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Susceptibility Pattern of Gram Negative Bacilli Isolated from Patients at Sobi Specialist Hospital, Ilorin to Imipenem

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Abstract

Over the past decade, carbapenems-resistant have been recognized in health-care settings as a cause of difficult-to-treat infections associated with increased morbidity, mortality and increasing healthcare costs. This study investigated the susceptibility of clinical isolates to Imipenem at Sobi Specialist Hospital, Ilorin, based on minimum inhibitory concentration (MIC) using the E-test method. Two hundred and sixty Gram negative bacilli from different clinical sample were obtained for this study. Antibiotic disc susceptibility testing of Imipenem was carried out using disc diffusion method and interpreted according to CLSI guidelines. Imipenem susceptibility categorization was carried out using E-test method following the manufacturer's instructions and CLSI guidelines. The isolates recorded from this study include, *Escherichiacoli* 24 (9.2%), *Klebsiella oxytoca* 113 (43.5%), *Proteus mirabilis* 6 (2.3%), *Pseudomonas aeruginosa* 43 (16.5%), *Klebsiella pneumoniae* 52 (20.0%), *Salmonella typhi*. *E. coli*, and *Proteus mirabilis* were all found in the same proportion of 22 (8.5%), and they had the highest susceptibility rate to Imipenem (100%) while *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* had 80% and 72.1% respectively. Whereas the least susceptibility rate of 59.3% was recorded for *Klebsiella oxytoca*. In conclusion all the isolates were highly susceptible to Imipenem, and this revealed that Imipenem has potent broad-spectrum activity against most common bacterial pathogens, Imipenem can be used in the treatment of infection caused by Gram negative isolates.

Keywords: Antimicrobial, drugs, sensitivity, resistance

Introduction

Carbapenems are a group of fused- β -lactam antibiotics (with wide spectrum antibacterial activity) that are used for the treatment of infections caused by multidrug resistant (MDR) Gram negative bacteria including those that produce extended spectrum β -lactamase enzymes (Walsh *et al.*, 2005). Carbapenems include imipenem,

meropenem, meropenem, and ertapenem. These antibiotics are known to be effective against infections from extended spectrum beta lactamase bacteria (Igbinoba *et al.*, 2012). However, there has been increasing rise in the prevalence of bacterial exhibiting antibiotic resistance to these groups of antibiotics (Chakaraborty *et al.*, 2010; Bashir *et al.*, 2011).

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Gram negative bacteria that are resistant to the carbapenems due to their inherent production of carbapenemas (carbapenems-hydrolyzing enzymes) have been increasingly reported (Chakraborty *et al.*, 2010). Different reports have shown that broad usage of carbapenems could lead to emergence of antibiotic resistance at varying levels (Jaccard *et al.*, 1998; Lepper *et al.*, 2002).

Also overuse of effective antibiotics over a long period of time have also been attributed to the development of antibiotics resistance. However, Imipenem has been very effected against Gram positive and negative bacteria, hence their use as antimicrobials of first choice in patients infected with these bacteria (Akhi *et al.*, 2009; Shahcheragi *et al.* 2008). Despite the high level of antimicrobial activity that has been shown by imipenem over the years, various incidence on the increasing antibiotic resistance organisms are developing against imipenem has been reported. Some studies have shown that this resistance could be attributed to loss of specific porin, OprD-2 (Quinn *et al.*, 1986; Bushcher *et al.*, 1987).

Despite this increasing resistance, Imipenem remains a drug of choice in the study environment with little information existing on the resistance pattern against this drug, hence this study was carried out to determine the susceptibility pattern of Gram negative bacilli to Imipenem at the Kwara State Specialist Hospital, Sobi.

MATERIALS AND METHODS

Study Area

Kwara State Specialist Hospital, Sobi, Ilorin. It is located in Ilorin South Local Government Area. It is a secondary Health care institution with about 250 inpatient and 450 outpatient capacities. Ilorin is the Kwara State capital and is situated 360KM North of Lagos, at 8°30N and 4°30N and 482KM South West from Abuja, the federal capital. It is the gate way between the Northern and Southern part of the country.

Ethical Consideration

Ethical clearance was obtained from the ethical committee of State Ministry of Health, before the commencement of this study.

Sample collection and Identification

Two Hundred and sixty (260) Gram negative bacilli bacterial isolated from different clinical specimens from the Medical Microbiology Laboratory of Sobi Specialist Hospital, Ilorin from October, 2013 to January, 2014, were collected and identity confirmed using standard microbiology identification techniques.

Determination of Minimum Inhibitory Concentration

This was carried out using Imipenem E-test strip in accordance with the manufacturer's instruction (Biomerieux, Marcy-1Etoile, France).

Data Analysis

Data generated in this study was entered into the computer and analyzed with Statistical package for social sciences (SPSS) software version 20.0 (SPSS Inc., Chicago III) and results were presented in tables and figures as applicable. Significant level was set at $P < 0.05$ and categorical data will be compared using chi-square (X^2). Continuous variables were described by means and mode.

Result

Two hundred and sixty isolates were recovered in the course of this study. Out of 260 isolates identified, *Klebsiella oxytoca* was (43.5%), *Pseudomonas aeruginosa* (16.5%), while *Salmonella species* was (8.5%), *E. coli* accounted for (9.2%), and *Proteus mirabilis* (2.3%). Results further showed that *Klebsiella species* is implicated in most of the clinical samples while *Proteus mirabilis* and *E. coli* having limited spread (Table 1). Susceptibility pattern of the isolates to Imipenem disc showed that *Klebsiella oxytoca* had the highest resistance while *E. coli*, *Proteus mirabilis* and *Salmonella typhi* were susceptible (Table 2 and 3). Table 4 shows the susceptibility pattern of the isolates from different clinical diagnosis to Imipenem disc, Isolates from Otitis media, Pneumonia, infertility, Chronic cough, Pyrexia and typhoid were 100% susceptible to Imipenem, while UTI has 82.4% susceptible, 11.8% intermediate and 5.9% resistance, sepsis is 67.4% susceptible, 18.5% intermediate and 14.1% resistance, Diabetic foot

ulcer has 44.7% susceptible and 55.3% intermediate.

DISCUSSION

In this study, *Klebsiella oxytoca* was found to be the most predominant isolate among the Gram negative bacilli isolated. Usually *E. coli* is a member of the normal intestinal flora, and contamination of the hand occurred so easily because of the poor hygiene in this environment.

From this study *E. coli*, *P. mirabilis* and *S. typhi* were found to show 100% susceptibility to Imipenem while *K. oxytoca*, *P. aeruginosa* and *K. pneumoniae* were found to show 18.6%, 7% and 15.4% resistance to Imipenem, respectively, using disc diffusion. This agrees with the findings of Boroumand *et al.* (2007) and Shahcheraghi *et al.* (2008) that *Pseudomonas* show 94.4% and 92% susceptibility to Imipenem respectively. Also the report that *E. coli* show 100% susceptibility to Imipenem (Shahcheraghi *et al.*, 2007) is in agreement with the finding of this study. The susceptibility pattern by disc diffusion as shown on Table 4 agrees with the susceptibility pattern by E-test except for the significant difference observed for *K. oxytoca*.

It has been shown that treatment with Imipenem, but not with other beta lactam drugs, is a major risk factor for the development of Imipenem-resistant *Pseudomonas* in hospitalized patients (Troillet *et al.*, 1997). It was also reported that there is a three-fold rise in the consumption of Imipenem worldwide. Given this substantial use of antibiotic, it is not surprising to note the change in the microbial ecology, with predominance of multi-resistant strains of *Pseudomonas*. It is also well documented that the indiscriminate use of Imipenem can lead to the selection and dissemination of antibiotic-resistant organism (Percival, 1997). But in the study area Imipenem is a very rare antibiotic which is just been introduced into the clinics as a result of resistance to the commonly used conventional antibiotics such as Ampicillin and the Cephalosporins. Imipenem is an injectable which cannot be easily abused compared to the oral group of antibiotics which are easily sold over the counter. The development of resistance to an

antibiotic is directly proportional to the usage of such antibiotic. Never the less Imipenem is not used alone in order to prevent the emergence of resistance, it is usually used in combination with Gentamycin and Cilastatin which is a peptidase inhibitor.

The value 0.5 μ g and 3.0 μ g observed as MIC 50 and MIC 90 respectively for *K. oxytoca*, 1.5 μ g and 4.0 μ g observed as MIC 50 and MIC 90 respectively for *P. aeruginosa*, and 0.38 μ g and 3.0 μ g observed for *K. pneumoniae* disagree with the report of Livermore (2001) which reported 0.25 μ g and 0.5 μ g as MIC 50 and MIC 90 respectively for *K. oxytoca*, 4.0 μ g and 16.0 μ g as MIC 50 and MIC 90 respectively for *P. aeruginosa*, and 0.25 μ g and 1.0 μ g as *K. pneumoniae*. While 0.25 μ g and 0.38 μ g observed as MIC 50 and MIC 90 for *E. coli* is in line with the work of Livermore, (2001) which reported 0.25 μ g and 0.5 μ g as MIC 50 and MIC 90.

The MICs observed in this study were higher than those reported by Livermore *et al.* (2001) this may be due to the difference in the period of study. Within the time lapse between the two studies there has been increase use of Imipenem as reported by Manguarkarasi *et al.* (2013). This might have resulted into the selection of the resistant strains, hence the recorded 7.7% recorded in this study.

In general, from this study 0.5 μ g and 4.0 μ g is recorded as MIC 50 and MIC 90 for all the Gram negative bacterial isolates from Sobi Specialist Hospital this implies that 0.5 μ g is required to inhibit the growth of 50% of Gram negative bacterial isolate from Sobi Specialist Hospital while 4.0 μ g is required to inhibit 90% of the Gram negative bacterial isolates from Sobi Specialist Hospital.

Imipenem remains a drug of choice in the treatment of infection caused by susceptible Gram negative bacteria but the efficacy of the drug is threatened by the emergence of resistant strains. Judicious use of this antibiotic is very essential, it should not be used as first line antibiotic but reserved; and can only be used when there is resistance to other first line antibiotics.

References

- Page | 3083
- Akhi, M.T., Farzaneh, F. and Oskouei, M. (2009). Study of enterococcal susceptibility patterns isolated from clinical specimens in Tabriz, Iran. *Pak Med*, 25(2): 211-216.
- Boroumand, M.A., Esfahanifard, P., Saadat, S., Sheihkvatan, M., Hekmatyazdi, S., Saremi M, *et al.* (2007). A report of *Pseudomonas aeruginosa* antibiotic resistance from a multicenter study in Iran. *Indian J Med Microbiol*, 25(4): 435-436.
- Bushcher, K.H., Cullman, W., Dick, W. and Opferkuch, W. (1987). Imipenem resistance of *pseudomonas aeruginosa* resulting from diminished expression of an outer membrane protein. *Antimicrobial Agent and Chemotherapy*, 32: 195-201.
- Chakraborty, D., Basu, S. and Das, S. (2010). A study on infections caused by Metallo Beta Lactamase Producing Gram negative Bacteria in Intensive Care Unit Patients. *Am. J. infectious Diseases*, 6(2):34- 39.
- Igbinoba, A.O. and Osazuwa, F. (2012). Zero resistance to the carbapenems among extended spectrum betalactamase producing *Klebsiella pneumoniae* in a Nigerian university hospital. *Int. J. Biol., Pharmacy and Allied Sciences*, 1(1):79-83.
- Jaccard, C., Troillet, N., Harbarth S *et al.* (1998). Prospective randomized comparison of Imipenem-cilastatin and Piperacillin-tazobactam in nosocomial pneumonia or peritonitis. *Antimicrob Agents Chemother*, 42: 2966–72.
- Lepper, P.M., Grusa, E., Reichl, H. *et al.* (2002) Consumption of Imipenem correlates with β -lactam resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother*; 46: 2920–2925.
- Livermore, D.M., Carter, M.W., Bagel, S. *et al.* (2001) In vitro activities of Ertapenem (MK-0826) against recent clinical bacteria collected in Europe and Australia. *Antimicrob Agents Chemother*; 45: 1860–1867.
- Manguarkkarasi, A., Erli, A., and Gopol, R. (2013). Antimicrobial Susceptibility pattern of clinical isolates of Gram-negative pathogens from a teaching hospital, Pondicherry, *Research Journal of pharmaceutical, Biological and chemical science*, 42: 664-667.
- Percival, A. (1997). Increasing resistance to antibiotics: A public health crisis? *Hosp pharm*, 4: 193-196
- Shahcheraghi, F., Nikbin, V.S. and Shooraj, F. (2008). PCR detection of PER & VEB & SHV and TEM N-lactamases in multidrug resistant *P. aeruginosa* isolated from wound infections in two hospitals of Tehran. *Iranian J Med Microbiol*, 4(1): 21-27.
- Troillet, N., Samore, M.H. and Carmali, Y. (1997). Imipenem resistant *P. aeruginosa*: risk factors and antibiotic susceptibility patterns. *Clin Infectm Dis*, 25(5): 1094-1098.
- Walsh TR, Toleman MA, Poirel L and Nordmann P (2005). Metallo β – lactamases: the Quiet before the storm? *Clinical Microbiology Review*. 18(2):306-325.
- Quinn, J.P., Dudck, E.J., DiVincenzo, C.A., Lucks, D.A. and Lerner, S.A. (1986) Emergence of resistance to Imipenem during therapy for *Pseudomonas aeruginosa* infections. *Journal of Infectious Diseases*, 154: 289-294.

Table 1: Distribution of isolates by Specimens.

Specimen	N	<i>Klebsiella oxytoca</i> (%)	<i>Pseudomonas aeruginosa</i> (%)	<i>E. coli</i> (%)	<i>Proteus mirabilis</i> (%)	<i>Salmonella typhi</i> (%)	<i>Klebsiella pneumoniae</i> (%)
Ear Swab	15	33.3	46.7	0.0	0.0	0.0	20.0
Wound Swab	122	65.6	3.3	0.8	4.9	0.0	25.4
Blood	45	0.0	6.7	44.4	0.0	48.9	0.0
Pus	6	66.7	0.0	0.0	0.0	0.0	33.3
Catheter Tip	12	66.7	33.3	0.0	0.0	0.0	0.0
Urine	17	52.9	29.4	17.6	0.0	0.0	0.0
Sputum	36	0.0	55.6	0.0	0.0	0.0	44.4
Semen	4	100.0	0.0	0.0	0.0	0.0	0.0
Joint aspirate	3	100.0	0.0	0.0	0.0	0.0	0.0
Total	260	43.5	16.5	9.2	2.3	8.5	20.0

$\chi^2 = 339.197$ P-value = 0.000

Table 2: Susceptibility pattern of the isolates to Imipenem Disc

Isolate	N	Susceptible (%)	Intermediate (%)	Resistance (%)
<i>Klebsiella oxytoca</i>	113	78.8	2.7	18.6
<i>Pseudomonas aeruginosa</i>	43	86.0	7.0	7.0
<i>E. coli</i>	24	100.0	0.0	0.0
<i>Proteus mirabilis</i>	6	100.0	0.0	0.0
<i>Salmonella typhi</i>	22	100.0	0.0	0.0
<i>Klebsiella pneumoniae</i>	52	78.8	5.8	15.4
Total	260	84.2	3.5	12.3

$\chi^2 = 17.889$ P-value = 0.085

Table 3: Susceptibility pattern of the isolates to E-test

Isolate	n	Susceptible	Intermediate	Resistance
<i>Klebsiella oxytoca</i>	113	59.3%	31.0%	9.7%
<i>Pseudomonas aeruginosa</i>	43	72.1%	18.6%	9.3%
<i>E. coli</i>	24	100.0%	0.0%	0.0%

<i>Proteus mirabilis</i>	6	100.0%	0.0%	0.0%
<i>Salmonella typhi</i>	22	100.0%	0.0%	0.0%
<i>Klebsiella pneumoniae</i>	52	80.8%	9.6%	9.6%
Total	260	73.8%	18.5%	7.7%

Page | 3085

$\chi^2 = 38.808$ P- value = 0.000

Highest resistance was observed among *Klebsiellaoxytoca* closely followed by *K. pneumoniae*

Table 4: Susceptibility pattern of isolates from different clinical diagnosis to Imipenem

Clinical diagnosis	N	Susceptible	Intermediate	Resistance
Otitis media	15	100.0%	0.0%	0.0%
Sepsis	135	67.4%	18.5%	14.1%
Urinary Tract Infection	17	82.4%	11.8%	5.9%
Pneumonia	35	100.0%	0.0%	0.0%
Infertility	4	100.0%	0.0%	0.0%
Diabetic foot ulcer	38	44.7%	55.3%	0.0%
Chronic cough	1	100.0%	0.0%	0.0%
Pyrexia	8	100.0%	0.0%	0.0%
Typhoid	7	100.0%	0.0%	0.0%
Total	260	73.8%	18.5%	7.7%

Table 5: Minimum Inhibitory Concentration of Imipenem for the Isolates showing MIC 50 and 90

MIC	Frequency	Percent	Cumulative Percent
.125	26	10.0	10.0
.190	22	8.5	18.5
.250	61	23.5	41.9
.380	20	7.7	49.6
.500*	19	7.3	56.9
.750	12	4.6	61.5
1.000	19	7.3	68.8
1.500	18	6.9	75.8
2.000	5	1.9	77.7
3.000	31	11.9	89.6
4.000**	6	2.3	91.9
32.000	21	8.1	100.0
Total	260	100.0	

*= MIC 50,

**= MIC 90