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Comparison of Disk Diffusion Test and Carba NP Test for Detecting Carbapenem Resistance in Gram Negative Bacilli in Tertiary Care Hospital

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Abstract: ***Introduction:*** *Antimicrobial resistance is a global health security threat that requires action across government sectors and society as a whole. Carbapenems, once considered the last line of defense against of serious infections with Enterobacteriaceae, are threatened with extinction.* ***Objective:*** *Comparison between disk diffusion method and Carba NP test for carbapenem resistance in gram negative bacilli.* ***Methods:*** *Various clinical specimens collected from indoor and OPD were processed. Identification of the Gram negative organisms and carbapenem resistance was done by standard bacteriology techniques. All isolates were detected for carbapenemase production by disk-diffusion and Carba NP test.* ***Results:*** *Out of 1670 samples, 935 (55.99%) were found to be culture positive of which 485 (51.87%) were gram negative bacteria. In our study 38 (7.83%) were resistance to carbapenem where as 447 (92.16%) were susceptible by disk diffusion method whereas 58 (11.96%) isolates were CNP positive and 427 (88.04%) were CNP negative.* ***Conclusion:*** *Although enormous advancements in medical technology and services have occurred within the last two decades, antibiotic resistance has dramatically increased worldwide. Therefore, simple and affordable screening methods are required.*

Keywords: carbapenemase production, Carba NP tests, AMR- antimicrobial resistance

1. Introduction

Antimicrobial resistance in gram-negative bacilli has become a major concern in healthcare settings worldwide[1]. Antimicrobial resistance to broad-spectrum agents, where resistance to multiple, or even all available antibiotic classes, is a key global healthcare problem and health security[2].

Antimicrobial therapy has allowed for rapid advancements in the field of medicine and has had a tremendous impact in patient survival from previously lethal infectious conditions[3]. Surveillance that generates reliable data is the essential foundation of global strategies and public health actions to contain AMR[4].

Carbapenems, once considered the last line of defense against of serious infections with Enterobacteriaceae, are threatened with extinction[5]. The detection of the activity of carbapenemases has a strong impact on hospital infection control, because the detection of their presence can initiate measures to avoid potential outbreaks and lateral spread of the resistance[6]. Early and rapid detection of carbapenemase-producing GNB helps in the containment of spread of resistance[7]. It occurs mainly among Gram-negative pathogens such as *Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter baumannii*, and may be intrinsic or mediated by transferable carbapenemase-encoding genes[8]. Recently, there has been a plethora of research information on carbapenem resistance, however, there are few comprehensive review papers discussing the research information[8]. Hence the present study was aimed to include phenotypic-based methods that detect the activity of carbapenemase enzymes such as growth-based assays and rapid colorimetric methods[1], also studied the sensitivity of both tests.

2. Material and Methods

The prospective study was conducted in the department of Microbiology, Government Medical College and Hospital, Amritsar. Various clinical specimens (blood, pus, urine, sputum and ET secretions) were collected from the OPD and indoor patients in various departments of Guru Nanak Dev Hospital, Amritsar.

Total 1670 samples were cultured on blood agar and Mac Conkey agar and incubated for 24 hours aerobically at 37°C. Identification of the organisms was made based on the colony characters, motility and gram staining. Final identification was made on the basis of biochemical reactions. Gram negative isolates were identified. The carbapenemase production by disk diffusion and Carba NP test was done as per CLSI guidelines[9].

3. Result

A total of 1670 clinical samples were collected. Out of them, 935 (55.99%) were found to be culture positive and 485 (51.87%) were gram negative bacteria (figure 1).

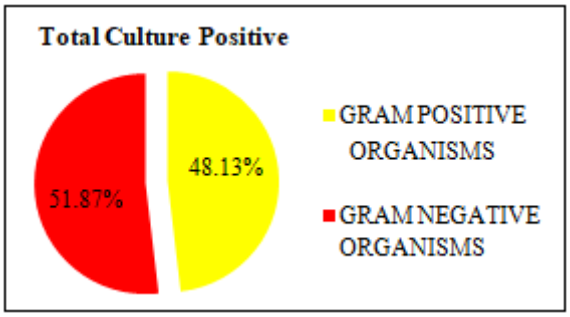

Figure 1: Distribution of Culture Positive Samples

In our study 38(7.83%) were resistance to carbapenem where as 447 (92.16%) were susceptible by disk diffusion method (figure 2).

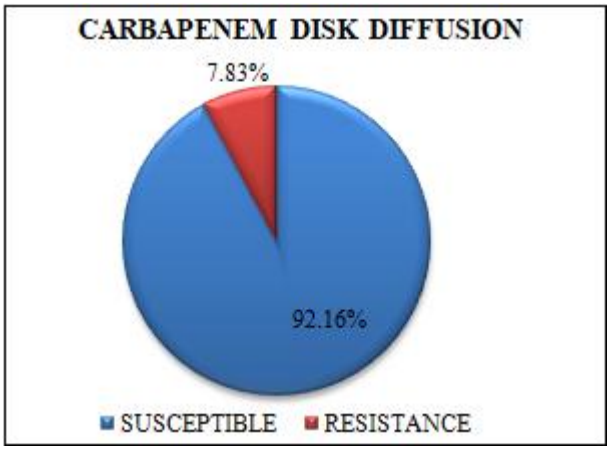

Figure 2: Carbapenem Resistance Detected by Disk Diffusion Method

Figure shows that 38/485 (7.83%) Gram negative organisms were carbapenem resistant by disk diffusion method. Amongst all isolates 58 (11.96%) were carbapenem resistant gram negative organisms by Carba NP test and 427 (88.04%) were CNP negative.

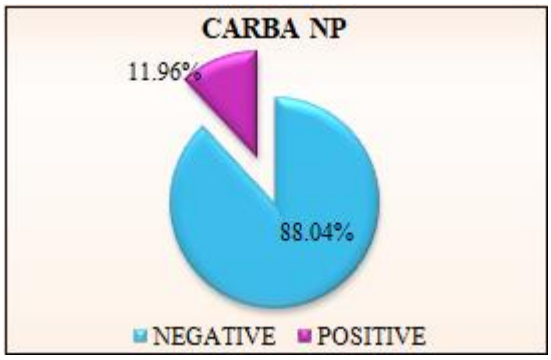

Figure 3: Percentage of Carna NP Test Positivity among Isolated Organisms

The above figure shows Carba NP test was positive in 58 (11.96%) and negative in 427 (88.04%) of the total 485 gram negative isolates.

4. Discussion

Phenotypic and molecular-based techniques are able to identify carbapenemase producers, although with variable efficiencies. Clinical and Laboratory Standards Institute (CLSI) recommended Carba NP test as the confirmatory test. Carba NP test has been added in CLSI 2015[9].

In present study 485 gram negative bacilli were isolated from various clinical samples (BAL, bile fluid, blood, endotracheal secretions, oral swab, pus, tracheal secretions, urine) received in the department of Microbiology, Government Medical College, Amritsar. Out of total 1670 samples received in the laboratory, 935 (55.99%) were culture positive and 735 (44.01%) were culture negative (table 1) which is in concordance with study done by Anitha M et al. in which 53.77% samples were culture positive[10].

Amongst amounts various Gram negative organisms, maximum isolates were *Escherichia* 148 (30.52%) followed by *Klebsiella* 123 (25.36%) which was in concordance with study done by Anitha M et al. in which most prevalent organism was *Escherichia*.

In our study 428 (88.25%) were indoor patients while 57 (11.75%) were outdoor patients. This was seen in study done b Ahmed M et al. which showed 69.46% indoor and 30.54% outdoor patients.

The present study showed 38 (7.83%) were resistant to carbapenem where as 447 (92.16%) were susceptible (figure 2), similar findings has been shown by Jan R et al which showed prevalence of 8.0% carbapenem gram negative isolates[11]. Also study done by Datta et al. reported 7.87%[12].

Out of 485, only 58 (11.96%) were Carba NP positive and 427 (88.04%) were Carba NP negative (figure 3). In our study most common carbapenem resistant isolate was *Klebsiella* 20 (16.26%) followed by *Escherichia* 6 (4.05%) in *Enterobacteriaceae*. Our results are reproducible to study done by Datta S et al showed maximum positivity of Carba NP was shown in *Klebsiella* (26.1%), *Escherichia* 18.18%[12].

5. Conclusion

CR-GNB is a growing threat to public health due to rapid spread into the community. In spite following infection control strategies, it has an increasing trend. Therefore, screening methods should be implemented worldwide for "at-risk" patients. The methods which are rapid, simple and in-expensive like Carba NP test should be introduced in bacteriology laboratories worldwide.

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