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ISOLATION AND GENETIC ANALYSIS OF MULTI-DRUG RESISTANT PSEUDOMONAS AERUGINOSA FROM UTI PATIENT

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ABSTRACT

Antibiotic resistance is a serious public health problem and the major scientific issues of modern time. Quick to mutate and adapt to counter different antibiotic treatments, Pseudomonas aeruginosa shows an innate ability to develop resistance to antibiotics. The study more than 100 patient's urinary tract infections caused by P. aeruginosa between January and December 2017. All isolates were determined antibiotic sensitivity by disc diffusion method. One isolate has been more resistance according to National Committee for Clinical Laboratory Standards (NCCLS). The isolated resistance P. aeruginosa was identified based on cultural, morphological and genetic (16S rRNA sequences) characteristic.

KEY WORDS

Urinary tract infection, Multi-drug resistant, Pseudomonas aeruginosa.

INTRODUCTION

Infectious diseases have been a main reason of morbidity and mortality throughout our history. With the development of the antibiotic era during the 20th century, there was a growing confidence that the need for infectious disease specialists would all but disappear. The global problem of antibiotic resistance is fast becoming one of the major scientific issues of modern times. The development of new antibiotics is slow and difficult work but bacterial resistance is decreasing our arsenal of existing drugs posing a catastrophic threat as ordinary infections become untreatable. Quick to mutate and adapt to counter different antibiotic treatments, *Pseudomonas aeruginosa* shows an innate ability to develop resistance to antibiotics. Described as 'opportunistic' because it primarily affects humans that are already critically ill, these bacteria can cause serious complications in the treatment of AIDS, cancer or cystic fibrosis patients. While it isn't a massive threat to humanity currently, these bacteria will become an increasing threat over the next few years.

Some of the more problematic drug-resistant pathogens encountered today include methicillin-resistant *Staphylococcus aureus*, multidrug-resistant *Streptococcus pneumoniae*, and vancomycin-resistant *Enterococcus spp*. among the gram-positive bacteria and multidrug-resistant *Acinetobacter baumannii, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* among the gram-negative bacteria. My research focuses specifically on the resistance problems associated with *P. aeruginosa*, with a special emphasis on the complexity by which key chromosomally encoded resistance mechanisms are regulated and coregulated to make *P. aeruginosa* one of our greatest therapeutic challenges. *P. aeruginosa* presents a serious therapeutic challenge for treatment of both community-acquired and nosocomial infections, and selection of the appropriate antibiotic to initiate

therapy is essential to optimizing the clinical outcome (Bisbe *et al.,* 1988). Even more problematic is the development of resistance during the course of therapy, a complication which has been shown to double the length of hospitalization and overall cost of patient care (Dimatatac *et al.,* 2003). Flamm et al. reported rates of multidrug-resistant *P. aeruginosa* ranging from 23 to 26% among 52,000 *P. aeruginosa* isolates collected in the United States from 1999 to 2002 (Flamm *et al.,* 2004). Present study was intended to isolation and genetic analysis of multi-drug resistant *Pseudomonas aeruginosa* from UTI patient.

MATERIALS AND METHODS

Sample Collection

The UTI infected urine samples were collected from various Hospitals at Thanjavur. The collected specimens were stored on specific aseptic container, for further study.

Isolation and Identification of Bacteria

The specimens were inoculated on Hifluoro Pseudomonas Agar Base (M1469, HiMedia, India) and incubated at 35-37°C for 18-24 Hrs (Cappuccino and Sherman, 1999). The test isolate were subjected to morphological, biochemical character (Hi25™ identification kit, Himedia Laboratories Pvt. Ltd., Mumbai, India).

Molecular characterization of antibiotic resistant *Pseudomonas aeruginosa*

The isolated antibiotic resistant *Pseudomonas aeruginosa* strain molecular characterization was analyzed. The 16S rRNA sequences was performed on an ABI 310 automatic DNA sequence (Applied Biosystems) using the primers by 27F and 1492R. The 16S rRNA sequences of isolate has been deposited in GenBank under submission ID is: 2137196. The 16s rRNA sequences analysis was performed using the BLAST (Altschul *et al.,* 1997) at NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) network services. After verifying and adjusting the alignments manually the phylogenetic tree was constructed using the neighbor-joining method and maximum parsimony algorithms contained in the PHYLIP package (Dereeper *et al.,* 2008).

Assay of Antibiotic Sensitivity

The commercially available antibiotic disc such as Amikacin (AK), Amoxyclav (AMC), Ampicillin/Sulbactam (A/S), Cefixime (CFM), Cefoperazone/Sulbactam (75/30mcg) (CFS), Ciprofloxacin (CIP), Cefalexin (Cephalexin) (CX), Cetriaxone (CTX), Cefotaxime (CN), Cefuroxime (CXM), Gentamicin (GEN), Levofloxacin (LE), Meropenem (MRP), Norfloxacin (NX), Ofloxacin (OF), Piperacillin/Tazobactam (100/10mcg) (PIT), Sparfloxacin (SPX), Tigecycline (TGC), Azithromycin (AZM), Nitrofurantoin (NIT), Linezolid (30mcg) (LZ), Roxithromycin (30mcg) (RO) and Co-Trimoxazole (Sulpha/Trimethoprim) (23.75/1.25 mcg) (COT) for bacterial culture. The antibiotic disc were purchased from High media chemical Pvt. Ltd, Mumbai. Antimicrobial activity test was carried out disc diffusion method originally described by Bauer *et al.,* (1996).

RESULTS AND DISCUSSION

The urinary tract infections patient urine specimens more than 100 UTI were collected. The 26 samples are isolated *Pseudomonas aeruginosa* (Fig-1). From the specimen four different bacterial colonies were noted after the incubation. The isolates were tentatively identified as *Escherichia Coli* (50%), *Staphylococcus aureus* (10%), *Klebsiella pneumoniae* (14%) and *Pseudomonas arruginosa* (26 %) on the basis of morphology ad biochemical reactions. Intracellular bacteria have been found in human bladder infection (Rosen *et al.,* 2007). They isolated Gram positive organism such as *Staphylococcus aureus, Streptococcus mitis, Streptococcus faecalis, Bacillus, Corynebacterium sp.* and the Gram-negative organisms such as *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Klebsiella ozaenae, Citrobacter sp. Serratia marcescens* and *Acinetabacter calcoacetius*.

The 26 isolated were analyzed sensitivity by disk diffusion method separately. Among the study all *Pseudomonas aeruginosa* bacterial isolates were more sensitive to Meropenem and Nitrofuration compared the other antibiotics. At the same time one *Pseudomonas aeruginosa* bacterial isolate have more resistance to all used antibiotics (Table – 1). According to Sirot *et al.* (1987), the Cephalosporins showed minimum zone of inhibition against *Klebsiella pneumoniae* and *Staphylococcus epidermidis*. Meropenem and Nitrofuration was the most effective agent tested in these studies of UTI and demonstrated the greatest reduction in mean bacterial numbers. The isolated resistant bacteria strain was characterized results were tabulated in 2. The present study based on partial 16S rRNA sequencing obtained from antibiotic

resistant bacteria strain when subjected to BLAST. The antibiotic resistant bacteria alignment sequencing showed sequence similarities 99% with *Pseudomonas aeruginosa*. The sequence has been deposited at GenBank Bethesda, MD, USA. After the alignment, the tree building option can be activated using Bioedit Software. The tree viewing software njplot is used to generate a cladogram the bacterial isolates as shown in Fig 2. In the light of this study, Antibiotic resistance is a serious public health problem. It can be prevented by minimising unnecessary prescribing and overprescribing of antibiotics, the correct use of prescribed antibiotics, and good hygiene and infection control.

Fig. – 1 Isolation of Bacteria

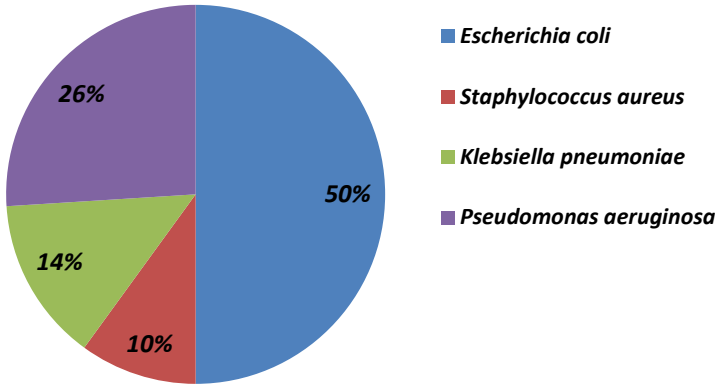


Table – 1 Isolated resistant *Pseudomonas aeruginosa* sensitivity batten

S. No.	Antibiotics	Zone of Inhibition (mm in diameter)	S/I/R
1.	Amikacin (30mcg)	22±1.21	S
2.	Ampicillin/Sulbactam (10/10mcg)	10±0.85	I
3.	Amoxyclav (30mcg)	-	R
4.	Cefixime (5mcg)	-	R
5.	Cefoperazone/Sulbactam (75/30mcg)	-	R
6.	Ciprofloxacin (5mcg)	-	R
7.	Cefalexin (Cephalexin) (30mcg)	-	R
8.	Ceftriaxone (30mcg)	-	R
9.	Cefotaxime (Cephotaxime) (30mcg)	-	R
10	Cefuroxime (30mcg)	-	R
11	Gentamicin (10mcg)	18±1.30	S
12	Levofloxacin (5mcg)	-	R
13	Meropenem (30mcg)		R
14	Norfloxacin (10mcg)	-	R
15	Ofloxacin (5mcg)	-	R
16	Piperacillin/Tazobactam (100/10mcg)	10±1.66	I
17	Sparfloxacin (5mcg)	-	R
18	Tigecycline (15mcg)		R
19	Azithromycin	10±1.34	R
20	Nitrofurantoin		R
21	Roxithromycin (30mcg)	-	R
22	Linezolid (30mcg)	-	R
23	Co-Trimoxazole (Sulpha/Trimethoprim) (23.75/1.25 mcg)	-	R

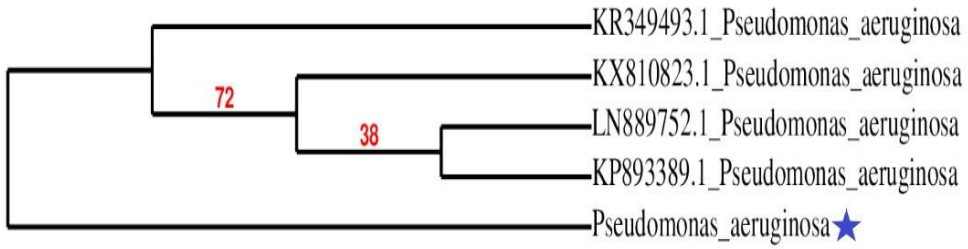
Values are expressed in Mean ± Standard deviation, n=3; S- Sensitive; I-Intermediate; R- Resistant. As per NCCLS

Table – 2 Identification of Isolated resistant *Pseudomonas aeruginosa*

S. No.	Morphological and Biochemical Characterization	Observation
1.	Gram staining	-
2.	Shape	Rod
3.	Motility Test	+
4.	Indole Test	–
5.	Methyl Red Test	-
6.	Voges Proskauer Test	-
7.	Citrate Utilization Test	+
8.	Catalase Test	+
9.	Coagulase Test	+
10.	Oxidase Test	+
11.	Ureas Hydrolysis Test	-
12.	Triple Sugar Iron Test	AG

"+" – Positive, "–" – Negative, "AG"- Acid/Gas "A"-Acid

Fig. – 2 Phylogenetic tree of isolated antibiotic resistant *Pseudomonas aeruginosa* strain IARUTIB1 based on partial 16S r RNA sequence



CONCLUSION

Antibiotic resistance is rising to dangerously high levels in all parts of the world. In the study 26 *Pseudomonas aeruginosa* bacterial strain were isolated from the UTI urine sample. Among the isolates one strain have more resistant as according to NCCLS. The resistant bacteria were characterized by cultural, biochemical and molecular (16S rRNA sequences). In all bacteria, the maximum inhibition was observed in antibiotics Meropenem and Nitrofuration. Other antibiotics should not be used because of low antibacterial activity against all isolated bacteria. The one *Pseudomonas aeruginosa* isolate has more resistance to all used antibiotics.

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REFERENCES

Altschul SF. Madden TL. Schaffer AA. Zhang J. Zhang Z. Miller W. and Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402 (1997).

Bauer AW. Kirby WM. Sherris JC. and Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. **45** (4):493–496 (1966).

Bisbe J. Gatell J. M. and Puig J. *Pseudomonas aeruginosa* bacteremia: univariate and multivariate analyses of factors influencing the prognosis in 133 episodes. Rev. Infect. Dis. 10:629–635 (1988.).

Cappuccino TG. and Sherman N. Microbiology, a laboratory manual. The Benjamin/Cummings publishing, Company Inc., California (1999).

Dereeper A. Guignon V. Blanc G. Audic S. Buffet S. Chevenet F. Dufayard JF. Guindon S. Lefort V. Lescot M. Claverie JM. and Gascuel O. Phylogeny.fr: robust phylogeneticanalysis for the non-specialist. Nucleic Acids Res. 1:36 (2008).

Dimatatac EL. Alejandria M. Montalban MC. Pineda C. Ang, C. and Delino R. Clinical outcomes and costs of care of antibiotic resistant *Pseudomonas aeruginosa* infections. Philipp. J. Microbiol. Infect. Dis. 32:159–167 (2003).

Flamm RK. Weaver MK. Thornsberry C. Jones ME. Karlowsky JA. and Sahm DF. Factors associated with relative rates of antibiotic resistance in *Pseudomonas aeruginosa* isolates tested in clinical laboratories in the United States from 1999 to 2002.

Antimicrob. Agents Chemother. 48:2431–2436 (2004).

Rosen DA. Hooton TM. Stamm WE. Humphrey PA. and Hultgren SJ. Detection of intracellular bacterial communities in human urinary tract infection. *PLoS Med.,* 4(12): e329 (2007).

Sirot D. Sirot J. Labia R. Morand A. and Courvalin P. Transferable resistance to third generation Cephalosporins in clinical isolates of Klebsiella Pneumoniae; Identification of CTX-1 a novel - B-Lactamase. Antimicrob Agents Chemother. 20: 323-324 (1987).

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