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PHENOTYPIC AND MOLECULAR CHARACTERISATION OF PSEUDOMONAS AERUGINOSA AMONG HOSPITAL ACQUIRED INFECTIONS IN A TERTIARY CARE HOSPITAL, TIRUPATHI

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ABSTRACT

Back ground: Nosocomial infections, being commonly caused by Pseudomonas aeruginosa, Klebsiella, Proteus, Staphylococcus etc., are responsible for prolonged hospital stay by the vulnerable hosts badly affecting the hospital economy **.**Multidrug resistance has become one among the great human life threatening problems, we may be left with no effective drug in near future if this phenomenon continues**.** It is an ongoing process, to curtail Hospital acquired infections to minimum possible ,consisting of effective surveillance of hospital hygiene through biohazardous waste management team, effective infection control team in order to know the emerging epidemiological strains,their sensitivity pattern ,awareness about cost effective drugs with minimal side effects all through effective practical implementation by the dedicated hospital authorities and their sub staff. Here a study is taken to know the prevalence of resistant strains of Pseudomonas aeruginosa in a tertiary care hospital, Tiupathi. **Materials & methods:** Nosocomial infections are identified by thorough clinical history. Further they were studied for specific pathogen by various biochemical tests and isolation by using appropriate culture media. Among them Pseudomonas aeruginosa is identified by using King's medium and by its specific bluish green pigmentation. (pyocyanin). Among 330 samples collected from different nosocomial infections such as P.O wound infection, burns infection, UTinfection, bronchial fluid, Otitis externa, Pseudomonas aeruginosa were 72,Klebsiella pneumonia were 58,Staphylococcus aureus were 50,Proteus were 28,other species 8 total being 218,culture negative samples were 112.Further Pseudomonas aeruginosa is studied for imipenem resistance using PCR . **Result:** Among 218

culture positive strains 72 were due to P.aeruginosa (33.02%), these infections were found to be more common in males age group 50 to 60 (37.2%) .i.e., 24 cases.Antibiotic senstitivity shows. Amikacin(58.33%) sensitive, Gentamycin(42%) Imipenem (84.72%) piperacil+ tazobactum (77.77%) Ceftazidime(54.16%) Cefotoxime(40%). Out of 72 pseudomonas aeruginosa strains 11 strains were resistant to imipenem they were further studied for molecular characterization by PCR. **Conclusion:** Because of rapidly emerging antibiotic resistance among different nosocomial pathogens there is essential requirement for antibiotic policy in hospitals. Knowledge of sensitivity pattern and molecular characterization of the resistance genes of nosocomial infections is useful for their epidemiological control.

KEYWORDS: Pseudomonas aeruginosa, Klebsiella, Proteus, Staphylococcus.

INTRODUCTION

Pseudomonas aeruginosa is an aerobic Gram negative bacilli motile,non-sporing,non-capsulated ,non-fastidious organism.[1] It's infection is common in patients with compromised host defence mechanisms,mucosal trauma, antibiotic mediated suppression of normal flora.[2] Pseudomonas aeruginosa is causative agent of burns wound sepsis,surgical wound infection,RTIs, ventilator associated Pneumonia (1st or 2nd common causative agent), Acute pneumonia,chronic RTI, endovascularinfection, keratitis, endophthalmitis, meningitis, Otitisexterna, UTIs, Pyodermagangrenosum, green nail syndrome. Hospital acquired infections include catheter induced UTI, infected ulcers, bed sores, burns and eye infections[2] Pseudomonas aeruginosa is inheritantly resistant to common antibiotics and hospital disinfectants and their ability to grow in the absence of nutrients[3] gives it opportunity to emerge as significant pathogen in various nosocomial infections.[4]

Pseudomonas aeruginosa is often being the commonest causative agent of nosocomial infections, and Multi drug resistant requires special antipseudomonadal drugs such as carbenicillins,3rd generation cephalosporins, Aminoglycosides,Fluoroquinalones for treating the nosocomial infections for which they are considered as primary pathogens.[5] Pan resistant strains were usually treated with Polymixins and colistins.[1,6,7,8]

Further molecular study on resistant strain is useful for epidemiological study. The strains producing metalloβenzymes are responsible for prolonged nosocomial out breaks and is are therapeutically often challenging and even to infection control management.[9] The detection of such strains is useful to limit their spread in hospital environment.

MATERIALS AND METHODS

In present study among total 1786 in-patients, Cases that were infected after more than 48 hours admission into hospital, either medical or surgical wards of tertiary care hospital ,were undertaken during October 2014 -september 2015.There were 330 samples diagnosed as nosocomial infections among which 218 were culture positive and 112 were culture negative. All the cases diagnosed as nosocomial infections were followed-up clinico-bacteriologically until they were discharged or until death during hospital. Nosocomial infections were diagnosed based on the criteria suggested by the Centre for Disease Prevention and Control.[10,11] All Pseudomonas aeriginosa strains isolated from different nosocomial infections were confirmed by different biochemical tests such as sugar fermentation tests, IMViC reactions, TSI agar, Mannitol motitity test, urease tests. Antibiogram was perfomed following Clinical Laboratory Standards Institute guidelines The antibiotics used were Amikacin, Gentamycin, PIT, Imipenem, Cefotoxime. A standard strain of Pseudomonas aeriginosa ATCC 27853 is used for positive control. Antibiotic pattern was known by the Kirby-Bauer disc diffusion method[11], Repeat cultures were made weekly for those with positive culture reports, in order to obtain whether there is an additional evidence of new infection till their discharge. The nosocomial infections with a single episode of infection were recognized based on the finding that the similar isolates with the same antibiogram at subsequent cultures. If there were than more than two isolates from a sample ,they were considered for chances of contamination, and the repeat sample was collected for those cases. The antimicrobial sensitivity was tested to the following antibiotics as per the relevance: Gentamycin(G), Ak – Amikacin,CZ-Ceftazidime, IMP- Imipenem, PIT-Piperacillin+Tazobactam, Piperacillin - tazobactam (100/10 µ-gm) Gentamicin (10 µ-gm) Amikacin (30 µ-gm)Ceftazidime (30 µ-gm) ,cefotoxime (30 µ-gm) Imepenem (10 µ-gm).

Kirby- Bauer disk diffusion method

Standard inoculum was inoculated by lawn culture method with the help of sterile cotton swab onto the surface of the Muller – Hinton agar plate & it was allowed to dry for 3-5 minutes.

Antibiotic discs required to be tested were applied to the surface of the agar plate seeded with desired strain i.e., Pseudomonas aeruginosa from nosocomial infections as mentioned above & incubated at 37°c. After 18 hours, the zones of inhibition were measured and the results were recorded by comparing with standard ATCC stains.

Minimum Inhibitory Concentration

Muller Hinton agar with 2% NaCl and inoculums of 10^4cfu/ml o.5 Mc Farland units were used to know MIC. Serial dilutions of antimicrobial agents were prepared by using standard test tubes, 1.5 ml of each dilution was added to 13.5ml of melted Muller Hinton agar suspension & poured into plates. Many strains isolated from hospital acquired infection such as urine, sputum, pus/ burns/ swab inoculated & incubated at 35°c. After 24 hours, the results were recorded. The MICs determining susceptibility thresholds for the different drugs were as follows ≤64 µg/ml for piperacillin, <16 µg/ml for ceftazidime, < 8 µg/ml for imipenem, and < 2 µg/ml for ciprofloxacin etc.[13,14]

Among the nosocomial infections pseudomonas aeruginosa were 72, Klebsiella pneumonia were 50, staphylococcus aureus were 58, Proteus mirabilis & vulgaris were 28,others were found to be 8.(Table1 & figure 1)

Table – 1 Hospital Acquired infections caused by different bacterial species

organism isolated	number
Pseudomonas aeruginosa	72
Klebsiella pneumonia	50
staphylococcus aureus	58
Proteus mirabilis & vulgaris	28
others	08
Total	216

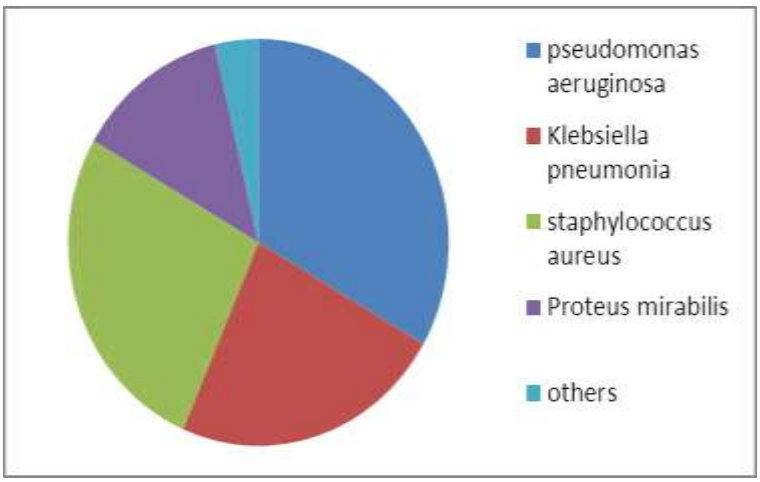

Figure - 1

Among the isolated strains of Pseudomonas aeruginosa 32 strains were from post operative wound infection,8 were from burns wound infection,23 were from urinary trat infection,4 were from bronchial fluid,5 were from Otitis externa.(table 2 and Figure 2)

Table – 2, Pseudomonas isolated aeruginosa from different nosocomial infections

site of infection	number of culture positives for Pseudomonas aeruginosa
PO wound infection	32
Bums	8
UT infection	23
Bronchial fluid	4
Otitis externa	5
Total number of cases	72

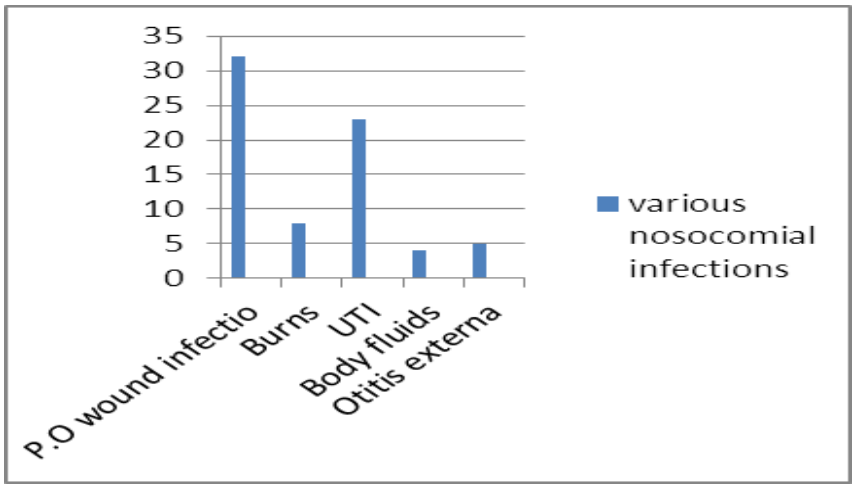

Figure - 2

In present study 42strains of P.aeruginosa were male patient and 30 were female patients.(figure – 3)

Males : Females

42 : 30

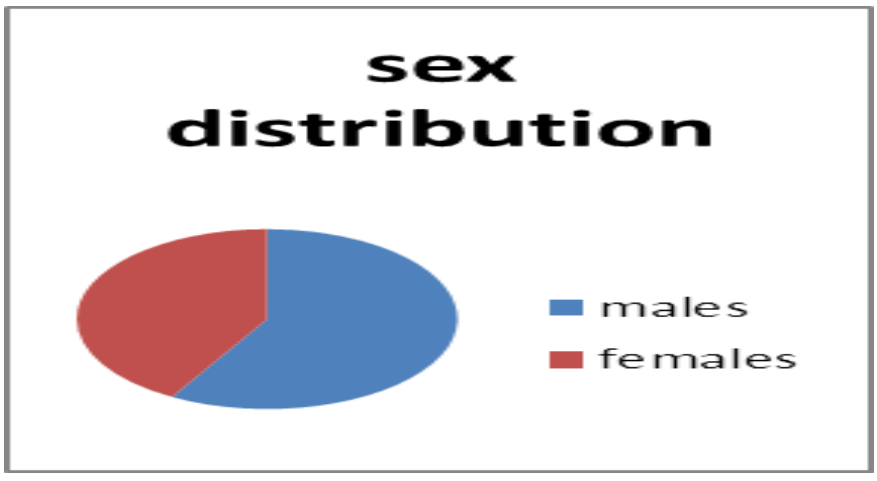

Figure - 3

There were 3 Panresistant strains that were resistant to all the antibiotics including both first line and second line and there were 9 strains that were sensitive to single antibiotic.(Table – 4)

Table - 4

Pan resistant strains – 3 → Resistant to all the antibiotics tested Single Antibiotic sensitive strains - 9 strains

The age incidence of various P.aeruginosa infections were as follows .There were 7 cases between 21 – 30,12 cases were between 31 – 40,22 cases were between 41 – 50, 24 cases were between 51 – 60,7 cases were between 61 – 70.(Table 5 and figure 4)

Table–5: Age distribution of Pseudomonas aeruginosa infections from nosocomial infections.

21 – 30	7
31 – 40	12
41 – 50	22
51– 60	24
61 – 70	7
Total	72

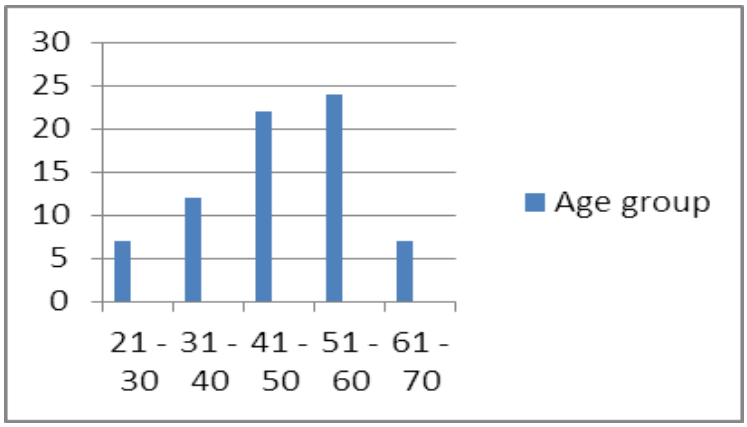

Figure - 4

The antibiotic sensitivity pattern of different strains of P.aeruginosa showed percentage of Amikacin resistance as 41.66%(AK),Gentamycin resistance as 58%(G),Imipenem resistance as 15%(IMP),PIT resistant were 22.22% Ceftazidime resistant were 45.83%(CZ) and Cefotoxime(CA) resistant strains were found to be 59.72%.(Table 6 and figure 5)

Table – 6, Antibiotic sensitivity of Ps.aeruginosa

Antibiotic	Sensitivity	Percentage of resistance R
AK – Amikacin	42	41.66%
G – Gentamicin	30	58 %
IMP – Imipenem	61	15%
PIT – piperacillin + tazobactum	56	22.22%
CZ – Ceftazidime	39	45.83%
CA – Cefotoxime	29	59.72%

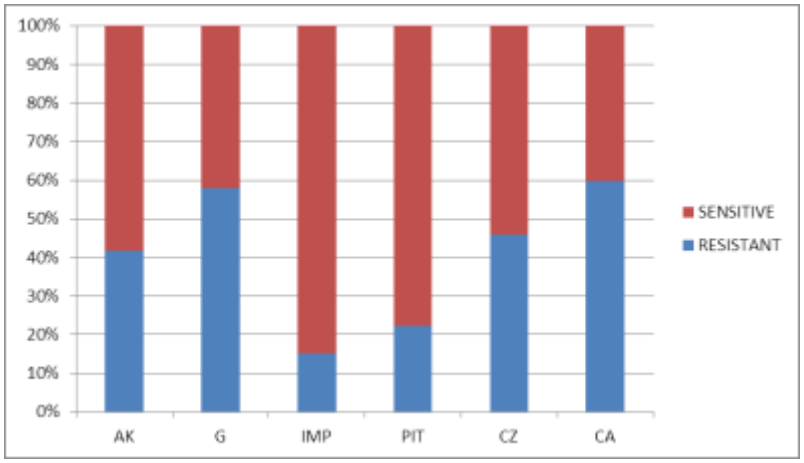

Figure - 5

Brief description PCR method for MBL

PCR was done for *bla* and *vim* genes.Extraction of DNA was done by boiling method.Brief description of procedure followed in PCR. Bacteria were subcultured onto MHA before DNA extraction. IMP-F: 5'-TCCCCACGTATGCATCTGAATTAAC-3', IMP-R: 5'-CGGACTTTGGCCAAGCTTCTATATT-3' with product size of 258 bp and annealing temperature at 61ºC. VIM-F: 5'- GGTCTCATTGTCCGTGATGGTG-3', VIM-R: 5'-GGAATCTCGCTCCCCTCTACCT-3' with product size of 242 bp and annealing temperature at 60ºC) were used as primers.Bacteria were grown on Muller - Hinton agar before DNA extraction.Into 500µl of 1 X Tris – EDTA freshly isolated 1 – 5 bacterial colonies were inoculated and heated at 95°c for 10 minutes and left at room temperature for 5 minutes.Again this was exposed to -20°c for 10 minutes and centrifuged at 14,000 rpm for 10 minutes at 4°c,2µl of supernatant was used as template .50µl of master mixture consisted of 2µl of 50 Mm $MgCl_2$,5µl of 10x reaction buffer,1µl of 2.5 mM dNTPs ,2µl of each 20p mol/µl primer,0.4µL Taq polymerase5U/µL and 35.6µL distilled water.Mater cycler Eppendorff was used for DNA amplification.Step wise initially denaturation for 3 minutes at 94°c for 45 seconds followed by 45 seconds at specific annealing temperature,then at 72°c for 45 seconds.Finally ,the last step ,primer extension was performed for 5 minutes at 72°c and the resultant products were kept at 4°c .Electrophoresis was done by using 1.5µg/Ml ethidium bromide in 1x Tris Borate EDTA buffer. Finally gels were visualized and photographed under UV illumination. (Figure 6)

Figure 6

PCR image for MBL in P.aeruginosa. Image showing strains positive for selected genes (*bla*VIM). Lane 1 is control band.

DISCUSSION

The preeminent role of Pseudomonas aeruginosa and its significant role in mortality and morbidity among various nosocomial infections has lead to many studies on its antibiotic sensitivity pattern.The organism is inheritantly resistant to several commonly used antibiotics by producing several enzymes as well as change in outer membrane permeability, multi – drug efflux mechanism. The imipenem resistance is due to class B metallo β lactamases,Class A clavulanic acid inhibitory enzymes or Class D oxacillinase or diminished expression of certain outer membrane proteins.[15,16,17]

The present study shows Pseudomonas aeruginosa infections are common among males with age 51 – 60.this finding is correlating with several studies.[18,19,20] In present study the maximum isolates were from post operative wound infection(pus sample.(32 i.e.,44.44%).This is correlating with studies such as[20,21] In their study P.aeruginosa was isolated maximum from pus samples. The resistance pattern in our study showed for different strains of P.aeruginosa as maximum percentage of resistance to Cefotoxime(CA) 59.72% next Gentamycin resistance as 58% followed by Ceftazidime resistant were 45.83%(CZ), then Amikacin resistance as 41.66%(AK), PIT resistant were 22.22%) and Imipenem resistance as 15%(IMP). In our study the maximum resistance was to cefotoxime 59.72%. This may be due to its routine use in pre and post operative wound management.Ceftazidime 45.83% was correlating with some of the studies such as Rajat Rakesh et al. (2012)[22] In some of the studies the resistant pattern was found to be maxium i.e., amikacin (70%), ceftazidime (68%), gentamicin 60% and imipenem (58%).[23] In another study it was found to be[24] | amikacin (67.09%), piperacillin(64.55%) carbenicillin

(62%), ceftazidime (59.5%) cephotaxime (53.16%), Gentamycin (31.65%) and Ceftriaxone (31.65%). Which suggests that the susceptibility patterns are highly variable according to geographical area and antibiotic usage.[25]

The prevalence of MBL resistant strains is world wide in distribution 10 – 30%.In present study MBL resistance is found to be 15%..This finding is nearly correlating with studies such as 11.5% in[20] in another study it was found to be 14%.[22] Though comparatively less resistance was detected for imipenem in our study,as this resistance is responsible for significant morbidity and mortality among nosocomial infections their use must be limited to only for treating life threatening infections.[26]

CONCLUSION

Comparing different studies from different geographical areas show that the antibiotic sensitivity is highly variable for P.aeruginosa strains isolated from various nosocomial infections.The increased inadvertent use of antibiotics leads not only to resistant strains but also to increased infections by opportunistic pathogen like P.aeruginosa**.**The detailed study on plasmid profile of resistant genes along with properly organized infection control team would provide us effective planning for limiting further spread of nosocomial infections caused by them.

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