“ISOLATION, IDENTIFICATION AND DRUG RESISTANCE TESTING OF GRAM-NEGATIVE BACILLI AT A TERTIARY-CARE HOSPITAL WITH SPECIAL EMPHASIS ON THE PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF CARBAPENEM RESISTANCE”

SUMMARY SUBMITTED

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SUMMARY

The study included specimens from OPD and IPD patients of all age groups and both sexes. Specimens were cultured on suitable media. Isolates were identified by biochemical methods, both in house and by VITEK 2. Susceptibility to carbapenem drugs (imipenem, meropenem and ertapenem), as well as ampicillin, amoxycillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam, cephalothin, cefoxitin, cefotaxime, ceftazidime, cefepime, aztreonam, gentamicin, amikacin, ciprofloxacin, levofloxacin, co-trimoxazole, tetracycline, tigecycline, chloramphenicol, and colistin performed by Kirby-Bauer disc diffusion method.

A total of 1544 isolates of Gram negative bacilli were studied from different specimens, of which 184 were resistant to one or more carbapenem drugs. *Acinetobacter calcoaceticus baumannii* complex (104 isolates; 52%) was the dominant species, followed by *Pseudomonas aeruginosa* (31 isolates; 15.5%). Among the 49 isolates of Enterobacteriaceae, *K. pneumoniae* was the commonest with 17 (34.6 %) isolates, followed by *E. coli* with 15 (30.6 %) isolates.

Most carbapenem-resistant GNB came from the intensive care units and the neurosurgery ward. The largest numbers of carbapenem-resistant isolates were isolated from pus, urine, and endotracheal tube aspirates.

Carbapenem-resistant isolates were generally also resistant to most other antimicrobials except tigecycline and colistin.

False-positive carbapenem-resistance

Six (10.9%) Enterobacteriaceae isolates appeared to be carbapenem-resistant upon testing with the Kirby-Bauer disc diffusion technique with discs procured from

HiMedia. These isolates later turned out to be sensitive when retested with the Kirby-Bauer disc diffusion technique with discs procured from Rosco Diagnostica, Denmark, and also by the Etest method, resulting in a false-positivity rate of 10.9% for disc diffusion.

In addition, four isolates of *Stenotrophomonas maltophilia* and six isolates of *Elizabethkingia meningoseptica* had to be left out of the study because they are intrinsically resistant to carbapenem

Minimum Inhibitory Concentration

The Etest method for minimum inhibitory concentration (MIC) showed that the carbapenem MICs of most resistant isolates were ≥32 μg/ml.

The remaining 184 isolates were then tested for carbapenemase enzymes and efflux pumps by phenotypic and genotypic methods.

PHENOTYPIC METHODS

Modified Hodge Test (MHT)

Of the 184 isolates tested by MHT, only 22 tested positive.

Rosco KPC and MBL detection kit

Of the 49 Enterobacteriaceae isolates, 40 were MBL positive, 2 were both MBL and KPC positive and the remainder did not produce any of the enzymes and were presumed to be resistant by other mechanisms.

Rosco Rapid CARB Blue kit

43 (41.3%) *Acinetobacte*r, 13 (41.9%) *P. aeruginosa* & 39 (79.5%) Enterobacteriaceae isolates showed the presence of carbapenemases by this kit.

Rosco Neo Rapid CARB kit

The Enterobacteriaceae and *Pseudomonas* isolates were then tested by Rosco neo rapid CARB kit. 40 (81.6%) *Enterobacteriaceae* isolates and 16 (51.6 %) *P. aeruginosa* isolates were tested positive as carbapenemase producers

Carba NP test, CarbAcineto NP and Blue Carba tests with modifications

Overall 45 (91.8%) Enterobacteriaceae and 19 (61.2%) *P. aeruginosa* isolates were found to be positive for carbapenemase production by Carba NP and Blue Carba tests

Overall 84.6% *Acinetobacter calcoaceticus baumannii* were positive by CarbAcineto NP and Blue Carba test for the carbapenemase detection**.**

In terms of modifications, no difference in results was found regarding the source of imipenem while in three (6.25 %) Enterobacteriaceae isolates carbapenemase production was found to be inducible by imipenem

Carbapenem inactivation method (CIM)

Of the resistant isolates, 73 (70.1%) *Acinetobacter* spp., 15 (48.3%) *P. aeruginosa* and 38 (77.5%) Enterobacteriaceae isolates were tested positive for carbapenemase activity by this test.

GENOTYPIC METHODS

Detection of different carbapenemase genes

Majority of the isolates showed the presence of NDM-1 carbapenemase gene. In addition to NDM-1gene, the isolates also showed the presence of VIM and OXA-48 genes. Majority (83.3 %) of the VIM positive were *P. aeruginosa*.

OXA-48 was detected in only *K. pneumoniae* isolates. None of the study isolate found to be positive for KPC gene.

EFFLUX PUMP ACTIVITY

The presence of efflux was detected by two methods namely ethidium bromide cartwheel method and agar dilution method using reserpine as efflux inhibitor

Ethidium bromide cartwheel method

On performing the efflux protocol 27 isolates were found to be positive. i.e. (the strains showing little or no fluorescens under UV light)

Reserpine as efflux pump inhibitor

Only 20 isolates showed a decrease in the MIC of meropenem when reserpine was added.