse findings. Kiratisin *et al*. found colistin and tigecycline to have comparable efficacy, however, Punpanich *et al*. found that meropenem demonstrated better antimicrobial activity against ESBL-producing Gram negative bacilli as compared to colistin.[19,20] In another study, Souli *et al*. compared the MIC_{90} value of tigecycline against ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* and found it to be 0.5 µg/ml and 2.0 µg/ml.[21]

In contrast to the present results, a study conducted by Naesens *et al*. found that tigecycline is not a reliable option for ESBL producing *Enterobacteriaceae* since MIC_{90} values of tigecycline for ESBL producing *Escherichia coli, Klebsiella pneumoniae* and *Enterobacter* spp. were found to be 1.5 µg/ml, 4 µg/ml and 12 µg/ml respectively.[22] In a study conducted by Jamal *et al*., MIC_{90} values of tigecycline and meropenem were found to be 0.25 and 0.05 µg/ml against the ESBL producing GNRs thus revealing a better efficacy of meropenem as compared to tigecycline.[23] In another study on ESBL producing *Escherichia coli*, Zhanel *et al*. found meropenem (MIC: ≤ 0.12 mg/dl) to have better *in vitro* efficacy as compared to colistin and tigecycline (MIC: 1.0 mg/dl both).[24]

Although the research is going on in developing newer antimicrobial agents yet their rate of development is quite slow.[6] Hence, judicious use of antibiotics and appropriate antiseptic measures are the prime requirements in order to curtail the ever increasing resistance.[6] The broad-spectrum antibiotics should be used empirically only in the serious infections and when the facility for susceptibility testing is not available.[6] The authors further recommend a large scale *in vivo* study in order to compare the *in-vivo* efficacy of these three antimicrobial agents against ESBL producing GNRs.

CONCLUSION

The lower concentration of tigecycline required to inhibit 90% of isolates (MIC_{90}) as compared to the colistin and meropenem indicates that tigecycline is superior in efficacy against the ESBL producing Gram negative bacilli as compared to colistin and meropenem.

REFERENCES

1. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. Global challenge of multidrug-resistant *Acinetobacter baumannii. Antimicrob Agents Chemother* 2007; **51**:3471-84.
2. Levinsen W, editor. Review of medical microbiology and immunology.10th ed. NewYork: *McGraw Hill;* 2008.
3. Morosini M, Garcia-Castillo M, Coque TM, Valverde A. Antibiotic coresistance in extended spectrum β-lactamase producing enterobacteriaceae and *in vitro* activity of tigecycline. *Antimicrob Agents Chemother* 2006; **50**:2695- 9.
4. Tsering DC, Das S, Adhiakari L, Pal R, Singh TS. Extended spectrum beta-lactamase detection in gram-negative bacilli of nosocomial origin. *J Global Infec Dis* 2009; **1**:87-92.
5. Clinical and Laboratory Standards Institute (CLSI)/NCCLS. Performance standards for antimicrobial susceptibility testing; twentieth informational supplement (M100-S20). Vol. 25. Wayne, PA: *CLSI/NCCLS;* 2010.
6. Gupta K, Kaushal S, Chopra SC. Tigecycline: a novel glycylcycline antibiotic. *Indian J Pharmacol* 2008; **38**:217-9.
7. Brooks GF, Carroll KC, Butel JS, Morse SA. Jawetz, Melnick and Adelberg's medical microbiology. 24th ed. New York; *McGraw Hill:* 2007.
8. Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. 6th ed. *Churchill Livingstone:* 2004.
9. Petersen PJ, Tuckman M, Jones CH. *In vitro* activity of tigecycline against patient isolates collected during phase 3 clinical trials for hospital acquired pneumonia. *Infect Dis Reports* 2010:**2**.
10. Bridson EY, editor. Oxoid manual. 9th ed. Hampshire England: *Oxoid Limited;* 2006.
11. Shoeb H. Bacterial identification by the analytical profile index system-analytical profile index E20 for Enterobacteriaceae [Internet]. 2006. Available from: http://www.microbelibrary.org
12. Hammam AS, Youssef MSK, Atta FM, Mohamed TA. Synthesis of new quinoxalinophenazinediones and tetrahydrobenzo-dipyrrolotetrones of biological interest. *Chem Papers* 2008; **62**:194-206.
13. Giriyapur RS, Nandihal NW, Krishna BVS, Patil AB, Chandrasekhar MR. Comparison of disc diffusion methods for the detection of extended-spectrum beta-lactamase-producing enterobacteriaceae. *J Lab Physicians* 2011; **3**:33-6.
14. Dashti AA, West P, Paton R, Amyes SG. Characterization of extended-spectrum beta-lactamase (ESBL)-producing Kuwait and UK strains identified by the Vitek system, and subsequent comparison of the Vitek system with other commercial ESBL-testing systems using these strains. *J Med Microbiol* 2006; **55**:417-21.
15. Galani I, Kontopidou F, Souli M, Rekatsina P, Koratzanis E, Deliolanis J, *et al.* Colistin susceptibility testing by Etest and disk diffusion methods. *Int J Antimicrob Agents* 2008; **31**:434-9.
16. Wyeth Pharmaceuticals. Tygacil (tigecycline) for injection [Package insert]. Philadelphia: *Wyeth Pharmaceuticals Inc.*; 2005.
17. Jones RN, Ferraro MJ, Reller LB, Schreckenberger BJ, Swenson JM, Sader HS. Multicenter studies of tigecycline disk diffusion susceptibility results for *Acinetobacter* spp. *J Clin Microbiol* 2007; **45**:227-30.
18. Gilbert DN, Moellering RC, Eliopoulos GM, Sande MA. The Sanford guide to antimicrobial therapy. Antimicrobial Therapy. 36th ed. USA: *INC;* 2006.
19. Kiratisin P, Tiengrim S, Yungyuen T, Thamlikitkul V. *In vitro* activity of colistin and tigecycline against extended-spectrum-beta-lactamase (ESBL)-Producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from patients in Siriraj Hospital. *J Infect Dis Antimicrob Agents* 2006; **23**:21-4.
20. Punpanich W, Tantichattanon W, Wongwatcharapaiboon S, Treeratweerphong V. *In vitro* susceptibility pattern of cephalosporin resistant Gram negative bacilli. *J Med Assoc Thai* 2008; **91**:S21-7.
21. Souli. *In vitro* activity of tigecycline against multiple-drug-resistant, including pan resistant, Gram-negative and Gram-positive clinical isolates from Greek hospitals. *Antimicrob Agents Chemotherap* 2006; **50**:3166-9.
22. Naesen R, Ursi JP, Schaeren JV, Jeurissen A. *In vitro* activity of tigecycline against multidrug-resistant Enterobacteriaceae isolates from a Belgian hospital. *Eur J Clin Micrbiol Infect Dis* 2008; **28**:380-4.
23. Jamal WY, Al Hashem G, Khodakhast F, Rotimi VO. Comparative *in vitro* activity of tigecycline and nine other antibiotics against Gram-negative bacterial isolates, including ESBL-producing strains. *J Chemother* 2009; **21**:261-6.
24. Zhanel GG, Adam HJ, Low DE, Blondeau J, Decorby M, Karlowsky JA, *et al*. Antimicrobial susceptibility of 15,644 pathogens from Canadian hospitals: results of the CANWARD 2007-2009 study. *Diagnostic Microbiol Infect Dis* 2011; **69**: 291-306.