to carbapenems, previously performed by Vitek 2 automated system (BioMérieux, Paris) and additionally by Etest (BioMérieux, Paris). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls. For Flow Cytometry analysis, bacterial cells were incubated in filtered Muller-Hinton broth until exponential phase and then exposed to different concentrations of meropenem, imipenem, doripenem and ertapenem for 1 hour and 2 hours and afterwards stained with Bis-(1,3-dibutylbarbituric acid) trimethine oxonol (DiBAC4(3)), a lipophilic anion able to diffuse across depolarized membranes. From Flow Cytometry suspensions, conventional Colony-Forming Units (CFU) assays were performed in order to establish a correlation between depolarized cells quantified by Flow Cytometry.

Results: A clear discrimination between susceptible and resistant strains was possible, soon after 1 hour of treatment with the studied carbapenems. An excellent correlation was obtained between the number of depolarized bacteria quantified by Flow Cytometry and the conventional CFU assays.

Conclusions: A novel, simple and fast assay is now available to detect carbapenem-resistant Gram-negative bacteria based upon Flow Cytometry.

P768 Detection of carbapenemase enzymes in clinical isolates of *Pseudomonas aeruginosa* by Re-modified Hodge Test and other phenotypic methods

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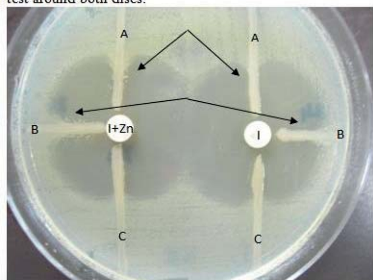
Objectives: Therapeutic failure to carbapenems is increasingly reported in *Pseudomonas aeruginosa* due to emergence and rapid spread of Metallo β Lactamase (MBL) enzymes which are present even in carbapenem sensitive isolates. Presently no standard guidelines for phenotypic testing are available for detection of carbapenemase enzymes in *Pseudomonas aeruginosa* and previous reports are variable. Timely and accurate laboratory detection and reporting of such isolates harboring these enzymes will indicate their prevalence pattern and will help advent appropriate and rational use of alternative antimicrobials agents. Aim of present study was to detect the presence of carbapenemase enzyme activity in carbapenem resistant and sensitive isolates of *Pseudomonas aeruginosa*.

Methods: Study was conducted in Department of Microbiology at Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, a 1000 bedded tertiary care hospital in North India, over a 2 month period (August–September 2008). We evaluated a Re-modified Hodge Test and compared it with conventional Modified Hodge Test, Double Disc Synergy Test (DDST) using ethylenediaminetetraacetic acid (EDTA) with imipenem, meropenem and ceftazidime and EDTA Imipenem Microbiological (EIM) assay among carbapenem resistant and sensitive isolates of *Pseudomonas aeruginosa*. We also extended principle of three dimensional enzyme extract test (3DT) for evaluation of MBL activity. 114 isolates of *Pseudomonas aeruginosa* were divided into two groups (resistant and sensitive) based on minimum inhibitory concentration values.

Figure 1- Modified and Re-Modified Hodge Test: Shows three strains plated as mirror images around an Imipenem disc on the right and an Imipenem plus zinc sulfate disc on the left. Strains A and B show enhancement of growth of indicator strain *E.coli* ATCC 25922 around 'I+Zn' disc compared to 'I' only disc. There is no change in case of strain C which shows an equally positive test around both discs.



Results: 83 isolates were carbapenem resistant and 31 were sensitive. Modified Hodge and re-modified Hodge tests were able to detect 58% and 82% of MBLs in carbapenem resistant, and 19% and 32% of carbapenem sensitive isolates respectively. Individually, EIM test and ceftazidime/EDTA DDST were the most sensitive tests detecting MBLs in 88% and 49% of carbapenem resistant, and 19% and 35.5% of carbapenem sensitive isolates respectively. 3DT for MBL detection had a poor sensitivity of 54% of carbapenem resistant and only 3% carbapenem sensitive strains.

Conclusion: This study validates the use of Re-Modified Hodge Test for routine detection of MBL activity in *Pseudomonas aeruginosa* as it is a sensitive and technically easy to perform phenotypic test. Its routine induction will maximize detection of MBL harboring *Pseudomonas aeruginosa* resulting in proper and effective antimicrobial guidance.

P769 Application of fluorescence in situ hybridisation using peptide nucleic acid probes in gastric samples for detection of *Helicobacter pylori* clarithromycin resistance

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Objectives: Microorganisms are responsible for several infectious diseases that can cause severe problems to patients and their treatment success is seriously correlated with the fast detection of the infectious agent. Some of the standard methods used, such as culturing methods are fastidious and time-consuming and do not give any information about the antibiotic resistance profile. Therefore, molecular methods have been developed during the last several years in order to overcome these shortcomings. In this work a new genotypic method that permits the identification of the microorganism in clinical samples in a prompt way is proposed. This technique is based on Fluorescence in situ hybridization with PNA probes that are synthetic molecules, complementary to a specific rRNA sequence of the microorganism.

Methods: A set of PNA probes were designed concerning *H. pylori* point mutations regarding clarithromycin resistance which is the main problem of gastric diseases treatment failure. An additional probe concerning susceptibility was also designed. After hybridization conditions optimization, probes were applied to *H. pylori* smears to achieve their practical sensitivity and specificity. At the end they were applied to gastric biopsies in a retrospective study for method validation in real samples. E-test and PCR-sequencing were used to evaluate the results.

Results: The probes concerning clarithromycin resistance hybridized only with the resistant strains that had the corresponding point mutations and as such presented 100% sensitivity (95% CI, 79.9–100) and 100% specificity (95% CI, 71.6–100). Results also showed that it is possible to discriminate susceptible from resistant *H. pylori* strains in gastric biopsy samples since it was presented similar results between the 3 tests used. Overall, the PNA-FISH method was in full agreement with PCR-sequencing although it was a little bit lower when compared to E-test that it was used as gold standard method in this retrospective study (86%).

Conclusion: PNA-FISH proved to be an important in situ method for detection of microorganisms in clinical samples in a more prompt way than the standard methods. Due to high *H. pylori* probes sensitivity and specificity it is proved the applicability of PNA-FISH methodology to clinical material, thus overcoming the need of culturing steps and/or PCR/sequencing procedures and enabling rapid initiation of appropriate antibiotic therapy until culture confirmation several days later.

P770 Comparison of the PBP2a latex agglutination assay, PBP2a rapid immunochromatographic assay and chromogenic medium for identifying methicillin-resistant *Staphylococcus aureus* directly from positive blood cultures

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Objectives: Rapid and precise molecular methods (eg., real time-PCR) have been developed for the detection of methicillin-resistant