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Occurrence of Esbl and Mbl in Clinical Isolates of Pseudomonas Aeruginosa – An Emerging Threat to Clinical Therapeutics

KEYWORDS: Pseudomonas aeruginosa - ESBL - MBL - Imipenem – EDTA Double Disk Synergy test

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ABSTRACT *The present study was undertaken to detect the Extended Spectrum β Lactamases (ESBL) and Metallo β Lactamases (MBL) in isolates of Pseudomonas aeruginosa which were isolated from wound infections and to evaluate their susceptibility patterns. The presence of the ESBL enzyme was detected by the phenotypic confirmatory test and the MBL enzyme was detected by the Imipenem – EDTA Double Disk Synergy test and Modified Hodge test. Out of 100 samples which were cultured, 26[26 %] yielded the growth of Pseudomonas aeruginosa.Among the 26 Pseudomonas aeruginosa isolates, 8 [30.76%] were ESBL producers and 4[15.4%] isolates were metallo β lactamase producers. The modified Hodge test detected 2 strains as metallo-β-lactamase producers (MBL).The EDTA disc synergy test detected the same 2 strains as well as an additional two strains as MBL producers.All the ESBL producing isolates were sensitive to Imipenem, while the MBL producing isolates showed widespread resistance to Aminoglycosides, Ciprofloxacin and the Piperacillin with Tazobactam combination. The present study underlines the unique problem that the presence of ESBL and MBL mediated resistance in Pseudomonas aeruginosa has created a therapeutic challenge for the clinicians and microbiologists. Hence, we suggest that the detection of ESBL and MBL in Pseudomonas aeruginosa should be a routine practice in the hospital.*

Introduction

Pseudomonas aeruginosa is reported to be amongst the leading causes of nosocomial infections. *P.aeruginosa is* physiologically versatile and flourishes as a saprophyte in multiple environments, including sinks, drains, respirators, humidifiers and disinfectant solutions. Infections due to *P. aeruginosa* are seldom encountered in healthy adults; but in the last two decades, the organism has become increasingly recognized as the etiological agent in a variety of serious infections in hospitalized patients.(1)

Pseudomonas aeruginosa is an opportunistic pathogen with innate resistance to many antibiotics and disinfectants. In addition to the intrinsic resistance of *P. aeruginosa*, it also produces the enzymes, namely ß-lactamase, which is responsible for the wide-spread ß-lactam resistance. These ß -lactamase hydrolyse the amide bond of the four membered characteristic ß - lactam ring, thus rendering the antimicrobial ineffective. [2]The ESBL enzymes encoded by the genes, SHV2a and the genes, SHV2a and TEM have been found in *P. aeruginosa and* the *Enterobacteriaceae* family, which suggests that these organisms are widespread reservoir of the ESBL.[3]

MBLs are class B enzymes which hydrolyze carbapenems and are encoded by genes like IMP, VIM etc [4].They have been described as the enzymes which require divalent cations,usually zinc, as metal co-factors for their enzymatic activity. In recent years, the MBL genes have spread from *P.aeruginosa* to *Enterobactericeae*.Given the fact that we are several years away from the implementation of a therapeutic inhibitor of MBLs their continued spread is going to be a major therapeutic challenge.[5]

Hence, the present study will be undertaken to detect the presence of ESBL and MBL producing *P. aeruginosa,* so as to help in formulating an effective antibiotic strategy and to plan a proper hospital infection control strategy to prevent the spread of these strains.

Materials and Methods

Study design and settings

This prospective study was conducted at the Microbiology Department at Tirunelveli Medical College Hospital, Tirunelveli, TamilNadu from April' 2012 to September' 2012. The study protocol was approved by the Institutional Scientific and Ethics Committee**.**

Study population:

A total of 100 samples (Pus, Blood, Urine and Sputum) sent to Clinical Microbiological Laboratory were included for this study.

Culture and Identification:

The obtained clinical specimens were plated on three different media namely Nutrient agar, Blood agar and MacConkey agar and were incubated at 37°C in an incubator. The isolates were subjected to a battery of tests like motility, oxidase, catalase, motility, indole, urease, nitrate, citrate tests and OF reactions of glucose(Hugh-Leifsons method),susceptibility to polymyxin (300U).

Antibiogram:

Antimicrobial sensitivity was determined using Kirby Bauer disc diffusion method. The antimicrobial agents used in the study are Amikacin, Gentamicin, Ciprofloxacin, Cotrimoxozole, Ceftazidime, Imipenem and Meropenem. The results were interpreted as per the CLSI guidelines. [7]

Extended spectrum β – lactamase (ESBL) detection

All the isolates of *P.aeruginosa which* showed resistance to ceftazidime were evaluated for ESBL production by using the phenotypic confirmatory test. [7]0.5 MacFarland's suspension of each isolate was spread on a Muller – Hinton agar (MHA) plate and ceftazidime (30 µg) and ceftazidime / clavulanic acid (30 µg/ 10 µg) discs were placed aseptically on the agar plate. A distance of about 15mm was kept between the two discs (edge to edge) and the cultures were incubated at 37°C overnight. The observation of a ≥ 5mm increase in the zone diameter for the antimicrobial agent which was tested

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in combination with clavulanic acid, versus its zone diameter when tested alone confirmed the presence of ESBL production by the organism. The increase in the zone diameter was due to the inhibition of the β lactamase by clavulanic acid.

The Metallo - β- Lactamase (MBL) Detection Method
Imipenem resistant strains were checked for production of carbapenemase and metallo β-lactamase by EDTA disc synergy test and the modified Hodge test.

Imipenem-EDTA double disk synergy test (DDST)
The Imipenem resistant isolates were tested by the Imipenem-EDTA double disk synergy test (DDST) as described by Lee et al. [7] The test organism were inoculated onto MHA plates as recommended by CLSI. An Imipenem 10 µg disk was placed 10mm edge to edge from a blank disc which contained 10 µl of EDTA (750 µg), with overnight incubation at 37°C. An enhancement in the zone of inhibition in the area between the Imipenem and the EDTA discs in comparison with the zone of inhibition on the far side of the drug were interpreted as a positive result.

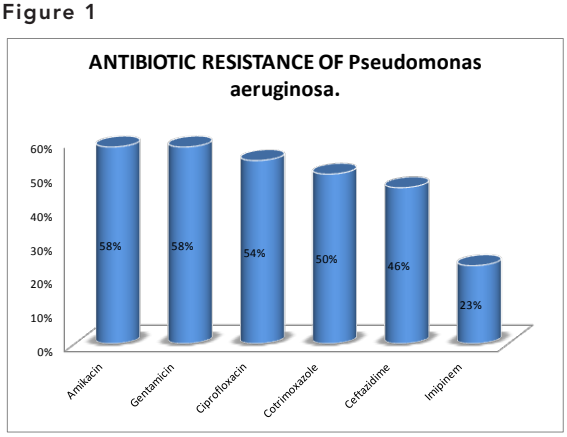
Modified Hodge test.
Modified Hodge test was carried out on Mueller-Hinton agar. The plate was inoculated using a cotton swab dipped in an overnight culture suspension of *E.coli* ATCC 25922.After brief drying, 10 µg imipenem disc was placed at the centre of the plate and test strains were streaked from the edge of the disc to the periphery of the plate in four different directions.

After overnight incubation the plates were observed for the presence of a 'cloverleaf shaped' zone of inhibition.[8]

Results
Out of 100 samples which were cultured, 26[26 %] yielded the growth of Pseudomonas aeruginosa. 20 samples yielded a pure growth of Pseudomonas aeruginosa, while 6 isolates were mixed cultures with E. coli, Staphylococcus aureus, Proteus species, Klebsiella pneumoniae. Out of the 26 subjects who showed the growth of Pseudomonas aeruginosa, 18[69.2 %] were male patients and 8 [30.8%] were female patients. Out of the 26 isolates, 4 [15.4%] isolates were from the Outpatients Department and 22 [84.6%] were from inpatients, In the present study, a majority of the P. aeruginosa isolates were obtained from pus samples [77%], followed by urine samples [23%].

The resistance pattern of P.aeruginosa was noted as follows, Amikacin-15(58%), Gentamicin-15(58%), Ciprofloxacin-14(54%), Cotrimoxozole-13(50%), Ceftazidime-12(46%), Imipenem-6(23%) and Meropenem-6 (23%).

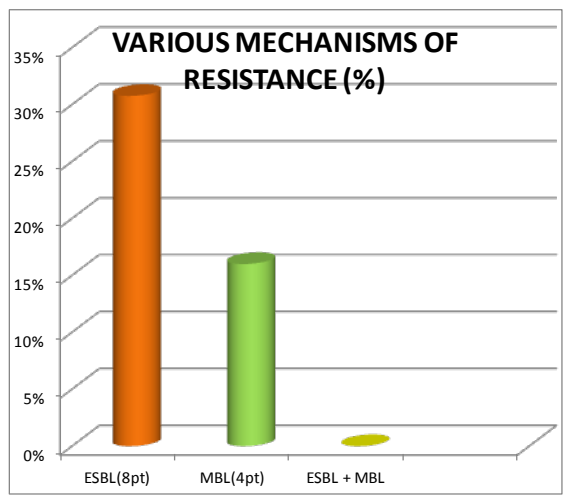
Figure 1



Among the 26 *Pseudomonas aeruginosa* isolates, 8 [30.76%] were ESBL producers. A total of twelve strains showed resistance to ceftazidime, of which 8 [66.66%] were found to be ESBL producers. Among the 26 isolates of *Pseudomonas aeruginosa*, 4[15.4%] isolates were metallo β lactamase producers and these were isolated from 6 imipenem resistant isolates. Hence, the percentage of MBLs in the imipenem resistant isolates was 66.6%. Also, those 4 MBL producing strains were also resistant to Amikacin, Gentamicin and Ciprofloxacin and Co-trimoxazole.

The modified Hodge test detected 2 strains as metallo-β–lactamase producers (MBL).The EDTA disc synergy test detected the same 2 strains as well as an additional two strains as MBL producers. None of the isolates showed the coexistence of ESBL and MBL.

Figure 2



The likely reason for the resistance in the remaining isolates may be mechanisms other than metallo-β-lactamase production such as decreased membrane permeability.

Discussion
The emergence of antibiotic resistant bacteria is threatening the effectiveness of many antimicrobial agents. It has increased the hospital stay of the patients, thus leading to an increased economic burden on them. In the present study, the rate of isolation of P. aeruginosa was higher in inpatients [84.6%] as compared to that in the outpatients [15.4 %].A similar observation was made by Shampa Anupurba et al, who reported the isolation of Pseudomonas aeruginosa to be more common in inpatients [73.42%] as compared to that in the OPD cases [26.57%].They expressed their view that the duration of the hospital stay was directly proportional to a higher prevalence of the infection, since the rate of isolation of the organisms was higher in inpatients than in outpatients[9,10]

In the present study, we observed an increased resistance of this organism to various antibiotics like Amikacin-58%, Gentamicin-58%, Ciprofloxacin-54%, Cotrimoxazole-50%,Ceftazidime-46%. A decreased susceptibility of P.aeruginosa to the commonly used antibiotics has already been noted by previous researchers.[4,11-13]Our study showed that among the 26 Pseudomonas aeruginosa isolates, 8 [30.76%] were ESBL producers, which was higher than 20.27 % ESBL producing isolates of P. aeruginosa which was reported by Agarwal et al.[11]

The ESBL mediated resistance of P. aeruginosa to the third Generation cephalosporins as reported by Uma et al [77.3%] was much higher than that reported in the present study.[14] We observed that 23.07% strains were resistant to carbapenem in our study, which was similar to Varaiya et al [25%][50]In contrast, Imipenem and Meropenem showed good antipsuedomonal activity in study by Jaykumar S. [4].This difference could be attributable to the study environment under which the study was performed.

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In our study, out of the 26 isolates of *Pseudomonas aeruginosa*, 4 [15.4 %] isolates were metallo β lactamases producers. The prevalence of MBLs in the present study was consistent with the findings of Ibukun et al, Navaneeth et al and others.[15-17]The percentage of MBLs in the imipenem resistant isolates was 66.6%. This suggested that the carbapenem resistance in *P. aeruginosa* was mediated predominantly via MBL production. A similar finding was observed by the SARI study group and by Behara et al.[17,18]

The EDTA disc synergy test was able to detect two more strains which was not detected by the modified Hodge test. However, the reason why there is better detection by EDTA disc synergy test as compared to modified Hodge test is unclear.[8]Among the 6 imipenem resistant isolates of *Pseudomonas aeruginosa*, the EDTA disc synergy test detected the 4 strains as MBL producers. The likely reason for the resistance in the remaining isolates may be mechanisms other than metallo-β-lactamase production such as decreased membrane permeability.[19]

In the present study, all the 4 MBL strains were resistant to all the antibiotics which were tested. The presence of MBLs in the pan drug resistant isolates was already observed by Jaykumar S [4]. In our study, none of the isolates had coproduced both ESBL and MBL. This was in agreement with the findings of Renata Picao et al [20]

Our study underlines the unique problem of ESBL and MBL mediated resistance, which has created a therapeutic challenge for the clinicians and microbiologists. To overcome the problem of emergence and the spread of multidrug resistant *P. aeruginosa*, a combined interaction and cooperation between the microbiologists, clinicians and the infection control team is needed. We recommend the routine surveillance of antibiotic resistance in the hospital.

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