ORIGINAL RESEARCH PAPER

Volume-7 | Issue-5 | May-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

PREVALENCE OF CARBAPENEM RESISTANT GRAM NEGATIVE BACTERIA IN CENTRAL REFERAL HOSPITAL, SIKKIM.

Microbiology

Pratibha Tamang Post Graduate Student, Department Of Microbiology, Sikkim Manipal Institute Of Medical Sciences.

T. Shantikumar Singh Professor & Hod, Department Of Microbiology, Sikkim Manipal Institute Of Medical Sciences.

Jyotsna Kapil* Professor, Department Of Microbiology, Sikkim Manipal Institute Of Medical Sciences. *corresponding Author

ABSTRACT

Carbapenems are beta-lactam antibiotics, often considered as drug of choice for treating infections caused by multi-drug resistant Gram negative bacteria. This study aimed in determing the prevalence of carbapenem resistant Gram negative bacteria and detecting the production of carbapenemase by Modified Hodge Test (MHT). Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method and carbapenemase detection by MHT. Out of the total 155 Gram negative bacterial isolates, the prevalence of carbapenem resistance was found to be 25.80% (40/155). The majority of the resistant isolates were detected among the ICU patients (53.13%), followed by ward patients (19.30%) and least among OPD patients (11.11%). Carbapenem resistant isolates were obtained mainly from urine sample (n=12), followed by pus (n=11), endotracheal secretion (n=10), blood (n=4) and sputum (n=3). Carbapenemase production was detected in 30% (12) of the resistant isolates by MHT with the carbapenemases production being highest in Escherichia coli (50%) and Proteus mirabilis (50%), followed by Klebsiella pneumoniae (42.9%), Pseudomonas aeruginosa (25%), Acinetobacter baumannii (25%) and not detected in any of the resistant isolates of Enterobacter cloacae, Burkholderia cepacia and Stenotrophomonas maltophila. The prevalence of carbapenem resistant Gram negative bacteria in our hospital is much less than that reported from other studies. Moreover, MHT lacks specificity, giving false positive results in case of AmpC producers and also lacks sensitivity as it weakly screens the New Delhi Metallo-beta-lactamase producers.

KEYWORDS

Carbapenems, Gram negative bacteria, Modified Hodge Test.

Carbapenems are a group of broad spectrum β-lactam antimicrobial agents (Nair & Vaz, 2013). They are stable even in response to extended spectrum and AmpC β-lactamases. However, the emergence and dissemination of carbapenem resistant bacteria in recent times is perilous to public health since it has led to limited treatment options associated with these organisms (Mate et al., 2014). The mechanism of carbapenem resistance that has been investigated in the most detail is the production of beta-lactamases. Other mechanisms include over-expression of efflux pumps, mutations altering the expression and/or function of porins and penicillin binding proteins (Papp-Wallace, Endimiani, Taracila & Bonomo, 2011).

Carbapenemases are specific β-lactamases capable of hydrolyzing carbapenems. They fall under the functional groups 2f, 2df, 3 and the molecular classes A, B and D (Bush & Jacoby, 2009). MHT is a phenotypic method used for the detection of carbapenemase activity, also recommended by the CLSI for screening purposes (Birgy et al., 2012). However, it cannot identify the type of carbapenemase involved (Miriagou et al., 2010).

Molecular techniques like polymerase chain reaction (PCR) can be used in identification and differentiation of carbapenemases (Queenan & Bush, 2007) but these methods may miss the novel gene types as they can detect only known carbapenemases genes (Kaase, Szabados, Wassill, & Gatermann, 2012).

This study was aimed in determining the prevalence of carbapenem resistant Gram negative bacteria in a tertiary care hospital in Gangtok, Sikkim. The growing antimicrobial resistance at an alarming rate with limited treatment options prompted us to conduct this study. This is the first study reporting the prevalence of carbapenem resistant Gram negative bacteria in Sikkim, to the best of our knowledge.

Materials and Methods

Bacterial Isolates

The study was conducted in the Department of Microbiology, Sikkim Manipal Institute of Medical Sciences (SMIMS), Tadong, Gangtok, Sikkim, during the period from 1st July 2016 to 30th June 2017. A total of 155 Gram-negative bacterial isolates obtained from different clinical samples (pus, sputum, stool, urine and vaginal swab) received for culture and sensitivity were included in the study. MacConkey agar, blood agar and cysteine lactose electrolyte deficient agar (CLED) media were used for isolation and identification was done by cultural characteristics, Gram stain, motility test by hanging drop preparation and other biochemical reactions. Those isolates which could not be identified to species level by conventional method were identified by VITEK 2 compact system.

Antiobiotic Susceptibility Testing

Antibiotic susceptibility testing was done employing Kirby-Bauer's disc diffusion method using Mueller Hinton agar medium. The antibiotics used were ampicillin, amoxyclav, amikacin, cefepime, ceftazidime, cefuroxime, ciprofloxacin, colistin, ertapenem, meropenem, imipenem, gentamicin, nalidixic acid, nitrofurantoin, piperacillin/tazobactum, cotrimoxazole and tigecycline. The antibiotics were placed and pressed gently on Mueller Hinton agar plates, already seeded with the bacterial suspension, whose turbidity was adjusted to 0.5 McFarland standard. The plates were incubated at 37ºC overnight. Zones of inhibition were measured on the next day and interpreted according to Clinical and Laboratory Standard Institute guidelines (CLSI, 2015).

Modified Hodge Test (MHT)

The carbapenem resistant Gram negative bacterial isolates were tested for carbapenemase production by Modified Hodge Test (Lee et al., 2001). A lawn culture of 1:10 dilution of an overnight culture suspension of *Escherichia coli* (ATCC25922), adjusted to a turbidity of 0.5 McFarland standard was made on Mueller Hinton agar plate. A 10 µg meropenem susceptibility disc was placed at the centre of the plate and the test organism was streaked from the edge of the disc to the edge of the plate in a straight line. The plate was then incubated overnight at 37ºC. Presence of a clover leaf-like indentation at the intersection of the test organism and *E. coli* (25922) within the zone of inhibition of meropenem susceptibility disc was interpreted as positive for carbapenemase production.

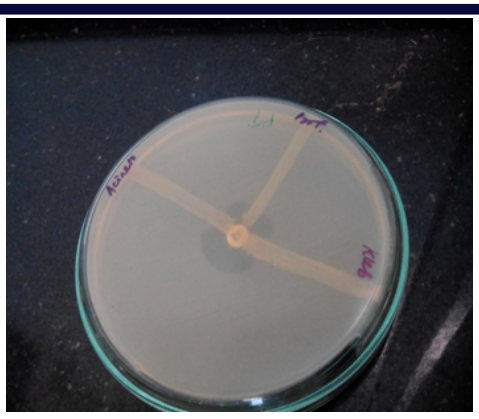
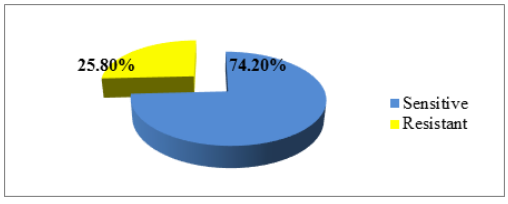


Figure 1. Positve Modified Hodge test showing clover leaf indentation

Result

The prevalence of carbapenem resistant Gram negative bacterial isolates was found to be 25.80% (40/155) and carbapenem sensitive organisms were 74.20% (115/155) as shown in Figure 2.

Figure2. Prevalence of carbapenem resistance in Gram negative bacterial isolates.



The majority of clinical isolates were from different wards of the hospital (73.55%), followed by ICUs (20.65%) and least from OPDs (5.80%). However, carbapenem resistance rate was highest among the ICU patients (53.13%), followed by ward patients (19.30%) and least among OPD patients (11.11%) (Table 1.).

Table1. Distribution of carbapenem resistant clinical isolates in different locations of the hospital.

Location	Carbapenem	Total	
	Resistant	Sensitive	
Wards	22(19.30%)	92(80.70%)	114(73.55%)
ICUs	17(53.13%)	15(46.87%)	32(20.65%)
OPDs	1 (11.11%)	8(88.89%)	9(5.80%)
Total	40(25.80%)	115(74.20%)	155

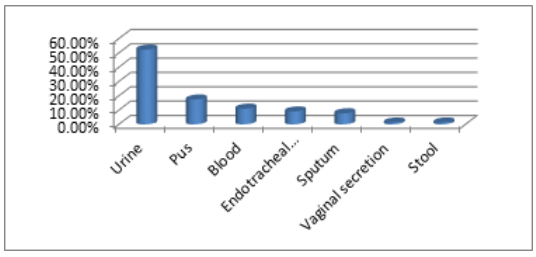
Out of the total 155 isolates, the majority of organisms isolated were Escherichia coli (56.12%), followed by Klebsiella pneumoniae (15.48%), Pseudomonas aeruginosa (11.61%), Enterobacter cloacae (5.81%), Acinetobacter baumannii (5.16%), Proteus mirabilis (3.23%) and Burkholderia cepacia (1.94%). Only one sample showed the growth of Stenotrophomonas maltophila (0.65%) (Table2).

Table 2. No. of Gram negative bacteria isolated (n=155)

Organism	No. of isolates	Percentage
Escherichia coli	87	56.12%
Klebsiella pneumoniae	24	15.48%
Pseudomonas aeruginosa	18	11.61%
Enterobacter cloacae	9	5.81%
Acinetobacter baumannii	8	5.16%
Proteus mirabilis	5	3.23%
Burkholderia cepacia	3	1.94%
Stenotrophomonas maltophila	1	0.65%
Total	155	100%

The highest percentage of clinical sample was urine 52.25% (n=81), followed by pus 17.42% (n=27), blood 10.96% (n=17), endotracheal secretion 9.03% (n=14) and sputum 7.74% (n=12) and least were vaginal secretion 1.30% (n=2) and stool 1.30% (n=2) (Fig.3).

Figure3. Percentage of the clinical samples.



Carbapenem resistant isolates were obtained mainly from urine sample (n=12), followed by pus (n=11), endotracheal secretion (n=10), blood (n=4) and sputum (n=3). No carbapenem resistant isolates were obtained from vaginal swab and stool (Table3).

Table 3. Distribution of carbapenem resistant isolates in different clinical samples.

Sample type	No. of resistant isolates (n)	Percentage
Urine	12	30%
Pus	11	27.50%
Endotracheal secretion	10	25%
Blood	4	10%
Sputum	3	7.50%
Vaginal swab	0	0%
Stool	0	0%
Total	40	100%

Out of the 40 carbapenem resistant isolates, 30% (12) was found positive for carbapenemase production by MHT, the enzyme production being highest in E.coli (50%) and P. mirabilis (50%), followed by K. pneumoniae (42.9%), P. aeruginosa (25%) and A. baumannii (25%). None of the resistant isolates of E. cloacae, B. cepacia and S. maltophila were found positive by MHT (Table 4).

Table4. Distribution of carbapenemases among the resistant isolates by Modified Hodge test.

Organism (No. of carbapenem resistant isolates)	Carbapenemase production by MHT
Escherichia coli (8)	4 (50%)
Pseudomonas aeruginosa (8)	2 (25%)
Acinetobacter baumannii (8)	2 (25%)
Klebsiella pneumoniae (7)	3 (42.9%)
Enterobacter cloacae (4)	0 (0%)
Proteus mirabilis (2)	1 (50%)
Burkholderia cepacia (2)	0 (0%)
Stenotrophomonas maltophila (1)	0 (0%)
Total (40)	12 (30%)

Discussion

In a study conducted by Mate *et al.* (2014), they found a prevalence of 30% among Gram negative bacterial isolates in a tertiary care hospital in Manipur. Wankhede *et al.* (2013), found 19.40%, Prakash (2006) found 15% and Shashikala *et al.* (2006) found 10.9% carbapenem resistance in their studies respectively. As compared to the above studies, the prevalence of carbapenem resistant Gram negative bacteria in the present study was found to be 25.80%.

The rate of carbapenem resistance in this study was highest among samples obtained from ICU patients (53.13%), followed by ward patients (19.30%) and lowest among OPD patients (11.11%). Similar findings were found by Mate *et al.*(2014), where the isolates obtained from ICU patients had the highest percentage of carbapenem resistance (57.1%).

In contrast to the present study, Nair and Vaz (2013) have

reported that most of the carbapenem resistant isolates were detected in samples obtained from ward patients (42%), followed by ICU (26%) and a significant number of isolates from the OPD patients (19%).

The carbapenem resistant isolates were obtained mainly from the urine samples 30% (12/40), followed by pus 27.5% (11/40), endotracheal secretion 25% (10/40), blood 10% (4/40) and sputum 7.5% (3/40) in the present study. Macaden *et al.* (2012) have also reported that the carbapenem resistant isolates were obtained mainly from urine samples upto 42%, followed by wound discharge (18%) and respiratory secretions (16%). Similar findings were also obtained by Mate *et al.* (2014), where the carbapenem resistant isolates were obtained mainly from urine samples (47.1%), followed by pus (27.1%). In most of the studies, urine has been the most frequently received sample, which may be because of urinary tract infection being the most common hospital acquired infection.

In the present study, out of 40 carbapenem resistant organisms, 30% (12/40) was found positive for carbapenemase production by Modified Hodge Test (MHT), with the enzyme detection being highest in *E. coli* (50%) and *Proteus* sp. (50%), followed by *K. pneumoniae* (42.9%), *P.aeruginosa* (25%) and *A.baumannii* (25%). Kumar and Mehra (2015), found that among 50 carbapenem resistant isolates only 34% were positive by MHT which is comparable to the present study. Mahajan *et al*. (2011) have reported that 47.6% of the carbapenem resistant isolates were found to produce carbapenemases by MHT. However, Singh *et al* (2014) have found 91.89% of the meropenem resistant isolates to be positive for carbapenemases production by MHT.

Conclusion

A significant rate of carbapenem resistant Gram negative bacteria was found in this study. To our knowledge, this is the first study documenting the scenario of carbapenem resistance prevailing in Sikkim. Modified Hodge Test may not be an ideal phenotypic confirmatory test for all types of carbapenemase producers due to its lack in specificity and sensitivity. As a result of which some potential drug resistant pathogens may be missed out. As for now, MHT along with other phenotypic tests such as double disk synergy test, combined disk test, Carba NP test, E-Test MBL strip test or molecular methods like polymerase chain reaction can be used to obtain reliable results which would again be time consuming and expensive.

Thus, a simple, efficient and cost effective testing method for all types of carbapenemase producers in routine laboratory is the need of the hour to detect carbapenem resistance accurately on time, which would also be an important step in curbing the spread of carpbapenem resistance.

References

1. Birgy, A., Bidet, P., Genel, N., Doit, C., Decre, D., Arlet, G., & Bingen, E. (2012). Phenotypic Screening of Carbapenemases and Associated β-Lactamases in Carbapenem-Resistant Enterobacteriaceae. *Journal of Clinical Microbiology, 50*(4), 1295-1302. doi:10.1128/jcm.06131-11
2. Bush, K., & Jacoby, G. A. (2009). Updated Functional Classification of β- Lactamases. *Antimicrobial Agents and Chemotherapy, 54*(3), 969-976. doi:10.1128/aac.01009-09
3. Clinical and Laboratory Standards Institute (2015). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-fifth Informational Supplement. CLSI M100-S25 USA. Clinical and Laboratory Standards Institute, Wayne, PA.
4. S., Devi, S., Kanungo, R., & Srinivasan, S. (2006). Emerging resistance to carbapenems in hospital acquired *Pseudomonas* infection: A cause for concern. *Indian Journal of Pharmacology,38*(4), 287. doi:10.4103/0253-7613.27029
5. Kaase, M., Szabados, F., Wassill, L., & Gatermann, S. G. (2012). Detection of Carbapenemases in Enterobacteriaceae by a Commercial Multiplex PCR. *Journal of Clinical Microbiology, 50*(9), 3115-3118. doi:10.1128/jcm.00991-12
6. Kumar, S., & Mehra, S.K. (2015). Performance of Modified Hodge Test and Combined Disc Test for Detection of Carbapenemases in Clinical Isolates of *Enterobacteriaceae*. *International Journal of Current Microbiology and Applied Sciences*, 4(5), 255-261.
7. Lee, K., Chong, Y., Shin, H., Kim, Y., Yong, D., & Yum, J. (2001). Modified Hodge and EDTA-disk synergy tests to screen metallo-β-lactamase-producing strains of Pseudomonas and Acinetobacter species. *Clinical Microbiology and Infection,7* (2), 88-91. doi:10.1046/j.1469-0691.2001.00204.x
8. Macaden, R., Chandran, S., Nagaraj, S., & Shamanna, P. (2012). Carbapenem resistance among Escherichia coli and Klebsiella pneumoniae in a tertiary care hospital in south India. *Indian Journal of Medical Microbiology,30*(1), 93. doi:10.4103/0255-0857.93054
9. Mahajan, G., Sheemar, S., Makhija, S., Chopra, S., Kaur, J., & Chowdhary, D. (2011). Carbapenem resistance and phenotypic detection of carbapenemases in clinical isolates of *Acinetobacter baumannii*. *Indian Journal of Medical Sciences, 65*(1), 18. doi:10.4103/0019-5359.103161
10. Mate, P. H., Devi, K. S ., Devi, K. M., Damrolien, S., Devi, N. L., & Devi, P. P. (2014). Prevalence of Carbapenem Resistance among Gram - Negative Bacteria in a Tertiary Care Hospital in North - East India. *IOSR Journal of Dental and Medical Sciences, 13*(12), 56-60. doi:10.9790/0853-131235660
11. Miriagou, V., Cornaglia, G., Edelstein, M., Galani, I., Giske, C., Gniadkowski, M., . . . Cantón, R. (2010). Acquired carbapenemases in Gram-negative bacterial pathogens: Detection and surveillance issues. *Clinical Microbiology and Infection,16*(2), 112-122. doi:10.1111/j.1469-0691.2009.03116.x
12. Nair, P. K., & Vaz, M. S. (2013). Prevalence of carbapenem resistant Enterobacteriaceae from a tertiary care hospital in Mumbai, India. *Journal of Microbiology and Infectious Diseases, 03*(04), 207-210. doi:10.5799/ahinjs.02.2013.04.0110
13. Papp-Wallace, K. M., Endimiani, A., Taracila, M. A., & Bonomo, R. A. (2011). Carbapenems: Past, Present, and Future. *Antimicrobial Agents and Chemotherapy, 55*(11), 4943-4960. doi: 10.1128/AAC.00296-11
14. Prakash, S. (2006). Carbapenem sensitivity profile amongst bacterial isolates from clinical specimens in Kanpur city. *Indian Journal of Critical Care Medicine,10*(4), 250. doi:10.4103/0972-5229.29844
15. Queenan, A. M., & Bush, K. (2007). Carbapenemases: The Versatile β -Lactamases. *Clinical Microbiology Reviews, 20*(3), 440-458. doi:10.1128/cmr.00001-07
16. Singh, R. M., Sarkar, S., Saha, P., & Gupta, M. S. (2014). Carbapenemase Producing Enterobacteriaceae Among Urinary Isolates: Scenario From A Tertiary Care Hospital In Eastern India. *Journal of Evolution of Medical and Dental Sciences,3*(6), 1323-1333. doi:10.14260/jemds/2014/1987
17. Wankhede, S. K., Iyer V., Ghadge, D. P., & Bhore, A.V. (2013). Hospital based infections of Gram- negative organisms. *Indian Journal of Basic & Applied Medical Research*, *2*(7), 797-800.