European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 7, Issue 11 , 2020

ISOLATION AND IDENTIFICATION OF PSEUDOMONAS AERUGINOSA FROM UROPATHOGENS

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ABSTRACT

Pseudomonas is a group of bacteria found in soil, water, skin flora and most man- made environments throughout the world. In this study, the resistance mechanisms of 16 clinical P. aeruginosa isolates from the Royal Care Hospital, Coimbatore city, Tamilnadu. All the samples were inoculated onto selective mediums for isolation of pseudomonas spp. and for isolation of pseudomonas aeruginosa. Various types of specific media were prepared to cultivate and characterize isolates, such as; Blood agar, Nutrient agar for all isolates. A pseudomonas aeruginosa isolate was selected for further studies taking one pseudomonas aeruginosa from each of the source and were identified by biochemical characteristics. Bacteria obtained from urine samples were cultured and tested for antimicrobial susceptibility to 16 kinds of antibiotics. Urine samples were cultured on different media and incubated, thereafter bacteria were isolated and purified by streaking four times on the same media; isolates were identified depending on morphological, microscopic, and biochemical characteristics. The isolated strains of bacteria were tested for their susceptibility to some antibiotics using disk diffusion method. The antagonistic activity was evaluated by observing a clear zone of inhibition growth. For all the three strains Antibiotic susceptibility testing (AST) was carried out to determine the antibiotic sensitivity and resistant in plate culture conditions. In antibiotic susceptibility testing, Gentamycin and Amoxicillin was sensitive against majority of isolates 80% effective, followed by Levofloxacin and Amikacin 70% and Chloramphenicol 50%, Nitrofurantoin 40% and marked resistance was observed to commonly used drugs like both Ceftriaxone and Amoxicillin 30%, less effectiveness showed by Cefixime 10%. Other Pseudomonad's also showed good sensitivity against antibiotics.

Keywords: Pseudomonas, Cetrimide agar, uropathogens, light microscope.

INTRODUCTION

Pseudomonas aeruginosa is one of the leading Gram-negative organisms associated with nosocomial infections. The increasing frequency of multidrug-resistant P. aeruginosa (MDRPA)

strains affects the efficacious antimicrobial options which are severely limited. Pseudomonas aeruginosa is an opportunistic pathogen. Its infections in hospitals mainly affect the patients in intensive care units and those having catheterization, burn, and/or chronic illnesses (Yetkin et al., 2006). The ability of P. aeruginosa to survive on minimal nutritional requirements and to tolerate a variety of physical conditions has allowed this organism to persist in both com-munity and hospital settings (Lister et al., 2009). Thou, it infects healthy tissues rarely but when defenses are compromised, it can infect different tissues. This explains why most infections are nosocomial (Mesaros et al., 2007). P. aeruginosa characterized by inherent resistances to a wide variety of antimicrobials. Its intrinsic resistance to many antimicrobial agents and its ability to develop multidrug resistance and mutational acquired resistance to antibiotics through chromosomal mutations imposes a serious therapeutic problem (Al-Grawi, 2011). A number of antimicrobial agents, including several Beta–lactams are active against P.aeruginosa. Extended-spectrum Penicillins, often used to treat infections caused by this bacterium.

However, P. aeruginosa adaptive ability causes difficulties for the sensitivity of microbial identification methods and it has become necessary to develop genotype-based characterization systems capable of accurately identifying these bacteria despite any phenotypic modifications. So, molecular identification eliminates the problem of variable phenotype and allows for more accurate identification of bacteria (Drancourt et al., 2000).16S rDNA genes are highly conserved among all organisms and they possess various unique species-specific regions that allow for bacterial identification. Polymerase chain reaction (PCR) is highly sensitive, specific and rapid method which vastly improved the detection of P. aeruginosa especially when using species-specific primer for 16SrDNA (Spilker et al., 2004).

MATERIALS AND METHODS

Sample collection

The study includes both no socomial and community- acquired isolates of P.aeruginosa. A total of 146 UTI (Urinary Tract Infection) cases were reviewed in this study from March 2017 to December 2017. Midstream specimen of urine, preferably of first morning void was collected of known UTI patients.

Urine culturing

Urine samples were cultured on Nutrient agar, blood agar and MacConkey agar medium and incubated over night at 37°C. Significant growth was evaluated as $\geq 10^5$ colony-forming units CFU/mL of midstream urine.

Microbiological features

Polymicrobial or monomicrobial flora, bacteremia by the same strain of P.aeruginosa isolated in the urine culture and resistance pattern to different antibiotics according to CMI criteria of CLSI. Strains with a pattern of antimicrobial multidrug resistance, those of which were insensitive to three or more antipseudomonal antibiotics were considered.

Isolation

Urine samples were cultured on different media including Blood agar, McConkey agar and Nutrient agar and incubated at 37˚C for 24 hours. Thereafter bacteria were isolated and purified by streaking four times on the same media (Spoorenberg et al., 2015).

Characterization and identification

Each of the color, size, elevation, margins and texture of colonies were screened. Pure isolates were examined microscopically, on the base of their cell wall composition and presence of capsule. According to gram staining technique; isolates were cultured on numerous selective and differential media to find out their color, colony morphology and ability of fermentation.

Microscopic examination

The suspected colonies were stained using gram stain method, and their shapes, colors and arrangements were observed under light microscope.

Biochemical tests

All bacteriological isolates were examined and confirmed by biochemical tests according to Baron and Bergey's manual of determinative bacteriology and other references(Difco's Manual,1984).Selected colonies were identified and differentiate according to the culture characteristics; microscopical examination and microbiological analysis were tested biochemically for further confirmation of isolated bacteria, such as; TSI,catalase,oxidase, Indole production test,MR-VP test, Citrate utilization test, motility test and Casein hydrolysis.

Antimicrobial sensitivity testing (Kirby-Bauer method)

The susceptibility of isolates to antibiotics was demonstrated by using nine specific antibiotics, including prescribed antibiotics that have been given by physician. Isolates were inoculated in peptone water and incubated in 37°C, 18-24 h. Next, they were re-cultured in broth and their turbidity compared to 0.5 Mcfarland standard solutions. More ever, new cultures were placed on Mueller-Hinton agar by swabbing. After drying for about 5-10 min, Plates were incubated for about 10-15 min at 37°C. Furthermore, interested antibiotic discs were adjusted on cultured plates using sterile forceps and incubated as inverted for 24 h at 37°C.

The adjusted suspension used within 15 minutes to inoculate the plates by dipping a sterile cotton-wool swab into the suspension and removing the excess liquid by turning the swab against the side of the container above the level of the liquid. The swab was streaked evenly over the entire surface of the plate by swabbing in three directions, rotating the plate through an angle of 60˚ after each application. Finally, the swab was passed round the edge of the agar surface. The plate was allowed to dry with the lid closed before applying discs. The antimicrobial discs were placed on the inoculated plates using a pair of sterile forceps. Plates were incubated at 35˚C - 37˚C for 18 - 20 hrs. After overnight incubation, the diameter of each inhibition zone (including the diameter of the disc) was measured and recorded in mm (Vandepitte et al., 2003). The antibiotics used were (Gentamycin, Amikacin, Chloramphenicol, Cefixime, Amoxicillin, Nitrofurantoin, Levofloxacin) . Statistical package used to analyze the data.

RESULTS

During this study period 146 samples were received, from these samples 69 bacterial isolates were obtained. Out of 69 isolates 16 were identified as Pseudomonas species. Out of this predominant numbers were that of P.aeruginosa 16 (84.25%), followed by other Pseudomonad's 3 (15.75%). Majority of the isolates of P.aeruginosa were isolated from urinary samples. P. aeruginosa was mainly isolated from cultures

From selected urine sample of sixteen patients were collected from Royal Care Hospital, Coimbatore city, Tamilnadu. Microscopically, they were examined and physical properties like pH, color, appearance, age, and sex recorded as a primitive stage of detecting inflammation, due to the presence of RBCs, crystal, epithelial cells, color, reaction and appearance as shown in Table - 1. Samples were cultured and obtained from different species. For this reason, all the colony characteristics were recorded to more recognize isolates and differentiation between them on various types of media (Table-2), because monitoring bacteria on media is an initial achievement to identify bacteria on the basis of colony appearance .

Various types of specific media were prepared to cultivate and characterize isolates, such as; Blood agar, Nutrient agar for all isolates. EMB and MacConky agar for gram negative bacteria, while MSA used as a selective and differential medium of P.aeruginosa.

Then, several biochemical tests were performed for gram negatives, such as Catalase, Citrate utilization test and TSI as the most special tests to identify them. Regarding present or absent of catalase and oxidase enzyme, there were only presence of P.aeroginosa (+) was respectively. Motility test was applied to all isolated bacteria, all gram negatives motile, while no gram positive motile. MR and VP tests were showed the absence of P.aeroginosa and the shape of P.aeroginosa was identified as rod shaped and it was used to differentiate P.aeruginosa from other gram negatives, which indicated their ability to casein hydrolysis was absent (Table-3).

In antibiotic susceptibility testing, Gentamycin and Amoxicillin was sensitive against majority of isolates 80% effective, followed by Levofloxacin and Amikacin 70% and Chloramphenicol 50%, Nitrofurantoin 40% and marked resistance was observed to commonly used drugs like both Ceftriaxone and Amoxicillin 30%, less effectiveness showed by Cefixime 10%. Other Pseudomonad's also showed good sensitivity against antibiotics.The obtained data of this study were represented that most of the people who are infected by UTI (Table-4).

DISCUSSION

Urinary tract infections (UTIs) are serious infections worldwide (Bano et al.,2012). The reasons for the enumerated resistance spread of antibiotics in the hospitals may be traced to the random and illogical use of these antibiotics by the temporary and permanent patients of the hospital (Chukwuani et al., 2002; Pinto-Pereria et al., 2004). The active way to prevent the enumerated resistance of the drug by pathogens is the logical use of antibiotics in addition to being restricted to the specialized physician orders which supplies with the best benefit of antibiotics, in addition to the financial expenditures (Valhovic-Palcevski et al., 2000).

Most of the studies state that there is a resistance of gram negative bacteria especially members of Enterobacteriaceae to antibiotics in their different kinds especially β-lactams

antibiotics (Belongia et al.,2005). This increases the importance of these bacteria and the infections they cause are often available at the hospitals with the patients who are inhibited immunologically. β-lactamases are regarded as one of the important and most common among members of this family for being able to move between the different species through plasmids that carry encoded genes of the enzymes. Moreover, the increased amount of these enzymes in quantity and quality had expanded and complicated the problem (Karlowsky et al., 2003; Orrett, 2004).

We found that E. coli is the main causative organism in urinary tract infections. This result is consistent with most of the previous studies (Hryniewicz, et al., 2001). The percentage of Proteus mirabilis conforms to what (Mohammed, 2010)) had come up. The reason behind spreading of Proteus mirabilis is because it has many pathogenicity factors like cilia that help it in adhesion, flagella that helps it to move, in addition to its outer membrane and capsule (Rozalski et al., 1997).The percentage of Citrobacter spp. exceeds that had come up with which was (3.7%). It also exceeds the percentage that (Al-Douri,2011) had found. They had reported the existence of C. diversus with a percentage of (4.8%) out of 517 samples that were collected from different parts of the body. These samples were expected to be infected in four different countries in Europe, Asia and two Americans.

The percentage of Klebsiella spp. is lower than that had got, and higher than (Mohammed, 2010)had found, his percentage was (3.7%). The results of Pseudomonas aeruginosa are consistent with what had found when he isolated this species from different infections and injuries. This result also conforms to what (Al-Tikrity, 2009)) had come up, he got a percentage of (2.4%). The results regarding Enterobacter aerogenes are close to those had come up with. The reason behind spreading of this species is its opportunistic normal existence in intestine and having many virulence factors like adhesion factors represented by cilia, in which the germs ability to adhere on cell's surfaces is regarded a necessary step to a successful colonization and then causing disease, in addition to having endotoxins represented by lipopolysaccharide that plays a big role to protect germs from phagocytosis (Saladin et al., 2002).

The results of susceptibility test are consistent with (Al-Jebouri and Mudish,2013))who found that bacteria isolates were most resistant to Ampicillin and have different resistance levels to different antibiotics (Chowdhury et al., 2013). Overall, our results indicated significant pathogenic bacteria counts in urine samples. Gentamycin and Amoxicillin was the most effective antibiotic in inhibiting the bacterial growth. These results have important clinical implications. Thus, these antibiotics do best in case of Urinary tract infections.

Table-1: Physical parameters of urine samples

Patient code	Age	Gender	Color	Appearance	pH
1	25	Female	white	Clear	Acidic
2	18	Female	Pale yellow	Turbid	Acidic

3	32	Female	White	Clear	Acidic
4	22	Male	Yellow	Clear	Acidic
5	30	Female	Yellow	Clear	Acidic
6	35	Male	Yellow	Turbid	Acidic
7	32	Female	Pale yellow	Clear	Acidic
8	40	Female	Red	Turbid	Acidic
9	65	Male	Yellow	Turbid	Acidic
10	27	Male	Yellow	Clear	Acidic
11	38	Male	Pale Yellow	Clear	Acidic
12	25	Female	Yellow	Turbid	Acidic
13	27	Female	White	Clear	Acidic
14	48	Male	Yellow	Clear	Acidic
15	21	Female	Yellow	Clear	Acidic
16	23	Female	Yellow	Turbid	Acidic

Table-2: Isolated bacteria on different media

Media	Uropathogen	Colony morphology
MacConkey agar	Pseudomonas aeruginosa	Flat, blue-green diffusible pigment, feathery
Blood agar	Pseudomonas aeruginosa	B-hemolysis Grayishcolonies, oblique lighting
Nutrient agar	Pseudomonas aeruginosa	Large, opaque, produce diffusible pigment

Table-3:Biochemical test for isolated bacteria

Biochemical Tests	P. aeruginosa
Gram stain	-
Shape	Rod
Capsule stain	-
TSI	K/K

Catalase	+
Oxidase	+
Indole production test	-
MR test	-
VP test	-
Citrate utilization test	+
Motility test	+
Casein hydrolysis	-

+ Presence - Absence

Table-4:Antibiotic sensitivity test Kerby-Bauer method

Antibiotic (mcg)	P. aeruginosa	
	Sensitive (S)	Resistance (R)
Levofloxacin (L) (5 mcg/10 mL	S	14
Amoxicillin (AMX) (25)	S	23
Gentamycin (G) (8000 mcg/2 mL)	S	23
Chloramphenicol (30)	S	12
Amikacin (AK) (10)	S	14
Nitrofurantoin (N) (50 mcg/mL)	S	11

Cefixime (CFM) (5)	R	-

REFERENCES

Al-Douri, A.Y.M. (2011) Diagnostic and Molecular Genetic Study of the Bacteria Causing Urinary Tract Infections toPatients in the Tikrit City. Ph.D. Thesis, College of Education, Tikrit University, Tikrit.

AL-Grawi IGA (2011). Expression of mexAB-oprM Operon of Septicemic Pseudomonas aeruginosa in Relation to Antibiotic Resistance [Doctor of Philosophy]. Al-Nahrain University.

Al-Jebouri, M.M. and Mudish, S. (2013) Antibiotic Resistance Pattern of Bacteria Isolated from Patients of Urinary tract Infections in Iraq. Open Journal of Urology, 3, 124-131.

Al-Tikrity, I.A.L. (2009) Bacteriological and Genetical Study of Pseudomonas aeruginosa Isolated from Different human Infections. M.Sc. Thesis, College of Science, Tikrit Universit, Tikrit.

Angoti, G., Goudarzi, H., Hajizadeh, M. and Tabatabaii, Z. (2016) Bacteria Isolated from Urinary Tract Infectionamong Patients and Determination of the Antibiotic Susceptibility Patterns of the Gram Negative Bacteria in Iran. Novelty in Biomedicine, 4, 1-4.

Bano, K., Khan, J., Begum, H., Munir, S., Akbar, N. and Ansari, J.A. (2012) Patterns of Antibiotic Sensitivity of Bacterial Pathogens among Urinary Tract Infections (UTI) Patients in a Pakistani Population. African Journal of Microbiology Research, 6, 414-420.

Belongia, E.A., Knobloch, M.J., Kieke, B.A., Davis, J., Janette, C. and Besser, R. (2005) Impact of a Statewide Programto Promote Appropriate Antimicrobial Drug Use. Emerging Infectious Diseases, 11, 912-920.

Buzayan, M. and Taher, I. (2009) Urinary Tract Infection among HIV-Infected Patients. The Libyan Journal of Infectious Diseases, 3, 19-24.

Chowdhury, F., Ahsan, S. and Kabir, M.D. (2013) Antibiotic Resistance Patterns of Pathogenic Gram Negative Bacteria Isolated from UTI Patients in Sirajganj District. Stamford Journal of Microbiology, 3, 17-20.

Chukwuani, C.M., Onifade, M. and Sumonu, K. (2002) Survey of Drug Practices and Antibiotic Prescribing Pattern at a General Hospital in Nigeria. Pharmacy World and Science, 24, 188-195.

Difco's Manual (1984) Manual of Dehydrated Culture and Reagents for Microbiology Detroit, Michigan. Vandepitte, J., Verhaegen, J., Engbaek, K., Rohner, P., Pitot, P. and Heuck, C.

(2003) Basic Laboratory Procedures in Clinical Bacteriology. 2nd Edition, World Health Organization, Geneva, 30-32.

Drancourt M, Bollet C, Carlioz A, Martelin R, Gayral JP, Raoult D(2000). (16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. J. Clin. Microbiol. 38: 3623-3630.

Gross, R.J. and Rowe, B. (1983) Citrobacter koseri (syn. C. diversus): Biotype, Serogroup and Drug Resistance Patterns of 517 Strains. Journal of Hygiene, 90, 233-239.

Hryniewicz, K., Szcypa, K., Sulikowska, A., Jankowski, K., Betlejewska, K. and Hryniewicz, W. (2001) AntibioticSusceptibility of Bacterial Strains Isolated from Urinary Tract Infections in Poland. Journal of Antimicrobial Chemotherapy,47, 773-780.

Karlowsky, J.A., Jones, M.E., Thornsberry, C., Friedland, I.R. and Sahm, D.F. (2003)Trends in Antimicrobial Susceptibilitiesamong Enterobacteriaceae Isolated from Hospitalized Patients in the United States from 1998 to 2001. AntimicrobialAgents and Chemotherapy, 47, 1672-1680.

Lister PD, Wolter DJ, Hanson ND (2009). Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin. Microbiol. Rev. 22:582-610.

Mesaros N, Nordmann P, Plesiat P, Roussel-DelvallezEL M, Vaneldere J, Glupczynski Y, Vanlaethem Y, Jacobs F, Lebecque P, Malfroot A, Tulkens PM, Vanbambeke F(2007). Pseudomonas aeruginosa resistance and therapeutic options at the turn of the new millennium. Clin. Microbiol. Infect. 13:560-78.

Mirzarazi, M., Rezatofighi, S.E., Pourmahdi, M. and Mohajeri, M.R. (2013) Antibiotic Resistance of Isolated GramNegative Bacteria from Urinary Tract Infections (UTIs) in Isfahan. Jundishapur Journal of Microbiology, 6, e6883.

Mohammed, S.A. (2010) Determination the Antibiotic Resistance of the Some Isolated Bacteria from Tikrit Teaching Hospital Infections and Their Relationship with Plasmids. M.Sc. Thesis, College of Science, University of Tikrit, Tikrit.

Orrett, F.A. (2004) Antimicrobial Susceptibility Survey of Pseudomonas aeruginosa Strains Isolated from Clinical Sources. Journal of the National Medical Association, 96, 1065-1069.

Pinto-Pereria, L.M., Phillips, M., Ramlal, H., Teemul, K. and Prabhaker, S. (2004) Third Generation of Cephalosporin Use in a Tertiary Hospital in Part of Spain, Trindad: Need for Antibiotic Policy. BMC Infectious Diseases, 4, 59.

Sabir, S., Ahmad, A.A., Ijaz, T., Asad, A.M., ur Rehman, K.M. and Nawaz, M. (2014) Isolation and Antibiotic Susceptibilityof E. coli from Urinary Tract Infections in a Tertiary Care Hospital. Pakistan Journal of Medical Sciences, 30,389-392.

Saladin, M., Van Thi Bao, C., Lambert, T., Donay, J.L., Herrmann, J.L., Ould-Hocine, Z., Verdet, C., Delisle, F., Phillippon,A. and Arlet, G. (2002) Diversity of CTX-M β-Lactamases and Their Promoter Regions from EnterobacteriaceaeIsolated in Three Persian Hospitals. FEMS Microbiology Letters, 209, 161-168.

Spilker T, Coenye T, Vandamme P, LiPuma JJ(2004). PCR-based assay for differentiation of Pseudomonas aeruginosa from other Pseudomonas species recovered from cystic fibrosis patients. J. Clin. Microbiol. 42: 2074–2079.

Spoorenberg, V., Geerlings, S.E., Geskus, R.B., de Reijke, T.M., Prins, J.M. and Hulscher, M.E.J.L. (2015) Appropriate Antibiotic Use for Patients with Complicated urinary Tract Infections in 38 Dutch Hospital Departments: A Retrospective Study of Variation and Determinants. BMC Infectious Diseases, **15**, 505.

Rozalski, A., Sidorczyk, Z. and Kotelko, K. (1997) Potential Virulence Factor of Proteus bacilli. American Society forMicrobiology, 61, 65-89.

Valhovic-Palcevski, V., Morovic, M. and Palcevski, G. (2000) Antibiotic Utilization at the University Hospital after Introducing an Antibiotic Policy. European Journal of Clinical Pharmacology, 56, 97-101.

Yetkin G, Otlu B, Cicek A, Kuzucu C, Durmaz R (2006). Clinical, microbiologic, and epidemiologic characteristics of Pseudomonas aeruginosa infections in a university hospital, Malatya, Turkey. Am. J. Infect. Control 34: 188-192.