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Bacteriological and molecular identification Of *Pseudomonas* Isolated From Sheep In Baqubah Province

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Abstract

Background: *Pseudomonas* cause a wide range of infections in animals.
Aims: Detection of P. aeruginosa from wounds cases of sheep by culture technique and PCR
Methods :forty wound swabs from sheep were collected and cultured on blood and MacConkey, pseudomonas agar . PASS primers used for (PCR) based identification
Results :
A total of 3/40 (7.5%), wound swabs were identified as *P. aeruginosa* after full morphological and biochemical tests and by PCR using the PASS primers for species level . The results pointed out that all 3 selected isolates gave positive results, and the gene size was ≈ 950 bp.
Conclusion: the PCR approach aids the rapid detection of pseudomonas to reduce financial loss. remarkable, and they were fast and accurate.
Keywords: *Pseudomonas*, Molecular diagnosis, Polymerase chain reaction

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Introduction

P.aeruginosa is an aerobic ,gram negative , opportunistic, ,have no ability to ferment glucose ,and resist to wide range of antibiotics which lead significant infections to humans and animals (1). It can be isolated from the tissues of healthy animals and has been determined to be the distinct cause of a number of different infections . (1)

Several factors such as "enterotoxins, exocytotoxins, and toxins produced by protein secretion systems" play a role in infection, septicemia, and fatal condition (2).Resistance to antibiotic is a worldwide problem. Numerous investigators concentrated on pathogenic microorganisms conflict against antibiotic ,that attitude direct hazards for social well-being (18)

P. aeruginosa have great ability for colonization and spreading via blood stream causing high range of mortality due to bacteremia compared with other Gram-negative (3) which become more serious with ability of resistance for wide range of antibiotics (2).

the present study aimed to isolate and diagnose P. aeruginosa from wounds cases of sheep in Baqubah-Diyala province by culture technique and PCR

Aim of the study:

Detection of P. *aeruginosa from wounds cases of sheep* by culture technique and PCR

Materials and Methods:

Sample collection and transport:

The study was conducted in "Department of Microbiology , College of Veterinary Medicine, University of Diyala, Iraq from February 2022 – March, 2023".

Forty wound swabs were collected from sheep and transferred with in less than 24hs. to the lab. using plain tubes with brain heart infusion broth .

Processing and identification

Upon arrival swabs were cultured on common media "blood agar, Mac-Conkey agar" and incubated at 37C˚ for 24hrs. Suspected colonies have characteristic colony morphology with pale color ,non lactose fermenter with dusty odor properties.(17)., microscopic examination and biochemical tests (4) were applied .

A. Microscopic examination:-

A single colony was picked up after the isolation of bacteria and stained with Gram stain, then examined under microscope to recognize their shape, length and gram reaction microscopically according to MacFaddin (5).

B. Biochemical tests:-

For the confirmation of any suspected isolate, numerous biochemical tests, urease , triple sugar iron and oxidase tests were achieved for all suspected isolates (6).

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Pseudomonas agar is clear from its name and thus its recommended selective isolation of *Pseudomonas* species.

Extraction of Bacterial DNA

Genomic DNA of pseudomonas isolates was extracted by using G- spin dna extraction kit according to the manufacturer's instructions. Electrophoresis has been done to determine DNA pieces after the process of extraction or to detect the result of the interaction of PCR during the presence of the standard DNA to distinguish the bundle size of the outcome of the interaction of PCR on the Agarose gel.

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 PASS-F (5′-GGGGGATCTTCGGACCTCA-3′) and PASS-R (5′-TCCTTAGAGTGCCCACCCG-3′), The PCR master mix reaction prepared according to kit instructions (GeNet Bio, Korea) .The reaction was performed in a thermocycler (BioRad, USA) for amplification by applied the following thermocycler conditions;. (Bio-Rad T100, USA)

Results and Discussion:

The results of current study showed that 31/40 collected wound swabs from sheep from Baqubah city showed positive growth on MacConkey agar . A total of (11/31) samples were lactose non-fermenter bacteria on MacConkey agar , and these isolates are subject to more identification. Microscopic examination showed that the bacterial isolates were gram negative coccobacilli, variable in length, arranged as single or as short chains without spores.

Because *P. aeruginosa* was non-lactose fermenter on MacConkey's agar, the plates appear yellow and the suspected colonies have pale color, with a little convex and smooth edges. MacConkey agar was used for growing *P. aeruginosa* strains because it differentiates it from other Gram-negative species and it contains all required nutrients for *P. aeruginosa* growth. These results are in agreement with (8)

Pseudomonas spp. were positive for "oxidase, catalase, and growth at 42ºC", while the negative for the "indole test, MR-VP test, gram stain, citrate utilization, and urease test" .

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Figure (1): *pseudomonas* isolates showed oxidase positive result

Culture on selective media (*Pseudomonas* agar).

The incorporation of tryptone and gelatin peptone provides nitrogenous and carbonaceous compounds, long chain amino acids, and other essential growth nutrients for the organisms. Magnesium chloride and potassium sulphate of medium enhance pigment production The presence of blue-green pigmentation may be considered as presumptive evidence of *P. aeruginosa. figure* (2).

The result of current study showed that 3/ 40, (7.5%) wound swabs were positive for *P. aeruginosa which come in line* with (2) how stated that " the difference in isolation rate of *P. aeruginosa* was according to the source of samples and according to species of animals. **Noomi ,(2018) stated that** *P. aeruginosa* isolated mainly from milk samples (26.8%) followed by ear samples(25.9%) and less frequently from wound (11.4%). This result was also agree with Dapgh,(9) Who stated that "*P. aeruginosa* was isolated from wound and abscess of sheep, 2/21, (9.5%) and goat 1/16 (6.25%)". Current findings come in accordance with (10), who stated that three strains *P. aeruginosa* was isolated from single infected sheep flock . Current findings come in agreement with (11) who obtained two *P. aeruginosa* from eight wound swabs of sheep.

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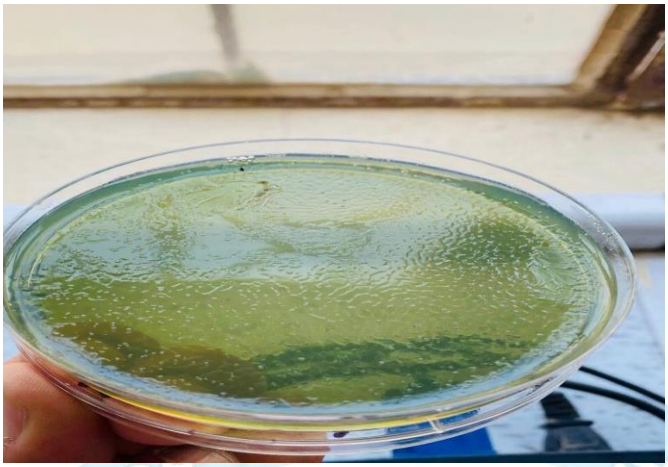

Figure (2): *pseudomonas* isolated showed blue-green pigmentation on pseudomonas agar

Molecular Identification of *Pseudomonas* spp. Isolates

DNA was extracted from all isolates by genomic DNA purification kit . After extraction ,DNA was applied for PCR and the final product processed by agarose gel electrophoresis. The length of amplified fragments was(956 bp of PASS primers) as showed in figure (3):

Figure (3) "PCR product the band size 956 bp. The product was electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1hr.. N: DNA ladder (100)".

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Table (1)The concentration and purity of DNA

NO.	Nucleic acid conc. (ng/ µl)	260/280 purity
1	6.6	2.095
2	15.2	1.974
3	46.4	1.878

Table (2): PCR result by PA-SS gene:

X	DNA Result	PCR Result PA-SS 956bp
P. aeruginosa	+	+
P. aeruginosa	+	+
P. aeruginosa	+	+

Because of accuracy and high sensitivity as well as minimum time requirement, the PCR-based detection and identification procedures for Pseudomonas preferred upon classical culture technique (12).

Spilker (13) use " PA-SS-F and PA-SS-R" for identification of *P. aeruginosa* based on the arrangement of 16S rDNA . Bosshard (14) used "16S rRNA gene sequence " for the identification of gram-negative bacilli while in local study, "16S rDNA gene amplification by PCR" was used for identifying P. aeruginosa. On the other hand ,(15) identified *P. aeruginosa* by using "16S rRNA amplification" . A local study by (16) used "PASS primers of 16S rDNA" for differentiation of *P. aeruginosa* .

Conclusion

P.aeruginosa still cause considerable infection in animals and further studies required to get ride serious effects of such opportunistic pathogens. PCR based detection approach aids the rapid detection of pseudomonas to reduce financial loss. remarkable, and they were fast and accurate.

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