SOME STRANGE FINDINGS: NON-INTERPRETABLE PATTERNS IN MODIFIED HODGE TEST

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ABSTRACT: Many clinically relevant species of Gram-negative bacilli are often resistant to β-lactam antibiotics even to carbapenems. According to CLSI guideline 2013, Carbapenemase producers are screened by zone size <21mm to Meropenem or Etrapenem and phenotypically confirmed by Modified Hodge test. The test of Hodge et al was modified by substituting Escherichia coli for penicillin-susceptible Staphylococcus aureus ATCC 25923, and 10-µg Imipenem disk for a 10-U Penicillin disk. While looking for the Carbapenemase producing strains, some astonishing, unexplainable facts were revealed in this study. Meropenem-resistant test strains were screened (zone diameter in Modified Kirby-Bauer technique<21mm) & for phenotypic confirmation of Carbapenemase production, modified Hodge test was performed. Thereafter the test strains were examined for production of Metallo β Lactamase using Imipenem (10µg) disk and combination of Imipenem and EDTA, followed by test for Bacteriocin production. Two Acinetobacter and one Pseudomonas aeruginosa strains were found to produce a Star like outward distortion. They did not produce Metallo β lactamase. Moreover, screening test for bacteriocin production was also found to be negative. The strains resistant to Carbapenem antibiotics but nonproducers of metallo β lactamase and/or Bacteriocins, have left behind a head twister for us.

INTRODUCTION: Many clinically relevant species of Gram-negative bacilli are often resistant to β-lactam antibiotics even to Carbapenems. Hodge et al developed a test to detect penicillinase-producing Neisseria gonorrhoea and other species of bacteria [1]. IMP-1 metallo-β-lactamase-producing Pseudomonas aeruginosa emerged in Japan [2], and then the resistance spread to other species. IMP-2-producing Acinetobacter baumannii [3] and VIM-1- and VIM-2-producing strains of P. aeruginosa have been reported in Europe [4, 5, and 6]. The metallo-β-lactamase-producing strains of P. aeruginosa was reported in 1998 [7] were subsequently identified as VIM-2 β-lactamase producers.

According to CLSI guideline 2013, Carbapenemase producers are screened by zone size < 21mm to Meropenem or Ertapenem and phenotypically confirmed by Modified Hodge test.

The test of Hodge et al [8] was modified by substituting Escherichia coli for penicillin-susceptible Staphylococcus aureus ATCC 25923, and 10-µg Imipenem disk for a 10-U Penicillin disk.

OBJECTIVE: While looking for the Carbapenemase producing strains, some astonishing Modified Hodge Test findings were there which could not be explained. To solve those unanswered facts, isolates producing such aberrant findings were further tested to verify whether they were Bacteriocin producers or not.

MATERIALS AND METHODS: The surface of a Mueller–Hinton agar plate was inoculated evenly using a sterile cotton swab with an overnight culture suspension of E. coli ATCC 25922, which was

adjusted to one-tenth turbidity of the McFarland no. 0.5 tube. After brief drying, Meropenem disk (Hi Media Lot-0000116959) was placed at the center of the plate, and Meropenem-resistant test strains (zone diameter in Modified Kirby-Bauer technique <21mm) from the overnight culture plates were streaked heavily from the edge of the disk to the periphery of the plate. The presence of a Clover leaf like distorted zone of inhibition after overnight incubation was interpreted as modified Hodge test positive i.e., phenotypic confirmation of Carbapenemase production.

There after the test strains were tested for production of Metallo β Lactamase using Imipenem (10µg) disk (Hi Media Lot-0000117308) and combination of Imipenem and EDTA (10 µg/750 µg)(Hi Media Lot-0000092893). Two disks were set on lawn culture of test strains keeping 20 mm distance (from centre to centre) between them. Zone Size difference between the zones surrounding the Imipenem only disk and the combination disk of more than 5mm indicated the production of Metallo β lactamase.

Strains producing the aberrant findings were further tested for production of Bacteriocin by following method:

On Muller Hinton Agar, test strain was streaked and incubated overnight. On the next day, the growth was scraped off and chloroform vapour was applied on the streak line for 20 to 30 minutes. There after E. coli ATCC 25922 strain was streaked (from 0.5 Mac Farland unit broth suspension) from the previous line at an angle of ninety degree and incubated at 37^0C for overnight. Next day, the growth of E. coli ATCC 25922 indicated that no Bacteriocin was produced or diffused in the media.

RESULTS & CONCLUSION: Two Acinetobacter strains and one Pseudomonas aeruginosa strain were found to produce a Star like distortion instead of expected cloverleaf distortion (Fig.1).

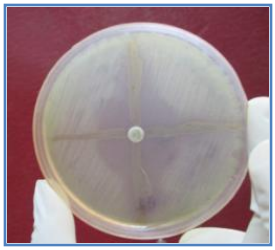

Fig. 1: Star like outward projection Produced by a Pseudomonal strain.

They did not produce Metallo β lactamase i.e., resistant to both Imipenem and Imipenem EDTA combination disk.

Inhibition of E. coli ATCC25922 around the streak line of those three test isolates was assumed to be due to production of Bacteriocin but phenotypic screening test for bacteriocin production was found to be negative (Fig.2).

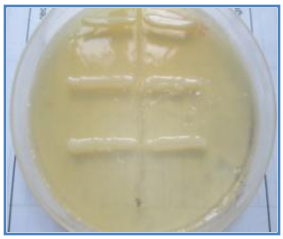

Fig. 2: Test strain was found to be a non producer of Bacteriocin.

DISCUSSION: We have found this strange finding among two Acinetobacter and one Pseudomonas isolate whereas some other workers have reported such a pattern in Modified Hodge test with

Enterobacteriaceae (Klebsiella spp.) They concluded that, possibly this was due to Bacteriocin production (9). On the contrary, our results do not corroborate these findings.

CONCLUSION: Thus, the strains resistant to Carbapenem antibiotics but nonproducers of Metallo β lactamase and/or Bacteriocins, have left behind a head twister for us.

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