East African Medical Journal Vol. 92 No. 8 August 2015
COMPARATIVE *IN-VITRO* ACTIVITY OF IMIPENEM AND DORIPENEM AGAINST ESBL PRODUCING KLEBSIELLA ISOLATES FROM A TERTIARY HOSPITAL IN ILORIN, NIGERIA
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ABSTRACT

***Background*: Doripenem is a recent carbapenem not commercially available in Nigeria with broad spectrum antibacterial activity against various clinical infections. Carbapenems have been shown to be the last line of agents against ESBL producing organisms.**
***Objective*: To determine the *in-vitro* activity of Imipenem and Doripenem against ESBL producing *Klebsiella spp*.**
***Design*: A cross-sectional laboratory based study.**
***Setting*: The University of Ilorin Teaching Hospital, a major tertiary hospital in Ilorin, the capital of Kwara state in Nigeria.**
***Subjects*: All strains of *Klebsiella spp* isolated from all clinical specimens collected at the hospital laboratory non– repetitively.**
***Result*: Doripenem had a superior *in-vitro* activity compared to imipenem with MIC 50/90 value of 0.0125/0.023 while imipenem was found to be 0.19/0.38 which was statistically different.**
***Conclusion*: The result obtained in this study is similar to those from other studies and therefore re-affirms the superior activity of doripenem compared to imipenem and should therefore be introduced as a better alternative to imipenem against ESBL producing organisms.**

INTRODUCTION

Carbapenems possess the broadest spectrum of activity and greatest potency against Gram-positive and Gram-negative bacteria of all the different β-lactam drugs available (1). Thus, they are often used as "last-line agents" or "antibiotics of last resort" when patients are seriously ill or are suspected of harboring resistant bacteria like those thatproduce Extended Beta Lactamases (ESBLs) (2,3). ESBLs break down the beta lactam ring of β-lactam antibiotics thereby rendering them useless for treatment of infection caused by ESBL organisms. Examples of carbapenems include imipenem, ertapenem, meropenem, doripenem among others. The carbapenems are similar in their antibacterial spectra with doripenem, ertapenem, and meropenem slightly more active against enterobacteriaceaethan imipenem (4). Because of their broad antibacterial spectrum covering gram-positive, gram-negative, and anaerobic bacteria, carbapenems are useful for treatment of a wide variety of infections, including bacteremia, bone and soft tissue infections, obstetric and gynecologic infections, complicated urinary tract infections, intra-abdominal sepsis, pneumonia and polymicrobial infections in which otherwise multiple-drug regimens of higher cost and potentially more adverse side effects would be necessary (5).

Doripenem is a recent broad- spectrum, intravenous carbapenem antibiotic that, to the best of my knowledge, is not commercially available in Nigeria. It may be the next choice in the treatment of infection caused by ESBL producing organisms. Unlike imipenem, it is not hydrolysed to nephrotoxic metabolites by renal dehydropeptidase-1 and does not require an inhibitor like cilastatin. It is also, the carbapenemwith the least susceptibility to hydrolysis by carbapenemases; hydrolysis of doripenem is 2 to

150-fold slower than that of imipenem (6). In vitro data indicate that doripenem combines the intrinsic activity of meropenem against gram-negative pathogens with the intrinsic activity of imipenem against gram-positive pathogens (7).

The choice of a carbapenem with a more favourable MIC as against one where the MIC is at the breakpoint of susceptibility with administration over an extended infusion time may be beneficial since the efficacy of carbapenems is associated with the percentage of the dosing interval during which the concentration of the drug is greater than the MIC of the drug (T>MIC) (8). Determination of MIC is important for the appropriate administration of a dosing regimen that will give the maximum concentration of the drug in the body in other to achieve complete bacterial eradication, thus inhibiting the development of resistance to the drug and prevent drug toxicity (9,10).E test method has been proven to be a reliable method for MIC determination (11). The E test method consists of a predefined and continuous concentration gradient of antimicrobial agents, which when applied to inoculated agar plates and incubated creates an ellipse of microbial inhibition and is read where the ellipse of inhibition intersects with the strip (12,13) .

The aim of this study is to determine and compare the MIC of imipenem and doripenem against ESBL producing strains of Klebsiellaspp in our enviroment.

MATERIALS AND METHODS

This study was done at the UITH Medical Microbiology laboratory. Ethical clearance was obtained from the Ethical committee of the institution. One hundred and eighty seven (187) clinical isolates of *Klebsiella* were obtained non-repetitively from different clinical specimens including blood, sputum, wound, urine, ear, eye and throat swabs between October, 2013 and February, 2014.

Re-characterisation and Storage of Isolates: The organisms were re-characterised using standard microbiological techniques (14). Identified *Klebsiella* isolates were stored at -20ºC using 20% Brain heart infusion (BHI)-glycerol broth until further processed.

ESBL Detection: ESBL production was assayed by the double disc synergy test (DDST) method. Briefly, 30 µg of ceftazidimedics (Oxoid, UK) was placed at a distance of 20 mm (edge to edge) from an amoxicillin-clavulinic acid disc (20/10 µg, Oxoid, UK) on Mueller-Hinton agar plate already inoculated with the test organism. Inoculum was prepared by suspending the test organism in sterile physiologic saline inside Bijou bottle and adjusting its turbidity to that of 0.5 McFarland standards. Inoculation was done by rolling the cotton swabs, previously dipped into the inoculum suspension with the excess fluid removed by compressing the swab against the inside wall of the container, on the surface of the Mueller-Hinton agar. The set-up was incubated at 35ºC for 16-18 hours. (15) Escherichiacoli ATCC 25922 was used as negative control. Positive test consisted of enhancement/ amplification of inhibition zone around Ceftazidime and towards Amoxicillin/Clavulinic acid disc giving a dump-bell appearance.

MIC Determination: MIC was determined using the Epsilometer test (E test) strip. ESBL producing Klebsiellaisolates were inoculated on Mueller hinton agar as described above. E- test strips of imipenem and doripenem were placed on the inoculated plate and incubated at 35ºC for 16 to 18 hours aerobically. The Minimum Inhibitory Concentration (MIC) was read directly from the scale in micrograms per milliliter (µg/ml) at the point where the inhibition ellipse edge intersects the strip according to manufacturer's recommendations and interpreted as susceptible, intermediate or resistant as recommended by CLSI standards (15). *E.coli* ATCC 25922 was used as control. The MIC50 and MIC90 which is the concentration that inhibits 50% and 90% of bacterial isolates will be determined for each carbapenem as the 50th and 90th percentile MIC respectively.

RESULTS

Figure 1 shows the line chart for carbapenem MIC and the cumulative percentage inhibition of EPK. Table 1 and 2 shows the MIC 50, MIC 90 and the mean MIC of ESBL producing *Klebsiella spp.* Doripenem had a much lower MIC compared with imipenem. The mean MIC for doripenem is significantly lower than that of imipenem. There is no significant difference in both imipenem and doripenem MIC values for *K.pneumoniae* and *K.oxytoca*.

Figure 1
Line chart for carbapenem MIC and cumulative percentage inhibition of ESBL producing Klebsiella spp.

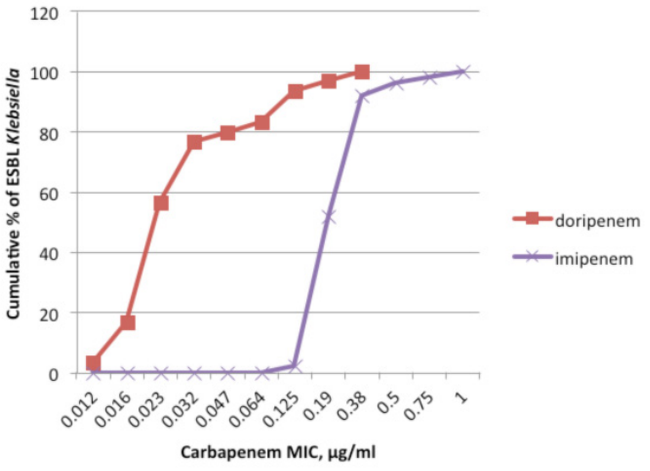


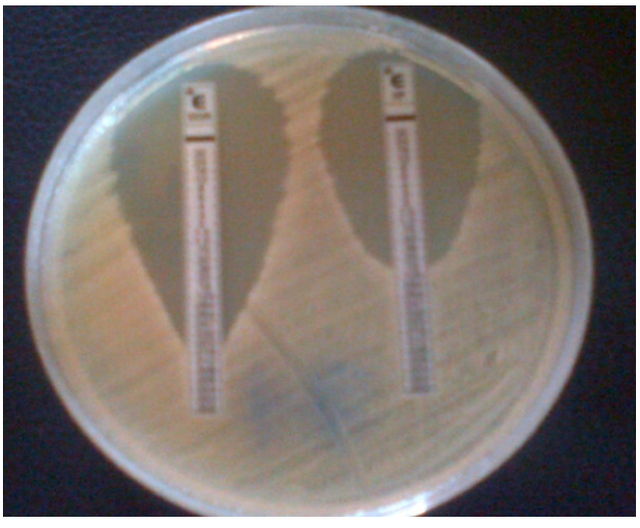
Table 1
Comparison of the Mean MIC, MIC50 and MIC90 for imipenem and doripenem

Category	Imipenem, n(%)	Doripenem, n(%)	T test	P value
Susceptible (≤1µg/ml)	50 (100)	30(100)		
Intermediate(2 µg/ml)	0(0)	0(0)		
Resistant (≥4 µg/ml)	0(0)	0(0)		
MEAN MIC	0.27	0.08	1.99	0.000
MIC50	0.19	0.023		
MIC90	0.38	0.125		

Table 2
Comparison of the mean MIC for imipenem and doripenem against species of Klebsiellae

Isolate	Carbapenem Mean Mic		T test	P value
	Imipenem	Doripenem		
K. oxytoca	0.23	0.05	1.67	0.000
K.pneumoniae	0.28	0.07	1.72	0.000
T test	2.02	2.05		
P value	0.223	0.671		

Figure 2
Photograph showing MIC of imipenem and doripenem using E test strip



Doripenem (left) with lower MIC than imipenem (right)

DISCUSSION

Although all the ESBL producing *Klebsiellae* were susceptible to both imipenem and doripenem, doripenem had a much lower MIC compared to imipenem. This is important as it is preferably to administer a drug with a much lower MIC in other to achieve the maximum concentration of the drug in the body so as to completely eradicate bacteria and prevent the development of resistance to the drug (8,9). Literature suggests that doripenem is the most effective carbapenem and the least hydrolysed by carbapenemases (6) and thus, therefore translates to the availability of an alternative to imipenem. In a study carried out in Spain, it was shown that doripenem with MIC50 of 0.12 mg/L was 2 to 8-fold more active than meropenem with MIC50 of 0.25 mg/L and imipenem with MIC50 of 1 mg/L (16).

Gimeno *et al*. (16), while comparing the activity of imipenem and doripenem reported a MIC50/90 for imipenem to be 0.25/1 and 0.03/0.12 for doripenem. The finding is comparable to the present study and might be because the two studies were conducted at a time when doripenem was novel to the environment when the research was performed.

Christensen *et al*. (11) also compared the activities of the two drugs for different countries. The MIC50/90 for imipenem and doripenem from various countries were as follows; Hongkong: imipenem 0.25/1, doripenem 0.03/0.12; India: IMP 0.12/0.25, DOR 0.015/0.06; Australia: IMP 0.25/0.25, DOR 0.015/0.06; Indonesia: IMP 0.25/1, DOR 0.06/0.25; Singapore: IMP 0.25/0.5, DOR 0.03/0.12; Thailand: IMP 0.12/0.25, DOR 0.03/0.06. The MICs of doripenem in these studies were much lower than that of imipenem in agreement with this study and thus emphasises the superior in-vitro activity of doripenemover imipenem.

In conclusion, doripenem had a much lower MIC50/90 value compared with imipenem and therefore remain a better option for treatment of ESBL producing organisms. Clinical studies need to be carried out to affirm the in-vitro report of doripenem as in vitro studies does not always translate to clinical efficacy.

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