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Research Article

Multidrug-resistant *Pseudomonas aeruginosa* Isolation and Identification from Clinical Samples

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ABSTRACT

Pseudomonas aeruginosa has become a prominent human opportunistic infection as it continues to develop drug resistance. Additionally, it was one of the most common and lethal pathogens that caused ventilator-associated pneumonia in patients receiving incubation, with a 38% direct attributable fatality rate. Conventional cystic fibrosis antimicrobial medications are insufficient to eradicate *P. aeruginosa* infections due to the rising prevalence of multidrug-resistant infections. Consequently, several approaches to tackle *P. aeruginosa* have drawn a lot of interest. Therefore, *P. aeruginosa* isolates from clinical samples were characterized using conventional techniques in the present investigation. By evaluating their antibiogram pattern, 15 multidrug-resistant bacteria were selected from a total of 52 isolated isolates. Quorum sensing (QS) is a cell-to-cell communication mechanism that *P. aeruginosa* uses to regulate the expression of many pathogenic genes. The increased transcriptional regulator LasR, which is amplified by quorum sensing, is seen as a potential substitute target when *P. aeruginosa* develops resistance to antibiotics. In order to investigate the occurrence of MDR strains of *P. aeruginosa*, the current investigation was conducted.

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Introduction

Twelve percent of urinary tract infections acquired in hospitals, ten percent of bloodstream infections, and eight percent of surgical site infections are caused by the ubiquitous human bacterium *Pseudomonas aeruginosa* [1]. Incubated individuals, it's also one of the most common and fatal illnesses; 38% of deaths are directly due to the illness [2]. Individuals suffering from cystic fibrosis are more susceptible to *Pseudomonas aeruginosa* infections, which lead to a significant illness and death rate in this group. *Pseudomonas aeruginosa*'s ability to control a number of its virulence traits via a mechanism that monitors cell density and permits bacterial communication is largely responsible for its ability to infect a potential host [3].

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The prevalence of multidrug-resistant (MDR) *Pseudomonas aeruginosa* infections is rising, and *P. aeruginosa* infections cannot be completely eradicated by standard antibiotic therapy for cystic fibrosis. Consequently, there is a great deal of focus on various approaches to attack *P. aeruginosa* [4]. Treatments that specifically target and inhibit QS in *P. aeruginosa* may lessen the pathogenicity of the bacteria and help the body's immune system eradicate the illness. Two QS networks, LasR and RhlR, are thought to provide attractive targets for future therapies [5]. The Las system is composed of the proteins LasI synthase and LasR transcriptional regulator. The production of LasI is necessary for the AHL signal molecule N-(3-oxododecanoyl)-L-homoserine lactone (3O-C12-HSL) [6]. For LasR to become a functional transcription factor, 3O-C12-HSL is necessary. According to current study, when 3O-C12-HSL is present, LasR forms multimers. Only this protein's multimeric form can bind DNA and regulate the transcription of many genes. *Pseudomonas aeruginosa*'s RhlI and RhlR proteins combine to generate a second QS system [7-8].

RhlI synthase is responsible for producing N-butyryl-L-homoserine lactone (C4-HSL), whereas RhlR functions as the transcriptional regulator. RhlR only controls the expression of many genes in combination with C4-HSL. It has been discovered that 3O-C12-HSL and C4-HSL both easily leave bacterial cells, however 3O-C12-HSL diffusion is slower than C4-HSL diffusion. Apart from LasR and RhlR, there is no equivalent acyl-HSL synthase gene for QscR, an orphan LasR-RhlR homolog [9–10]. A mutation of qscR is highly contagious. It has been studied how QscR affects the expression of a few genes controlled by the LasR-I and RhlR-I systems. Premature activation of genes in a qscR mutant is seen in the phenazine synthesis operons *phz1* and *phz2*, *hcnAB*, the hydrogen cyanide synthesis operon, *lasB*, which codes for elastase, *rhlI*, and *lasI*. The mechanism via which QscR inhibits these genes temporarily is not known. At low acyl-HSL concentrations, QscR may form heterodimers with LasR and RhlR. This might lead to the inactivation of LasR and RhlR [11–12].

Public health concerns have escalated due to the emergence and dissemination of germs that are resistant to drugs, creating an uneasy situation. Treating MDR strains seems to have serious limitations given the present treatment choices; thus, it is necessary to investigate new targets and create new antimicrobials [13–17]. It is well known that a variety of gram-negative bacterial pathogens communicate with one another by minuscule chemical signal molecules, which modify the expression of many genes and synchronize virulence components that cause illness in the host [18]. A new and innovative potential therapeutic target for preventing the virulence system and the expression of many genes that improve pathogen-host interactions is quorum sensing, a chemical communication mechanism among bacteria. Another possibility is that QscR binds to acyl-HSL signals, which postpones the expression of genes controlled by LasR and RhlR [19]. In order to better understand QscR's role in *P. aeruginosa* gene regulation, we focused on it. In light of the aforementioned, we postulate that QscR may directly affect certain genes in response to the 3OC12-HSL signal generated by LasI. In this study, *P. aeruginosa* was isolated from clinical samples, and an antibiogram pattern was used to screen for multidrug-resistant bacteria.

RESOURCES AND TECHNIQUES

Sampling and processing Medical laboratories provided samples, which were then brought to the lab [20]. The nutrient agar plates were streaked with the obtained samples and incubated for a full day at 37 degrees Celsius. The purported single colonies were identified by Gram's staining and then subcultured on Mac Conkey agar [21–23]. For later usage, *P. aeruginosa* isolates were moved to a 1% nutritional agar slant and stored in a refrigerator at 4°C.

Identification using biochemistry and morphology

The subcultured isolates in the selective and differential media were identified using Bergey's handbook of determinative bacteriology [24], which included morphological characterisation using techniques such as Gram staining, motility, catalase, and oxidase. To verify the identification, biochemical tests were performed, such as the fermentation tests for glucose, lactose, maltose, sucrose, and mannitol, the Indole production test, the Triple

Sugar Iron agar test, the methyl red test, the Voges-Proskauer test, the citrate utilization test, the urease test, and the nitrate reduction test [25].

Sensitivity to antibiotics

P. aeruginosa isolates are subjected to antibiotic sensitivity testing (AST) to determine their resistance to several potential therapy medications. The experiment was carried out using the Kirby-Bauer test technique, often known as disc diffusion [26]. Commercially available antibiotic discs (Himedia) were used to evaluate the antibiotic sensitivity pattern. After selecting and injecting around 4–5 bacterial colonies into the Nutrient broth, the mixture was incubated for 2–5 hours at 35°C and adjusted to the 0.5 McFarland turbidity standard, yielding a total count of 1×10^8 CFU/mL. Muller-Hinton agar was produced, poured into sterile Petridishes, and allowed to harden while the apparatus was sterilized. After being dried in an incubator for thirty minutes to remove excess moisture from the surface, the plates were swabbed and subsequently infected.

The disc diffusion method was used to assess each isolate's susceptibility to several antimicrobial medications, including Ampicillin (10µg), Chloramphenicol (30µg), Streptomycin (10µg), Tetracycline (30µg), Cefuroxime (30µg), Ceftriaxone (30µg), Ofloxacin (10µg), and Ciprofloxacin (10µg). The antibiotic discs were carefully placed on the plates under sterile conditions, let to stand for 30 minutes (the pre-diffusion time), and then incubated for 24 hours at 37°C. Without opening the lid, the diameter of the inhibitory zones was measured at the conclusion of the incubation period using a zone measuring ruler (Hi-Media) [27].

Identifying strains resistant to several drugs

The multidrug-resistant *Pseudomonas aeruginosa* was identified by measuring the zone of inhibition in accordance with the CLSI standard chart for Enterobacteriaceae [28]. It was shown that isolates of MDR *Pseudomonas aeruginosa* were resistant to three or more medications [29–30].

END RESULTS AND TALK

An significant opportunistic human infection that affects those with cystic fibrosis and impaired immune systems is *pseudomonas aeruginosa*. The rise in *Pseudomonas aeruginosa* strains that are resistant to drugs is responsible for both the notable worsening of the illness and its treatment, which is likely fatal [31]. Thus, to manage *P. aeruginosa* infection in the public health sector, a prevalence investigation and the suppression of *P. aeruginosa*'s multidrug resistance by blocking the QS enhanced transcriptional regulator are required.

Gathering, analyzing, and identifying *Pseudomonas aeruginosa* samples

From medical labs, 189 samples in total were gathered. Based on the MacConkey Agar enrichment medium's growth properties, 116 samples (61.3%) showed signs of turbid growth. Of the 116 samples, 52 (45.21%) were determined to be *Pseudomonas aeruginosa* based on growth characteristics and biochemical assays (Table 1).

19.2% of *P. aeruginosa* from 445 clinical samples from Minia, Egypt, were reported by Gad et al. [32] in 2007. Shenoy et al. [33] released data on 495 *P. aeruginosa* isolates and 1548 clinical samples from Mangalore. At a tertiary care hospital in Andhra Pradesh, 290 *P. aeruginosa* were identified in 2012 by Ramana et al. [34] from illnesses linked to healthcare. According to research by Swetha et al. [35], 39 *P. aeruginosa* isolates were found in human samples taken in Uttar Pradesh, India. Bangalore has produced a large number of *P. aeruginosa* isolates, according to Rakesh et al. [36]. In New Delhi, India, Wattal et al.'s research [37] revealed *P. aeruginosa* in 85 patients.

***Pseudomonas aeruginosa*'s antibiotic susceptibility and multidrug resistance were determined.**

All 52 *Pseudomonas aeruginosa* isolates underwent antimicrobial susceptibility testing using eight different classes of antibiotics: ampicillin (10µg), cefuroxime (30µg), ceftriaxone (30µg), ciprofloxacin (10µg), ampicillin (30µg), streptomycin (10µg), Tetracycline (30µg), and cefuroxime (30µg). The isolate was classified as

resistant, intermediate, or susceptible to each antibiotic based on its zone of inhibition against it, as per the NCCL standard chart for Enterobacteriaceae. The isolate was deemed resistant when the zone of inhibition diameter was less than 13 mm for ampicillin, less than 12 mm for chloramphenicol, less than 11 mm for streptomycin, less than 11 mm for Tetracycline, less than 14 mm for cefuroxime, less than 13 mm for ceftriaxone, less than 12 mm for ofloxacin, and less than 15 mm for ciprofloxacin (Table 2).

Table 1 Biochemical tests for the identification of *Pseudomonas aeruginosa*

Name of the test	Observations
Gram's staining	G-ve rod
Motility test	Positive
Catalase test	Positive
Oxidase test	Positive
Glucose fermentation	Negative
Sucrose fermentation	Negative
Lactose fermentation	Negative
Maltose fermentation	Negative
Mannitol fermentation	Positive
Indole production test	Negative
Methyl red test	Negative
Voges-Proskauer test	Negative
Citrate utilization test	Positive
Urea hydrolysis test	Negative
Nitrate reduction test	Positive

Table 2 NCCL Guidelines to classify *Pseudomonas aeruginosa* as resistant/ intermediate / sensitive to antibiotics

Type	Antibiotics	Zone of inhibition in mm		
		Resistant	Intermediate	Sensitive
Pencillin	Ampicillin	≤ 13	14 – 16	≥ 17
Phenicols	Chlorempenicol	≤ 12	13 – 17	≥ 18
Aminoglucoisides	Streptomycin	≤ 11	12 – 14	≥ 15
Tetracyclins	Tetracycline	≤ 11	12 – 14	≥ 15
CEPHEMS	Cefuroxime	≤ 14	15 – 22	≥ 23
	Ceftriaxone	≤ 13	14 – 20	≥ 21
Fluoroquinolones	Ofloxacin	≤ 12	13 – 15	≥ 16
	Ciprofloxacin	≤ 15	16 – 20	≥ 21

Isolates that shown resistance to every antibiotic were noted in the current investigation. There was less resistance to cefuroxime (3.15%) and more resistance to ampicillin (87.3%). Table 3, Figure 1 lists the number of isolates that were susceptible, intermediate, and resistant to each drug. Multidrug-resistant strains were defined as isolates that showed resistance to three or more drugs. Of these MDR isolates, 100% of them showed resistance to ampicillin (Fig 2).

Greater worry for public health arises from the advent of drug-resistant isolates. The fact that 15 isolates in this investigation were found to be multidrug resistant suggests that *Pseudomonas aeruginosa* is present in large quantities. Over the last several decades, there has been an increase of multidrug-resistant bacterial species due to the extensive use of antibiotics. Most of the identified multidrug-resistant (MDR) isolates in this investigation showed resistance to tetracycline, ampicillin, and chloramphenicol. As a result, the isolates' pattern of antibiotic sensitivity was examined, and the MDR strain of *Pseudomonas aeruginosa* was found for more research.

Table 3 Antibiogram pattern of 15 MDR *P. aeruginosa* isolates against eight antibiotics, with zone of inhibition in mm

Isolate	Antibiotics - Zone of inhibition (mm)								No. of resistance
	Ampicillin (13)	Chloramphenicol (12)	Streptomycin (11)	Tetracycline (11)	Cefuroxime (14)	Ceftriaxone (13)	Ofloxacin (12)	Ciprofloxacin (15)	
Pae 1	10 (R)	10 (R)	12 (I)	10 (R)	25 (S)	14 (I)	14 (I)	13 (R)	4
Pae 3	11 (R)	11 (R)	11 (R)	14 (I)	14 (R)	20 (I)	19 (S)	24 (S)	4
Pae 4	10 (R)	10 (R)	16 (S)	10 (R)	12 (R)	22 (S)	18 (S)	22 (S)	4
Pae 5	10 (R)	11 (R)	17 (S)	11 (R)	14 (R)	13 (R)	19 (S)	24 (S)	5
Pae 7	10 (R)	12 (R)	15 (S)	11 (R)	20 (I)	30 (S)	18 (S)	26 (S)	3
Pae 11	12 (R)	12 (R)	18 (S)	14 (I)	13 (R)	18 (I)	19 (S)	25 (S)	3
Pae 13	11 (R)	12 (R)	18 (S)	11 (R)	15 (I)	25 (S)	16 (S)	21 (S)	3
Pae 19	12 (R)	10 (R)	14 (I)	11 (R)	19 (I)	22 (S)	21 (S)	20 (I)	3
Pae 22	10 (R)	11 (R)	12 (I)	14 (I)	14 (R)	22 (S)	17 (S)	24 (S)	3
Pae 27	11 (R)	15 (I)	11 (R)	13 (I)	14 (R)	23 (S)	10 (R)	15 (R)	5
Pae 28	11 (R)	22 (S)	19 (S)	11 (R)	21 (I)	22 (S)	12 (R)	15 (R)	4
Pae 34	10 (R)	12 (R)	18 (S)	15 (S)	13 (R)	12 (R)	21 (S)	24 (S)	4
Pae 36	11 (R)	11 (R)	18 (S)	11 (R)	15 (I)	14 (I)	14(I)	21 (S)	3
Pae 42	10 (R)	12 (R)	18 (S)	11 (R)	13 (R)	23 (S)	16 (S)	29 (S)	4
Pae 46	11 (R)	11 (R)	13 (I)	11 (R)	13 (R)	17 (I)	15(I)	27 (S)	4

R: Resistance; I: Intermediate; S: Sensitive

FINAL VERDICT

Pseudomonas aeruginosa has become an important human opportunistic infection as it continues to develop drug resistance. Using growth characteristics, biochemical tests, and pus samples collected in the Salem area, *Pseudomonas aeruginosa* isolates were discovered. The MDR strain of *P. aeruginosa* was identified after the antibiotic sensitivity patterns of these isolates were examined. 52 strains of *P. aeruginosa* are isolated from samples and characterised using standard morphological, physiological, and biochemical methods in order to study *P. aeruginosa* surveillance. Fifteen *P. aeruginosa* MDR strains were identified using the antibiogram pattern. Most of the strains exhibited resistance to tetracycline, ampicillin, and chloramphenicol.

References

1. Taylor RF, Gaya H, Hodson ME. 1993. *Pseudomonas cepacia*: Pulmonary infection in patients with cystic fibrosis. *Respir. Med.* 87: 187-192.
2. Whiteley M, Lee KM, Greenberg EP. 1999. Identification of genes controlled by quorum sensing in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Science* 96: 13904-13909.
3. Chugani SA, Whiteley M, Lee KM, D'Argenio, Manoil C, Greenberg EP. 2001. QscR, a modulator of quorum sensing signal synthesis and virulence in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Science* 98: 2752-2757.
4. Pesci EC, Pearson JP, Seed PC, Iglewski BH. 1997. Regulation of *las* and *rhl* quorum sensing in *Pseudomonas aeruginosa*. *Journal of Bacteriology* 179: 3127-3132.
5. Rakesh KC, Chandrashekar MR, Nagesha CN. 1994. Multidrug resistant *P. aeruginosa* in Bangalore, South India. *Indian Jr. Med. Sciences* 48: 85-88.
6. Hassett DJ, Ma JF, Elkins JG, McDermott TR, Ochsner UA, West SE, Huang CT, Fredericks J, Burnett S, Stewart PS. 1999. Quorum sensing in *Pseudomonas aeruginosa* controls expression of catalase and superoxide dismutase genes and mediates biofilm susceptibility to hydrogen peroxide. *Mol. Microbiology* 34: 1082-1093.
7. Latifi A, Fogliano M, Tanaka K, Williams P, Lazdunski A. 1996. A hierarchical quorum-sensing cascade in *Pseudomonas aeruginosa* links the transcriptional activators LasR and RhIR (VsmR) to expression of the stationary-phase sigma factor RpoS. *Mol. Microbiology* 21: 1137-1146.
8. Holden MTG, Chhabra SR, deNys R. 1999. Quorum-sensing cross talk: isolation and chemical characterization of cyclic dipeptides from *Pseudomonas aeruginosa* and other Gram-negative bacteria. *Molecular Microbiology* 33: 1254-1266.
9. Pesci EC, Milbank JBJ, Pearson JP. 1999. Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Science.* 96: 11229-11234.

10. Baburam S, Ramasamy S, Shanmugam G, Mathanmohun M. 2022. Quorum sensing inhibitory potential and molecular docking studies of *Phyllanthus emblica* phytochemicals against *Pseudomonas aeruginosa*. *Applied Biochemistry and Biotechnology* 194: 434-444.
11. Boca Raton FL, Dong YH, Xu JL, Li XZ, Zhang LH. 2000. An enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. *Proc. Natl. Acad. Science* 97: 3526-3531.
12. Pesci EC, Milbank JB, Pearson JP, McKnight S, Kende AS, Greenberg EP, Iglewski BH. 1999. Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Science* 96: 11229-11234.
13. Ansari S, Bari A, Ullah R, Mathanmohun M, Veeraraghavan VP, Sun Z. 2019. Gold nanoparticles synthesized with *Smilax glabra* rhizome modulates the anti-obesity parameters in high-fat diet and streptozotocin induced obese diabetes rat model. *Journal of Photochemistry and Photobiology B: Biology* 201: 111643. <https://doi.org/10.1016/j.jphotobiol.2019.111643>.
14. Vakayil R, Muruganantham S, Kabeerdass N, Rajendran M, Ramasamy S, Alahmadi TA, Almoallim HS, Manikandan V, Mathanmohun M. 2021. Acorus calamus-zinc oxide nanoparticle coated cotton fabrics shows antimicrobial and cytotoxic activities against skin cancer cells. *Process Biochemistry* 111: 1-8. <https://doi.org/10.1016/j.procbio.2021.08.024>.
15. Maghimaa M, Alharbi SA. 2020. Green synthesis of silver nanoparticles from *Curcuma longa* L. and coating on the cotton fabrics for antimicrobial applications and wound healing activity. *Journal of Photochemistry and Photobiology B: Biology* 204: 111806. <https://doi.org/10.1016/j.jphotobiol.2020.111806>.
16. Abirami K, Karthikeyan M, Maghimaa M. 2021. Phytochemical screening and antibacterial potential of *Piper nigrum* seed extract against the bacterial isolates. *Research Journal of Agricultural Sciences* 12(6): 2029-2033.
17. Vakayil R, Krishnamoorthy S, Anbazhagan M, Kumar NS, Mathanmohun M. 2021. Antibacterial Potential of Acorus Calamus Extracts Against the Multi-Drug Resistant Nosocomial Pathogens. *Uttar Pradesh Journal of Zoology* 24: 144-150.
18. Pearson JP, Pesci EC, and Iglewski BH. 1997. Roles of *Pseudomonas aeruginosa las* and *rhl* quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. *Journal of Bacteriology* 179: 5756-5767.
19. Shenoy S, Baliga S, Saldanha DR, Prashanth HV. 2002. Antibiotic sensitivity patterns of *Pseudomonas aeruginosa* strains isolated from various clinical specimens. *Indian Jr. Med. Sciences* 56: 427-430.
20. Kabeerdass N, Krishnamoorthy S, Anbazhagan M, Srinivasan R, Nachimuthu S, Rajendran M, Mathanmohun M. 2021. Screening, detection and antimicrobial susceptibility of multi-drug resistant pathogens from the clinical specimens. *Materials Today: Proceedings*. 47: 461-467. <https://doi.org/10.1016/j.matpr.2021.05.018>
21. Vakayil R, Kabeerdass N, Kuppusamy A, Mathanmohun M. 2019. Phytochemical screening and antibacterial properties of *Punica granatum* extracts against gastrointestinal infection an in-vitro study. *Uttar Pradesh Journal of Zoology* 26: 25-32.
22. Abirami K, Maghimaa M. 2019. Phytochemical screening and bioactivity of *Zingiber officinale* to combat the multidrug-resistant bacterial pathogens using foldscope. *Uttar Pradesh Journal of Zoology* 40(2): 67-74.
23. Vakayil R, Abdul Nazeer T, Mathanmohun M. 2021. Evaluation of the antimicrobial activity of extracts from *Acorus calamus* rhizome against multidrug-resistant nosocomial pathogens. *Research Journal of Agricultural Sciences* 12(5): 1613-1617.
24. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. 1994. Bergey's manual of determinative bacteriology. Ninth Edn. Lippincott Williams & Wilkins. pp 242.
25. Vakayil R, Kabeerdass N, Srinivasan R, Shanmugam G, Ramasamy S, Mathanmohun M. 2021. Invitro and in silico studies on antibacterial potentials of phytochemical extracts. *Materials Today: Proceedings* 47: 453-460. <https://doi.org/10.1016/j.matpr.2021.05.017>
26. Bauer AW, Kirby MDK, Sherris JC, Turck M. 1966. Antibiotic susceptibility testing by standardized single disc diffusion method. *Am. Jr. Clin. Pathology* 45: 493-496.
27. Vakayil, R, Krishnamoorthy S, Gnanendra S, Senthil Kumar N, Ramasamy S, Mathanmohun M. 2020. Screening and identification of multi-drug resistance nosocomial infection, isolates from clinical specimen: A cross-sectional study. *Plant Archives* 20(2): 7247-7251.
28. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; Seventeenth Informational Supplement. *CLSI document M100-S17*. ISBN 1-56238-625-5.
29. Vakayil R, Anbazhagan M, Shanmugam G, Ramasamy S, Mathanmohun M. 2021. Molecular docking and in vitro analysis of phytoextracts from *B. serrata* for antibacterial activities. *Bioinformation* 17(7): 667-672. [doi:10.6026/97320630017667](https://doi.org/10.6026/97320630017667)

30. Kabeerdass N, Al Otaibi A, Rajendran M, Manikandan A, Kashmery HA, Rahman MM, Madhu P, Khan A, Asiri AM, Mathanmohun M. 2021. *Bacillus*-mediated silver nanoparticle synthesis and its antagonistic activity against bacterial and fungal pathogens. *Antibiotics* 10(11): 1334. <https://doi.org/10.3390/antibiotics10111334>.
31. Showalter RE, Martin MO, Silverman MR. 1990. Cloning and nucleotide sequence of *luxR*, a regulatory gene controlling bioluminescence in *Vibrio harveyi*. *Jr. Bacteriology* 172: 2946-2954.
32. Gad GF, El-Domany RA, Zaki S, Ashour HM. 2007. Characterization of *Pseudomonas aeruginosa* isolated from clinical and environmental samples in Minia, Egypt: prevalence, antibiogram and resistance mechanisms. *Jr. Antimicrob. Chemother* 60(5): 1010-1017.
33. Shenoy S, Baliga S, Saldanha DR, Prashanth HV. 2002. Antibiotic sensitivity patterns of *Pseudomonas aeruginosa* strains isolated from various clinical specimens. *Indian Jr. Med. Sciences* 56: 427-430.
34. Ramana BV, Chaudhury A. 2012. Antibiotic resistance pattern of *Pseudomonas aeruginosa* isolated from healthcare associated infections at a tertiary care hospital. *Jr. Sci. Soc.* 39: 78-80.
35. Swetha, Rajesh Kumar A, Tiwari SC, Singh H. 2011. Antibiotic resistance pattern among the *P. aeruginosa* isolated from human, and animal in India. *Trop. Anim. Health Prod*, DOI 10.1007/s 11250-011-9953-7.
36. Rakesh KC, Chandrashekar MR, Nagesha CN. 1994. Multidrug resistant *P. aeruginosa* in Bangalore, South India. *Indian Jr. Med. Sciences* 48: 85-88.
37. Wattal C, Kaul V, Chugh TD, Kler N, Bhandari SK. 1995. An outbreak of multidrug resistant *P. aeruginosa* in Delhi (India). *The Indian Journal of Medical Research* 100: 266-267.