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Detection of *Pseudomonas aeruginosa* infection from the water bottles of immune-compromised mice by conventional and PCR based microbiology

Shinde A.D and Ingle A.D

Laboratory Animal Facility, ACTREC, Tata Memorial Centre
Kharghar, Navi Mumbai- 410210

Corresponding author:
Dr. Arvind Ingle,
Officer-in-Charge, Laboratory Animal Facility, ACTREC, Tata Memorial Centre,
Kharghar, Navi Mumbai- 410210.
Phone: +91 022-27405047, Fax: +91 022-27405116, Email: aingle@actrec.gov.in

Abstract

Microbiological control of laboratory animals is important in any animal facility for production of microbiologically clean animals. It is possible through accurate diagnosis of the rodent pathogens in the laboratory. *Pseudomonas aeruginosa* is a ubiquitous and free living organism which infects the gastrointestinal tract through water. Normal animals do not show clinical signs in infection but immuno-compromised/ irradiated mice show varied symptoms of the disease. In this study, *Pseudomonas aeruginosa* was isolated from drinking water provided to the laboratory animals. The *P. aeruginosa* grown on the Nutrient and MacConkey agar plates were subjected for identification by using colony characters, odor and biochemical tests. DNA was isolated and PCR was carried out by using positive DNA as positive control. PCR product of 726 bp pairs was developed on 2% agarose gel for confirmation of the organisms. PCR method along with conventional method may offer sensitive and rapid detection of *P. aeruginosa* in water as well as clinical specimens.

Key words: *Pseudomonas aeruginosa,* PCR, mice

Introduction

Demand for microbiologically free laboratory animals is ever growing. Accurate and economical diagnosis of pathogenic bacteria is a key for the production of microbiologically free laboratory animals. *Pseudomonas aeruginosa* is a gram-negative, non-spore forming, motile, aerobic bacterium belonging to the family Pseudomonadaceae and responsible for gastric diseases in laboratory animals (CRL technical sheet, 2009). It is found in moist, warm environments and can often be isolated from soil, water, sewage, and occasionally from human skin. *P. aeruginosa* is transmitted to the laboratory animals via contact with contaminated water, feed, bedding, and infected rodents and humans (Baker, 1998). It was isolated in the nasopharynx and the lower digestive tract of laboratory mice and is considered to be an opportunistic agent in immune-deficient, immuno-suppressed or stressed mice (Mahabir *et al.*, 2009; Jeong*et al.,* 2011) and humans as well (Meynard *et al*., 1999). Such mice are septicemic, show a hunched posture, rough hair coat, emaciation and torticollis (head tilting and circling) due to infection and inflammation of the inner or middle ear. *P. aeruginosa* infection in mice and rats could affect a variety of research projects, depending upon the organ systems affected and was found to be a major gastro enteric bacterium in mouse and rat facilities (Urano *et al.*, 1995; Baker, 1998). The bacterium is resistant to mechanical cleansing and flushing, to disinfectants and to antibiotics. In a mouse facility, the normal route of infection is orally via drinking water. The treatment of the bacterial diseases is also undertaken through drinking water (Homberger *et al*., 1993). Such treatments not only get rid of the water source but also take care of the gastrointestinal tract. The standard method for detecting *P. aeruginosa* is by isolation and identification on agar media in microbiology laboratory by conventional method. However, a Polymerase Chain Reaction (PCR) method is a choice for confirming the diagnosis of infection. In any case, routine program of checking the rodent pathogens is the only way to produce microbiologically clean laboratory animals which yield reproducible results (Ingle and Shinde, 2014). In this paper, we report the diagnosis of *Pseudomonas aeruginosa* from the water bottles of immune-compromised mice using conventional agar media, biochemical tests as well as PCR method.

Materials and methods

Conventional microbiological detection of *P. aeruginosa* :

Nude and SCID mice were bred and maintained in IVC system at ACTREC, Laboratory Animal Facility under strict conditions as per CPCSEA guidelines. The filtered and autoclaved drinking water, sterile feed were provided. Water samples from the water bottle of these animals were collected for microbiological monitoring at an interval of 3, 5 and 7 days from offering water to these animals.

Direct water samples as well as swabs were collected from water bottles from animal cages and cultured on Nutrient agar (HiMedia) and MacConkey agar (HiMedia) and incubated at 37°C for 24 hours. *P. aeruginosa* colonies were diagnosed by growth appearance, colony characteristics from their green color pigmentation and odor and by performing biochemical tests.

PCR detection of *P. aeruginosa*

1. DNA isolation:

DNA was isolated from the confirmed *P. aeroginosa* organisms by using DNA Sure Mini Kit (Nucleo-pore Genetix Brand). Isolated colonies were collected in to sterile centrifuge tube (15 ml capacity) containing 5 ml 0.9 % sterile normal saline (NS) and centrifuged at 4000 rpm for 5 minutes at 4°C. The sediment was placed in a micro centrifuge tube, washed thrice with NS and processed with the steps as per the instructions of the manufacturers for extraction of the DNA.

2. DNA quantification and PCR:

The isolated DNA was quantified by using Nanodrop (ND-1000 v 3.5.2) machine. The final concentration was adjusted for PCR purpose at 100 ng/ul. The primers were selected with length 23 and 20 bases respectively for forward and reverse, PA f: 5`-TAT TTC AAG GAT GAT GGC TCC AC-3', r: 5'-GCG TTG GTT GTC CAA GTT TA-3' (Jeong *et al*., 2011). The PCR was performed with 1 µl DNA in a final reaction volume of 15 µl with final concentrations as 1X PCR buffer (MBI Fermentas), 3 mM $MgCl_2$ (MBI Fermentas), 0.4 uM deoxynucleoside triphosphate mix (10 mM each, MBI Fermentas), 10uM mix of each single primer at 0.4 µM, 0.5U of Taq DNA Polymerase (5U/µl, MBI Fermentas) and desired quantity of dH_2O. Using the PA F and PA R primers, the PCR started with an initial denaturation step at 94°C for 5 min followed by 35 cycles each of 94°C (20 sec), 72°C (30 sec) and 72°C (20 sec). The last cycle was followed by a 3-min extension period at 72°C. Ten ul of the PCR product was mixed with 2 µl loading buffer (MBI Fermentas), electrophoresed on a 2.0% agarose gel (SRL), stained with ethidium bromide, visualized under UV light and photographed. The PCR was expected to yield a productsize of 726bp.

Results

For conventional microbiology, the isolated colonies were subjected for identification by using colony characters like size, shape, consistency and finally confirmed by using biochemical tests. Same colonies were sent to the Microbiology Department, Tata Memorial Hospital, ACTREC for double confirmation. Both the reports showed growth of *P. aeruginosa* colonies on the Nutrient agar (Fig-1).

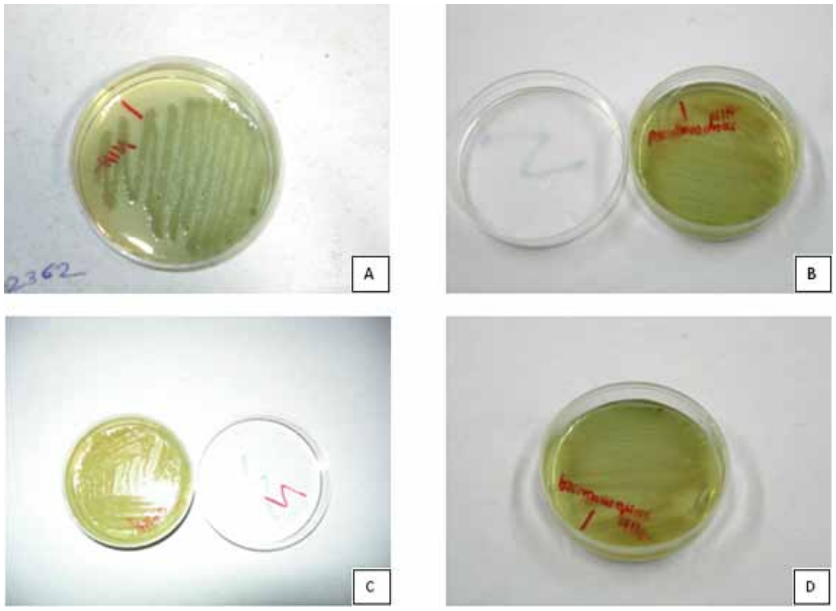

Fig-1: Green pigmented colonies on Nutrient Agar plates.

Fig-2: Biochemical test results

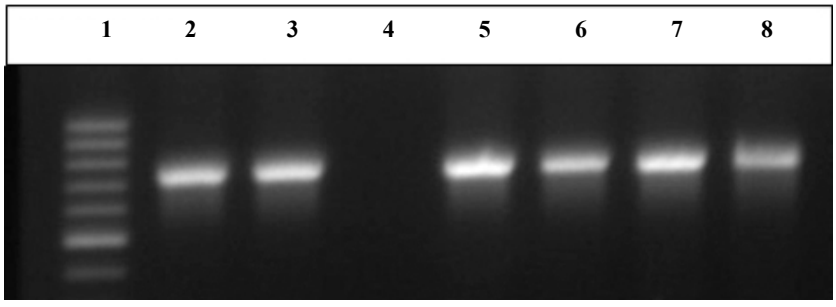

Fig-3: PCR panel showing positive control (lane 2 and 3), negative control (lane 4) and positive test samples (lane 5-8).

The colonies grown on the Nutrient agar were subjected to the biochemical tests. Results showed typical biochemical tests positive for *P. aeruginosa* (Fig-2).

To confirm again by using molecular biology technique, the samples were subjected for DNA extraction. PCR was performed on the extracted DNA using primers PA F and PA R which yielded amplified PCR product of 726 bp for *P. aeruginosa* (Fig-3).

Discussion

P. aeruginosa found abundant in soil and water and is an opportunistic organism. Although it is sporadic cause of natural infection, *P. aeruginosa* poses a mortality rate of up to 85%. *P. aeruginosa* has been reported for tissue invasion leading to septicemia, wasting disease and death (Dietrich *et al.,* 1995). Presumptive diagnosis of *P. aeruginosa* is based on clinical signs in the immune-compromised/irradiated mice but definitive diagnosis can be made on culture and isolation of the organisms from the animals with septicemia. The cultures of *P. aeruginosa* have a characteristic fruity odour but pigment production is not a characteristic phenotype (Holcombe & Schauer, 2007; Mahabir*et al.*, 2009). The aim of the present study was to demonstrate rapid, reproducible and reliable technique for the detection and identification of *P. aeruginosa* in clinical specimens. The classical microbiological techniques currently used for the detection of *P. aeruginosa* and identification are satisfactory in most situations but remain necessary for drug susceptibility testing. However, quicker tests may be useful in some specific situations like quarantine period or transport phase.

Critical animals like irradiated and immunosuppressed mice are maintained in IVC of isolators. Every cage in an IVC rack is considered as a separate microbiological unit. Every possible care is taken to provide sterile air to each cage but comprehensive microbiological monitoring of animals kept in IVCs cages become a challenging task. Use of appropriate sentinel mice is used to screen the microbiological status. However, water as a source is difficult to monitor as is replaced at a very short interval. Therefore, chances of introducing the

unwanted or pathogenic organisms through water/bottles are very high. Screening of such water/water bottles by conventional microbiology followed by PCR confirmations offers a reliable means of checking the contaminations. Infection of the SCID mice by *P. aeruginosa* is well documented (Dietrich *et al*., 1996). Monitoring program should therefore take both equipment-related and infectious agent-related parameters into consideration. Water provided to the laboratory animals is therefore a foremost source of infection and assumes importance especially in immuno-compromised animals. The method employed in this paper thus helps to detect and subsequently eradicate pathogenic bacterial infection from laboratory animal facilities and improves quality control of laboratory animals and research. However, full proof managemental practices, equipment maintenance, personnel management and implementation of routine microbiological quality control are key factor for keeping the *P. aeruginosa* at bay.

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