The Prevalence of *Pseudomonas aeruginosa* among Baghdad Hospitalised Patients

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Abstract

In order to determine the prevalence of *Pseudomonas aeruginosa* among hospitalised patients, 156 specimens were collected from hospitalized patients referring Baghdad, Iraq hospitals. Bacterial isolates were identified using conventional biochemical tests and then identification was confirmed by the locating of 16SrRNA. *Pseudomonas aeruginosa* constitutes 30.05%. In conclusion, the attention should be paid toward the infections of this opportunistic pathogen.

Keywords: *Pseudomonas aeruginosa, hospital, 16SrRNA, PCR.*

Introduction

Pseudomonas aeruginosa is a Gram-negative bacterium that is ubiquitous in different ecosystems and involves in numerous forms of relations with eukaryotic host. It is an opportunistic pathogen extensively spread in humans and animals [(1)], leading to a wide range of infections in community and hospitals [(2, 3)]. Due to the extended spread of *P. aeruginosa* habitat, the control of the organism in a hospital setting is very difficult, and makes it practically impossible to prevent contamination [(4)]. The major threat is the infection of immunocompromised patients or those in burns, neonatal and cancer wards [(5)]. Infection of *P. aeruginosa* is still one of the main causes of death among the critically ill and patients with impaired immune systems in spite of the development of newer and stronger antibiotics [(6)].

Due to the extended spread of *P. aeruginosa* habitat, the control of the organism in a hospital setting is very difficult, and makes it practically impossible to prevent contamination. The major threat is the infection of patients who are immunocompromised or those in burns, neonatal, cancer wards, it is the main cause of morbidity and mortality in cystic fibrosis patients and one of the leading nosocomial pathogens affecting hospitalized patients [(7)].

Due to the innate capacity of resistance to antimicrobial agents, this bacterium is greatly difficult to treat. What's more, such resistance is being progressively a problematic issue because of increasingly development of resistance to agents regarded as powerful therapeutic options [(8)].

In this study we have used a genus specific bacterial 16S PCR to investigate the prevalence and diversity of *P. aeruginosa* among Iraqi hospitalised patients.

Materials and Method

Ethical Statement: All participants agreed to provide the investigator with the specimens. Informed consent according to the Declaration of Helsinki was obtained from all participants.

Pseudomonas aeruginosa isolation and identification: One hundred and fifty-six specimens included mid-stream urine, burn swabs, wound swabs, and blood were collected from hospitalized patients referring Al-Yarmouk teaching Hospital and Baghdad Medical City in Baghdad, Iraq. All specimens were cultured on enrichment media such as blood agar and transferred onto MacConkey agar. Pale colonies on MacConkey agar (lactose non-fermenter) were assayed for the conventional morphological and biochemical characterization comprising Gram stainability was conducted alongside the activities of oxidase and

catalase. Thereafter the primarily identified *P. aeruginosa* isolates were submitted to molecular identification by polymerase chain reaction (PCR) technique.

Detection of 16SrRNA

Extraction of Bacterial DNA: Genomic DNA was extracted using Presto™ Mini gDNA Bacteria (Geneaid, Thailand). Upon the procedure itemized by the manufacturing company, DNA was extracted from overnight cultures of the carefully chosen staphylococcal isolates. Purified DNA concentration was measured using Biodrop (Biodrop, Canada).

PCR: To confirm the identification of *P. aeruginosa* isolates, conventional PCR technique was carried out in accordance to Spilker et al. [9] to amplify a fragment of *16SrRNA* (956 bp). Two microliters of each primer PA-SS-F (5′-GGGGGATCTTCGGACCTCA-3′) and PA-SS-R (5′-TCCTTAGAGTGCCCACCCG-3′), different concentrations of DNA (depending on DNA yield) extracted from each *P. aeruginosa* isolate and deionized D.W. were added to PCR premix tubes (Bioneer, Korea) in order to reach 20 µl as a final volume. The thermocycling conditions (Bio-Rad T100, USA) set at 94°C for 2 min, followed by 25 cycles of 94°C for 20s, 58°C for 20s, and 72°C for 40s. A final extension of 1 min at 72°C was applied. PCR products were visualized using 2% agarose gel stained with diamond nucleic acid dye (Promega, USA).

Results

Isolation and identification: A total of 52 (33.33%) isolates developed a growth on cetrimide agar, pale colonies on MacConkey agar, and succeeded to grow on nutrient agar at 42°C. Moreover, they were oxidase and catalase positive. Hence, these isolates were primarily identified as *P. aeruginosa.*

DNA extraction and preparation: After DNA extraction by Presto™ Mini gDNA Bacteria Kit, DNA concentration was between 24 and 78 ng/ml; whereas, purity was about 1.82- 1.99. A ratio of 1.8 -2.0 is generally accepted as "pure" for DNA [10]. Gel electrophoresis was done to confirm the integrity of extracted DNA.

Molecular Detection: The current results revealed that *16SrRNA* was located in 50 (96.1%) out of 52 biochemically *P. aeruginosa* isolates. Correspondingly, two isolates were identified using traditional method as *P. aeruginosa*, they did not have this gene.

Discussion

Pseudomonas aeruginosa is an opportunistic pathogen capable of causing a wide array of life threatening acute and chronic infections particularly in patients with compromised immune defense [11]. Earlier reports have shown that the antibiotic resistance of bacteria due to biofilm formation contributes to the persistence of bacterial cells and causes problems in the complete eradication of infection [12, 13].

The present work is in agreement with [14] as they found that the results of *16S rRNA* showed that all *P. aeruginosa* isolates were resistance to gentamicin harbored this gene. Al-Derzi [15] stated that out of 8038 and 1878 clinical specimens submitted for culture in Mosul and Duhok, respectively, 180 and 21 clinically significant isolates of P. aeruginosa were isolated, resulting in a prevalence of 5.2% and 1.6%, respectively. Moreover, cultural and biochemical identification revealed that 63 *P. aeruginosa* isolates were recovered from 158 samples in Karbala, Iraq [16]. Also Hasan et al. [17] reported that 21.6% *P. aeruginosa* isolates were collected form 185 swab samples in Kirkuk City, Iraq.

The variability in *P. aeruginosa* isolation percentage may be attributed to geographic, climatic, and hygienic factors among different areas. As well as, the high prevalence of *P. aeruginosa* in our community may be related to the rise of burn and wound patients than other samples in our population; which may be the result of different increased kitchen accidents, terrorist incidents, and electrical fire [18].

Conclusion

Pseudomonas aeruginosa was isolated in relatively considerable number; consequently, much work is needed to overcome and control this opportunistic pathogen.

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Funding: Self

Ethical Clearance: Not required

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