



Original Research Article

Immuno and Molecular screening of *Pseudomonas aeruginosa* from selected infection

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ABSTRACT

Keywords

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samples;
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P.aeruginosa.

About 100 samples such as Blood, pus from wound, urine, sputum were collected to isolate bacteria from patients admitted in different hospitals in and around vellore. The nature of bacterial isolates was identified with standard morphology, biochemical and cultural characteristics, *Staphylococcus aureus*, *E.coli*, *Bacillus* sp. *P.aeruginosa*. *P.aeruginosa* were predominant ones in almost of the samples. The positive *P.aeruginosa* sample in pus were (20) 49, Urine 32(58), sputum 26(49), blood 14(44). The serotype of *P.aeruginosa* were identified using serotype methods. Susceptibility test was also carried out and results were noted. Sulbactam and clavulanic acid was used to overcome the penicillin and cephalosporin derivative resistant problem. This study clearly revealed that the sulbactam and clavulanic acid has anti beta lactamase activity and resistant to ones which are beta lactamase produces become susceptible to same antibiotic.

Introduction

Pseudomonas aeruginosa is a gram negative aerobic, rod shaped bacterium with unipolar flagella. It secretes variety of pigments including pyocyanin (blue-green), fluorescein (yellow-green) now also known as pyoverdinin and pyorubin (red-brown). *Pseudomonas aeruginosa* is often preliminarily identified by its pearlescent appearance and grape like odour invitro. The word pseudomonas means "False unit" from the Greek (pseudo-false; Latin-monas-"A single unit"). In hospital 1018 strains were isolated in one year from an intensive care

P.aeruginosa. (Roussel-Delvallez *et al.*, 1996). Sulbactam, clavulanic acid beta lactamase inhibitor showed the bound action to PBS 1 and 2 at clinically achievable level in both susceptible / resistance strains (Urban *et al.*, 1995). Non classic antibiotic combinations such as ticarcillin with clavulanic acid and sulbactam seem to show promise for treating systemic infection caused by multiresistant strains (Towner *et al.*, 1997).

The overall problem of antibiotic resistance is one of the genetic ecology

and a better understanding of the contributing parameters is necessary to reduce the development and the spread of antibiotic resistance (Mazel and Davis *et al.*, 1999). Antimicrobial biocides are widely used in critical human health situations in which rigorous infection control is needed. Biocide resistance mechanisms share many by themes with antibiotic resistance mechanisms (White and Mc Dermott *et al.*, 2001). *Pseudomonas* species showed 25% increase in resistance to piperacillin-tazobactam and an 18% increase to ciprofloxacin which was correlated with the increased use of these antimicrobial agents (82%) in the ICU during the 3 years (Hariharan *et al.*, 2003).

Materials and Methods

Collection of sample

About 100 samples such as blood, pus from wound, urine and sputum (Bronchial secretions) were collected to isolate bacteria from hospitals in and around Vellore.

Isolation of Bacteria from samples

The swabs were touched from the individual clinical samples and inoculated on nutrient agar, blood agar and Mannitol salt agar plates. The isolated colonies were allowed to grow at 37°C for 24 hrs. The colonies grown on the plates were examined for the morphology, haemolysis and other cultural characters. From the above testing the organisms were identified as *Staphylococcus aureus*, *E. coli*, *Bacillus sp.*, and *P. aeruginosa*. The special concentration upon the *P. aeruginosa* was given because it is the most predominant organism isolated from the clinical samples.

Test for *P. aeruginosa*

The samples were inoculated over the selective medium for the identification of *P. aeruginosa*. The slide agglutination test and the ONPG test also performed positive result by yellow colour appearance in the ONPG disc.

Test for isolates Resistant to antibiotics

The MHA plates were prepared and a pinch of ceftazidime was added to the plates. After solidification the clinical sample was inoculated. The resistant colonies were grown in the plates. The colonies were streaked in sulbactam with ceftazidime in MHA plates to identify the resistant colonies. The same procedure was followed in amoxicillin.

Resistant Sulbactam with Ceftriaxone / Clavulanic Acid

The MHA plates were prepared and a pinch of ceftriaxone was added to the plates. After solidification the clinical sample was inoculated. The resistant colonies were grown in the plates. The colonies were streaked in sulbactam with ceftriaxone in MHA plates to identify the resistant colonies. The same procedure was followed in Clavulanic acid.

Results and Discussion

About 100 samples such as blood, pus from wound, urine, sputum were collected to isolate bacteria from different patients admitted in different hospital in and around Vellore. The positive *P. aeruginosa* samples were 20 (49), urine 32 (58), sputum 26 (49), blood 14 (44). The test for *P. aeruginosa* was performed by slide agglutination test. It shows positive result by agglutination.

Table.1 Antibiotics sensitivity of Amoxycillin with Sulbactam medium

S.No	Name of the organism	Amoxycillin medium	Amoxycillin +Sulbactam
1.	<i>Staphylococcus aureus</i>	Resistant	Sensitive
2.	<i>Escherichia coli</i>	Resistant	Intermediate
3.	<i>Bacillus subtilis</i>	Resistant	Intermediate
4.	<i>Pseudomonas aeruginosa</i>	Resistant	Intermediate

Table.2 Antibiotics sensitivity of Amoxycillin with Clavulanic acid

S.No	Name of the organism	Amoxycillin medium	Amoxycillin + Clavulanic acid
1.	<i>Staphylococcus aureus</i>	Resistant	Sensitive
2.	<i>Escherichia coli</i>	Resistant	Intermediate
3.	<i>Bacillus subtilis</i>	Resistant	Intermediate
4.	<i>Pseudomonas aeruginosa</i>	Resistant	Intermediate

Table.3 Antibiotics sensitivity of Ceflazidime with Sulbactam medium

S.No	Name of the organism	Ceflazidime medium	Ceflazidime +Sulbactam
1.	<i>Staphylococcus aureus</i>	Resistant	Sensitive
2.	<i>Escherichia coli</i>	Resistant	Intermediate
3.	<i>Bacillus subtilis</i>	Resistant	Intermediate
4.	<i>Pseudomonas aeruginosa</i>	Resistant	Resistant

Table.4 Antibiotics sensitivity of Ceflazidime with Clavulanic acid

S.No	Name of the organism	Ceflazidime medium	Ceflazidime +Sulbactam
1.	<i>Staphylococcus aureus</i>	Resistant	Sensitive
2.	<i>Escherichia coli</i>	Resistant	Intermediate
3.	<i>Bacillus subtilis</i>	Resistant	Intermediate
4.	<i>Pseudomonas aeruginosa</i>	Resistant	Intermediate

shows positive result by yellow colour appearance in the task tube *P.aeruginosa* were also identified using serotyping methods.

The resistant pattern were identified particularly to penicillin derivative such as Amoxyllin, ceflaxidime and cephalosporin

derivative ceftriazone, for that Sulbactam and clavulanic acid was used. The study has clearly revealed that the sulbactam and clavulanic acid has antibeta lactamase activity and resistant to o's which are become susceptible to same Antibiotics (Tables 1- 4).

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