ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 07, Issue, 10, pp.16428-16431, October, 2017

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

MICROBIOLOGICAL CHARACTERIZATION AND ANTIBIOTIC SENSITIVITY PATTERN OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM CLINICAL SAMPLES

***,[1]Sukesh, K. and [2]Prakash Vincent, S. G.**

[1]Department of Microbiology, Malankara Catholic College, Kaliakkavilai, Tamilnadu-629 153
[2]Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam, Tamil Nadu 629 502

ARTICLE INFO

Article History:
Received 08th July, 2017
Received in revised form
28th August, 2017
Accepted 14th September, 2017
Published online 30th October, 2017

Keywords:

Pseudomonas aeruginosa, Characterization, Antimicrobials, Clinical samples, Antibiotic sensitivity pattern, Beta-lactam antibiotics.

ABSTRACT

Pseudomonas aeruginosa is intrinsically resistant to several antimicrobials and rapidly develop resistance to other drugs during treatment and make it difficult and ineffective. In this study *Pseudomonas aeruginosa* was isolated and clinically characterized from clinical samples and its antibiotic sensitivity pattern towards commonly prescribed Beta-lactum antibiotic disc. Analysis by Gram staining and culturingon nutrient agar, blood agar and MacConkey agar plates revealed that among the 154 sputum samples, only eight were with *Pseudomonas aeruginosa* whereas 11 out of 462 urine samples and 13 of 100 pus samples were positive to *Pseudomonas aeruginosa*. The isolates were identified as *Pseudomonas aeruginosa,* by clinically standard confirmative protocols. The isolates are motile, Gram negative bacilli, citrate, catalase and oxidase positive, indole and urease negative, triple sugar iron test, Mannitol non-fermentative and motile. Antibiotic sensitivity pattern of the characterized isolates were by Kirby-Bauer disc diffusion method against beta-lactam antibiotic discs revealed that all the *Pseudomonas aeruginosa* isolates are resistant to antibiotics other than gentamicin. Thus the present study indicates the prevalence of multi drug resistant *Pseudomonas aeruginosa* strains, among clinically ill cases and implies the necessity of an immediate solution or alternative to overcome any possible *Pseudomonas aeruginosa* out breaks.

****Corresponding author***

Citation: Sukesh, K. and Prakash Vincent, S. G. 2017. "Microbiological characterization and antibiotic sensitivity pattern of *Pseudomonas aeruginosa* isolated from clinical samples", *International Journal of Development Research,* 7, (10), 16428-16431.

INTRODUCTION

Microbial infections are the principal health problems causing serious illness and death in the developing countries (Black *et al.,* 1982). *Pseudomonas aeruginosa* accounts for almost 80% of opportunistic infections and 50% death in patients hospitalized with cancer, cystic fibrosis and burns (Cragg and Newman, 2001). Major infections caused by *Pseudomonas* sp*ecies* include endocarditis, pneumonia and infections of the urinary tract, central nervous system, wounds, eyes, ears, skin and musculo-skeletal system (Levin and Bonten, 2004). It is occasionally pathogenic for plants as well as animals too (Pollack, 2000). The high incidence of *Pseudomonas aeruginosa* in healthcare institutions is due to poor health status of the patients, the high carriage rate of often multi-drug resistant strains in hospital wards, and prior use of broad spectrum antibiotics (Otter *et al.,* 2011). Once chronic infection is established, *Pseudomonas aeruginosa* is virtually impossible to eradicate and is associated with increased mortality and morbidity in patients (Levin and Bonten, 2004). Biofilm formation, quorum sensing and secretion of many proteases imparts strength to resisting mechanical forces and penetration of toxic chemicals like antibiotics, and host defense molecules (Bjarnsholt *et al.,* 2010; Lieleg *et al.,* 2011; Waite *et al.,* 2006). Epidemiological studies specify that antibiotic resistance is increasing in clinical isolates of *Pseudomonas aeruginosa and* more than 70% of pathogenic bacteria were estimated to be resistant to at least one of the currently available antibiotics (Koneman, 2006; Katz *et al.,* 2006). The so-called superbugs, the organisms that are resistant to most of the clinically used antibiotics are emerging at a rapid rate (Balaban and Dell, 2005). Considering the severity of *Pseudomonas aeruginosa* infections, a major problem to be resolved for the healthy human survival, the current topic was selected.

MATERIALS AND METHODS

The clinical samples such as sputum (deep cough sputum: 154 samples); pus (infected and surgical wound site swabs: 100 samples) and urine (mid-stream urine: 462 samples) were collected through the microbiology laboratories of SK Hospital, Edapazhanji, NIMS Medicity, Aaralumoodu, Neyyatinkara, and Dr. Somervell Memorial CSI Medical College, Karakkonam of Thiruvananthapuram District, Kerala, India. The collected samples were analysed for the isolation of *Pseudomonas aeruginosa*, by direct Gram staining and culturing it on Nutrient Agar, Blood Agar and MacConkey Agar plates.

Biochemical characterization *Pseudomonas aeruginosa*

Suspected *Pseudomonas aeruginosa* colonies were identified according to standard microbiological methods (Koneman *et al.,* 2006) such as Indole, Citrate, Triple sugar iron (TSI), Catalase, Oxidase and physiologically by Urea hydrolysis and Mannitol motility.

Antibiotic sensitivity pattern of *Pseudomonas aeruginosa*

Overnight broth cultures of *Pseudomonas aeruginosa* were swabbed (horizontally, vertically, clock-wise and anti-clock-wise directions) on Muller Hintonagar plates. The plates were kept undisturbed for 20 minutes in upright position.Antibiotic discs such as Piperacillin, Piperacilin tazobactum, Imipenem, Gentamicin, Amikacin, Ciprofloxacin, Levofloxacin, Ampicillin, Penicillin, Cefotaxime, Ceftazidime, and Ceftazidime clavulanic acid (Himedia, India) were placed aseptically on inoculatedagar surface using disc dispenser. The plates were incubated at 37.00C for 24 hours andobserved for the zone of growth inhibition (Bauer *et al.,* 1966). The diameter of zone ofgrowth inhibition was measured using a millimeter ruler from the underside of the plate and the results were recorded.

RESULTS

Of the 154 sputum samples analysed, only eight (5.2%) samples showed the presence of *Pseudomonas aeruginosa*. Among the 8 positive samples 6 (75%) were from male patients and the remaining 2 (25%) from female. The age-wise prevalence of clinical isolates showedthat most of the patients (25%) were aged between 31 to 45 years. Among the 462 urine samples analysed only 11 (2.4%) samples were positive to *Pseudomonas aeruginosa.* Of the 11 positive samples 7 (63.6%) were form females and 4 (36.4%) from male patients. In case of age-wise frequency 72.72% fall in the 7 to27 years. In case of the 100 pus samples analysed 13 (13%) were positive and 87 (87%)showed negative response. Within these 13 positive samples 9 (69.2%) were from males and the remaining 4 (30.8%) from females. Regarding the age wise occurrence 9 (69.2%) were ranging between 41 to 77 years. The positive response wasre ported based on the presence of Gram negative bacilli upon Grams staining of the samples, formation of bluish green diffusible pigmented colonies on nutrient agar, beta hemolytic colonies on blood agar and non-lactose fermenting colonies on Mac-Conkey agar plates**.** Among the different samples analysed maximum number of *Pseudomonas aeruginosa* was obtained from pus samples 40.6% followed by urine samples 39.4% and 25% from sputum.

Table 1. Characterization properties of *Pseudomonas aeruginosa*

S.No.	Tests	Characters
1	Gram staining	Gram negative bacilli
2	Motility test	Motile
3	Indole production test	Negative
4	Citrate utilization test	Positive
5	Triple sugar iron (TSI) test	Alkali/alkali with no gas and hydrogen sulphide production
6	Catalase test	Positive
7	Oxidase test	Positive
8	Urea hydrolysis test	Negative
9	Mannitol motility test	Mannitol non-fermentative and motile

Upon incubation for 24 hours on nutrient agar plates *Pseudomonas aeruginosa* colonies were characterized by one to two mm circular, smooth, low convex, opaquecolonies with diffusible blue-green pigment. On blood agar plates colonies exhibited oneto two mm circular, smooth, low convex, opaque colonies with beta-haemolysis whereason Mac-Conkey agar the colonies produced two to three mm circular, smooth, lowconvex, non-lactose fermenting colourless colonies. Characterization of the selected *Pseudomonas aeruginosa* isolates was made through standard protocols adopted in clinical laboratories such as macroscopic, microscopic, biochemical and physiological properties followed by its comparison with Bergy's manual of determinative bacteriology (Table1). Microscopic analysis revealed that they are gram negative rod shaped cells with motility. Biochemically these isolates were negative towards indole and positive towards citrate utilization, catalase and oxidase production. In case of triple sugar iron test allisolates showed alkali/alkali stab and slant with no gas and Hydrogen Sulphide production. Physiologically the isolates were negative to urease test and were mannitolnon-fermentative and motile. In order to evaluate the antibiotic sensitivity and resistance pattern of the clinicalisolates of *Pseudomonas aeruginosa* towards commonly prescribed beta-lactamantibiotics, antibiotic sensitivity study by Kirby- Bauer disc diffusion method was carried-out. All the clinically isolated and characterized *Pseudomonas aeruginosa* and *Pseudomonas aeruginosa* ATCC27853 strain (control) exhibited resistance to all the antibiotic discs, evidenced by the absence of growth inhibitory zone around them except the antibiotic disc gentamicin, in which sensitivity was noticed with a zone of growth inhibition, measured 15mm in diameter.

DISCUSSION

Present study dealt with the isolation and characterization of *Pseudomonas aeruginosa* from clinical samples and evaluation of its antibiotic sensitivity pattern. As part of it a sum of 716(154 sputum, 462 urine and 100 pus) clinical samples were tested. Outof 716 samples, 32samples were with *Pseudomonas aeruginosa* which was evidenced by direct Gramstaining of the samples followed by Nutrient agar, Blood agar and MacConkey agarplating. (Sara, 2013) isolated and selected 92 clinical *Pseudomonas aeruginosa* strains from pediatric patients and detected that it varies widely. Of the eight *Pseudomonas aeruginosa* positive sputum samples 6(75%) were from male patients and the remaining 2 (25%) female. The age-wise prevalence of clinical isolates showed that most of the patients (25%) were aged between31 to 45 years. Among the 11 *Pseudomonas aeruginosa* positive urine samples 7 (63.6%) were females and 4 (36.4%) males. In case of age-wise frequency 72.72% fall in the 7-27years. In case of

13 *Pseudomonas aeruginosa* positive pus samples 9 (69.2%) were from males and the remaining 4 (30.8%) females. Regarding the age wise occurrence 9 (69.2%) was ranging 41 to 77. The results obtained are very similar to the previous studies in this field. The low number of *Pseudomonas aeruginosa* positive to that of the more number of samples analysed may be due to the health awareness or concerns of the population, thus they may approach the physician for any discomfort. (Rakesh, 2012) a total of 630 samples collected from hospital surgical wards, paediatric ward, medical ward, orthopaedic and gynecology and obstetrics ward and Intensive Care Units were tested, in which 321 samples showed growth of bacteria. Out of 321 samples, 100 clinical isolates of *Pseudomonas aeruginosa* were isolated. Out of 100 clinical isolates of *Pseudomonas aeruginosa*, maximumisolates (71%) are isolated from pus/swab followed by16% from urine, 12% from sputum and 3% from other samples. Out of 100, 61% are from males and 39% are females. Mostof the patients were aged between 31 to 45 years. (Ahmed, 2013) analysed samples from287 inpatients and 283 clinical isolates of *Pseudomonas aeruginosa* was recovered. *Pseudomonas aeruginosa* was mostly isolated from burn unit (32.3%), ICU (16.7%) the nurology department (18.75%). *Pseudomonas aeruginosa* accounted for 19 % (54 of 283)of nosocomial infections.

From the positive samples prominent isolates of *Pseudomonas aeruginosa* were selected based on Gram staining and colony characters for further studies. The selected isolates were further characterized by standard protocols adopted in clinical laboratories and tested for their antibiotic sensitivity. In the present study the isolation rate of *Pseudomon asaeruginosa* was comparable with other studies. While comparing with the data of (Gales*et al.,*2002) the result of this study correlated directly with the presence of *Pseudomon asaeruginosa* as one of the main etiological agents of infectious diseases. *Pseudomon asaeruginosa* remains as an important pathogen and responsible for multiplicity ofinfections very particularly listed as cause of nosocomial infections. Their predominancein health care facilities results from its resistance properties towards common antibioticsand antiseptics. Being an extremely adaptable organism, it can survive and proliferateeven in minimum nutrients even in conditions adverse to organisms of same category. Upon 24 hours of growth on nutrient agar plates *Pseudomonas aeruginosa* colonies were characterized by one to two mm circular, smooth, low convex, opaquecolonies with diffusible blue-green pigment. On blood agar plates colonies exhibited oneto two mm circular, smooth, low convex, opaque colonies with beta haemolysis whereas on MacConkey agar the colonies produced two to three mm circular, smooth, lowconvex, non-lactose – colourless colonies.(Rakesh *et al.,* 2012) characterized bacterial isolates on the basis of their growth on routine MacConkey medium which showed lactose non-fermenting pale colonies which were oxidase test positive and on nutrientagar pigmented and non-pigmented colonies with oxidase positive as *Pseudomon asaeruginosa.* In the current study characterization of the selected *Pseudomonas aeruginosa* isolates was made through macroscopic, microscopic, biochemical and physiological characters on comparison with Bergy's manual of determinative bacteriology, second edition. (Sara, 2013) characterized 92 clinical *Pseudomonas aeruginosa* strains isolated from pediatric patients with the *Vitek system* and conventional biochemical tests. In biochemical characterization, production of pigment was observed in 79 of 81 isolates, most of them (82.7%) displayed characteristic green colour. All isolates were hemolytic and most of them demonstrated beta-haemolysis (91.3%).The results of carbohydrate utilization were the same for both the OF and ammonium saltsugar media, although the time needed to obtain positive results was not always the same. Most isolates were positive for galactose (99%), and only a small number metabolized rhamnose (1%). The results of these tests provided 10 distinct biotypes. Isolates that usedurea, galactose, mannose and mannitol that displayed a green pigment and betahaemolysis belonged to the more frequent biotype (63% of the cases).

The antibiotic susceptibility pattern of clinical isolates of *Pseudomon asaeruginosa* towards commonly prescribed beta-lactam antibiotics revealed that not only the clinical isolates but the ATCC strain27835 also resistant to all the antibiotics exceptgentamicin. (Sara, 2013) isolated *Pseudomonas aeruginosa* from various clinical samples. Maximum isolates of *Pseudomonas aeruginosa* isolated from various samples are resistant to to bramycin (68%) followed by gentamicin (63%), piperacillin (50%), ciprofloxacin 49%) and ceftazidime (43%). It is evident from the study that now adays *Pseudomonas aeruginosa* is becoming less sensitive to cephalosporins, aminoglycos idesand beta-lactamase inhibitors. (Hammami *et al.,* 2011); (Vitkauskiene *et al.,* 2011) described the presence of clinical strains of *Pseudomonas aeruginosa* resistant to carbapenems in various hospital intensive care units. (Ranjbar *et al.,* 2011) studied a sum of seventy clinical samples with positive culture results for *Pseudomonas aeruginosa* forits antimicrobial susceptibility test according to the standard CLSI (2009) guidelines. The relationship between the strains was also determined using antimicrobial drug resistancepattern analysis and plasmid profiling. All strains were multi-drug resistant. The percentage of resistance to tested antibiotics was: imipenem 97.5%, amikacin 90%,piperacillin 87.5%, ceftizoxime 72.7%, gentamicin 67.5%, ciprofloxacin 65%, ceftriaxone 60%, and ceftazidime 57.5%. The study showed an increased rate ofresistance for some antibiotics tested among *Pseudomonas aeruginosa* strains isolated. (Piyatip *et al.,* 2012) isolated two-hundred and sixty-one clinical isolates of MDR *Pseudomonas aeruginosa*from eight tertiary hospitals across Thailand. Antibiotic resistance rates of MDR *Pseudomonas aeruginosa* revealed ceftazidime with highest resistance rate of about 95.79%, followed by ciprofloxacin and gentamicin which were 92.34% and 87.36%resistance respectively. The carbapenem resistance rate among MDR *Pseudomon asaeruginosa* isolate was the highest for meropenem (about 65.52%) and the resistancerates for imipenem and doripenem were about 44.44% and 36.02%, respectively. (Ahmed*et al.,* 2013) evaluated the resistance patterns of 57 *Pseudomonas aeruginosa* strains (54clinical and three environmental isolates). Amikacin was the most effective drug againstall *Pseudomonas aeruginosa* isolates showed maximum sensitivity (80.5%) followed byimipenem (66.7%) and gentamicin (56.1%). Allisolates were totally resistant to Carbenicillin and tetracycline. Although many strains aresusceptible to gentamicin, to bramycin and amikacin, resistant forms have also developed. It is desirable that the antibiotic susceptibility pattern of bacterial pathogens like *Pseudomonas aeruginosa* in specialized clinical units to be continuously monitored and the results readily made available to clinicians so as to minimize the resistance. The solution can be planned by continuous efforts of microbiologist, clinician, pharmacist and community to promote greater understanding of this problem. Frequent hand washing to prevent spread of

organism should be encouraged. Better surgical and medical care should be provided to patients during hospital stay.

Conclusion

By means of the results obtained, it was concluded that clinical isolates of *Pseudomonas aeruginosa* developed resistance towards commonly prescribed beta-lactum antibiotics against them. Since *Pseudomonas aeruginosa* causes serious infections and multi-drug resistance, several studies should be carried out to detect its antibiotic susceptibility pattern for the various drugs available. Such study helps clinicians for the better management of patients and researchers to device novel drugs to its control.

Acknowledgement

The authors express their gratitude to the Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalamand the Malankara catholic college, Management for providing research facilities.

REFERENCES

Ahmed, B.M., Wafaa, A.Z., Ghada, R.H., Aza, Z.L. and Rasha, G. 2013. Prevalence of Multidrug Resistant *Pseudomonas aeruginosa* in Patients with Nosocomial Infections at a University Hospital in Egypt, with Special Reference to Typing Methods, *J. Virol. Microbiol*., 1-13.

Balaban, N. and Dell' Acqua, G. 2005. Barriers on the road to new antibiotics. *Scientist*, 19: 42–43

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

Bjarnsholt, T., Tolker-Nielsen, T., Hoiby, N. and Givskov, M. 2010. Interference of *Pseudomonas aeruginosa* signalling and biofilm formation for infection control. *Expert Rev. Mol. Med*., 12: Pp: 11.

Black, R.E., Brown, K.D., Becker, S. and Yuns, M. 1982. Longitudinal studies of infectious diseases and physical growth of children in rural Bangladesh. Am. J. Epidemiol., 115: 305 -314.

Cragg, G.M. and Newman, D. J. 2001. Medicinals for the millennia. The historical record. *Ann. NY. Acad. Sci*., 953a: 3–25.

Gales, A.C., Sader, H.S. and Jones, R.N. 2002. SENTRY Participants Group (Latin America). Urinary tract infection trends in Latin American hospitals: report from the SENTRY antimicrobial surveillance program. Diagn. *Microbiol. Infect. Dis*., 44:289-299.

Hammami, S., Boutiba-Ben, Boubaker, I., Ghozzi, R., Saidani, M., Amine, S. and Ben Redjeb, S. 2011. Nosocomial outbreak of imipenem resistant *Pseudomonas aeruginosa* producing VIM-2 metallo- β-lactamase in a kidney transplantation unit. *Dia. Pathol*., 6:106.

Katz, M.L., Mueller, L.V., Polyakov, M. and Weinstock, S.F. 2006. Where have all the antibiotic patents gone? *Nat. Biotechnol*., 24: 1529–1531

Koneman, E., Winn, W. J., Allen, S., Janda, W., Procop, G., Woods, G. and Schreckenberger, P. 2006. 'Koneman's Color Atlas and Textbook of Diagnostic Microbiology,' *Sixth Edn*., 7: 264-269.

Koneman, Koneman's 2006. Color Atlas and textbook of diagnostic Microbiology. The Non fermantative Gram-Negative Bacilli, 6: 303-391.

Levin, B.R. and Bonten, M. J. M. 2004. Cycling antibiotics may not be good for your health. *Proc. Natl. Acad. Sci. USA*., 101: 13101–13102.

Lieleg, O., Caldara, M., Baumgartel, R. and Ribbeck, K. 2011. Mechanical robustness of *Pseudomonas aeruginosa* biofilms. *Soft Matter*, 7: 3307–3314.

Otter, J.A., Yezli, S. and French, G.L. 2011. The role played by contaminated surfaces in the transmission of nosocomial pathogens. Infect. *Control Hosp. Epidemiol*., 32: 687–699.

Piyatip, K., Preecha, M., Piroon, M., Visanu, T., Mullika, T. and Chomnawang, I. 2012. Prevalence and genotypic relatedness of carbapenem resistance among multi drug resistant *P. aeruginosa* in tertiary hospitals across Thailand., *Ann. Clin. Microbiol.andAntimicrob*., 11: 25 1-7

Pollack, M. 2000. *Pseudomonas aeruginosa*; Principles and practice of infectious diseases. In: Mandell, G.L., Bennett, J.E. and Dolin R., Eds. 2:2310–2335.

Rakesh, R.M., Ninama, G.L., Mistry, K., Parmar, R., Patel, K. and Vegad., M.M. 2012. Antibiotic resistance pattern in *Pseudomonas aeruginosa* species isolated at a tertiary care Hospital, Ahmadabad. *Nat. J. Med. Res*., 2(2): 156-159.

Ranjbar, R., Parviz, O., Horie, S., Sadegh, M., Nematollah, J., Morteza, I., Shohreh, F. and Mohammad, A. 2011. Characterization of *Pseudomonas aeruginosa* Strains Isolated from Burned Patients Hospitalized in a Major Burn Center in Tehran, Iran; *Acta. Medica.Iranica*., 49(10): 675-679.

Sara, A., Ochoa, Fernanda L.-M., Gerardo, E., Ariadnna C.C., Leticia, B. D., Briseida, L.M., Yolanda, J.T., Silvia, G., Carlos, E., Rigoberto, Juan, H.C. and Xi, C. 2013. Pathogenic characteristics of *Pseudomonas aeruginosa* strains resistant to carbapenems associated with biofilm formation. *Bol. Med. Hosp. Infant. Mex*., 70(2):133-144.

Vitkauskiene, A., Skrodeniene, E., Dambrauskiene, A., Baksyte, G., Macas, A. and Sakalauskas, R. 2011. Characteristics of carbapenem resistant *Pseudomonas aeruginosa* strains in patients with ventilator-associated pneumonia in intensive care units. Medicina (Kaunas), 47: 652-656.

Waite, R.D., Paccanaro, A, Papakonstantinopoulou, A. Hust, J.M., Saqi, M. Littler, E. and Curtis, M.A. 2006. Clustering of *Pseudomonas aeruginosa* transcriptomes from planktonic cultures, developing and mature biofilms reveals distinct expression profiles. *BMC Genomics*, 7: 162-171.

****