Detection of virulence and multidrug resistance operons in Pseudomonas aeruginosa isolated from Egyptian Baladi sheep and goat

A. N. Dapgh¹, A. S. Hakim², H. A. Abouelhag³, A. M. Abdou⁴ and E. A. Elgabry⁵

1. Department of Bacteriology, Animal Health Research Institute, Dokki, Giza, Egypt; 2. Department of Microbiology and Immunology, National Research Centre, 33 Bohouth Street, 12622 Dokki, Cairo, Egypt.

Corresponding author: A. S. Hakim, e-mail: migris410@yahoo.com

Co-authors: AND: amanydapgh@yahoo.com, HAA: drabouelhag@yahoo.com, AMA: amrkheir@yahoo.com, EAE: elgabry7373@yahoo.com

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Abstract

Background: Pseudomonas aeruginosa is a pit of an enormous group of free-living bacteria that are able to live everywhere and suggested to be the causative agent of great scope of acute and chronic animal infections.

Aim: The current study was carried out to illustrate the prevalence of P. aeruginosa in small ruminants and existence of some virulence operons as well as its antimicrobial resistance.

Materials and Methods: A total of 155 samples from sheep and 105 samples from goats (mouth abscesses, fecal swabs, nasal, tracheal swabs, and lung tissue) were collected for bacteriological study, existence of some virulence expression operons with the study of their sensitivity to the antimicrobials using disc diffusion and presence of mexR operon which is responsible for multidrug resistance (MDR).

Results: The bacteriological examination revealed that P. aeruginosa was isolated from nine out of 155 samples from sheep (5.8%) and four isolates out of 105 samples from goat (3.8%). It is found that 12 (92.3%), 10 (76.9 %), and 8 (61.5%) of P. aeruginosa isolates harbored hemolysin phospholipase gene (pc/H), gene (exoS), and enterotoxin gene (touA), respectively. The results of antibiotic sensitivity test showed that all tested isolates were resistant to ampicillin, bacitracin, erythromycin, streptomycin, tetracycline, trimethoprim-sulfamethoxazole, and tobramycin but sensitive to ciprofloxacin and norfloxacin. The MDR (mexR) operon was existed in all isolates.

Conclusion: There is a growing risk for isolation of virulent MDR P. aeruginosa from sheep and goat illness cases, and this should be regarded in the efficient control programs.

Keywords: drug resistance, goat, Pseudomonas aeruginosa, sheep, virulence.

Introduction

Pseudomonas aeruginosa is a Gram-negative, encapsulated, nonsporulated, and strict aerobic motile rod. It is an opportunistic pathogen, widely exists in various ecosystems and believed to be implemented in several serious human and animal diseases [1,2]. P. aeruginosa causes numerous diseases in sheep and goats; respiratory illness, which is one of the major issues particularly pneumonia, associated with physical and physiological stress, leading to significant mortality rates, and increased economic loss [3]. A number of mastitis cases and the pathogen can reside in the udder for many years [4]. Moreover, P. aeruginosa infection may lead to urogenital disorders, gastrointestinal illness sinusitis, and osteomyelitis [5-8].

The organism declares plenty of virulence agents, which share in its pathogenicity. These comprise enterotoxins, exocytotoxins, and toxins produced by protein secretion systems, as a result of expression of certain virulence operons. Consequently, many of these have been implemented in infection, septicemia, and fatal condition [9,10]. The mortality rate is usually higher than bacteremia sourced with other Gram-negative pathogens due to its ability to secrete these several products that after successive colonization can induce extensive tissue damage, bloodstream invasion, and dissemination [11]. Through all resistant pathogens, the condition is most significant for P. aeruginosa as its incidence of resistance to antimicrobial agents in continuous increasing and has been accounted worldwide [12]. This critical ability of the pathogen could be attributed to the existence of the unusually restricted outer membrane permeability which acts as a safeguard barrier for antibiotics to overcome. Besides that, there were other secondary intrinsic factors as energy-dependent multidrug efflux and chromosomally encoded periplasmic beta-lactamase [13].

This study was conducted to address its isolation and identification from ovine and caprine population, with stressing on its toxigenic expressed operons as well as the associated multidrug resistance (MDR) property.
Materials and Methods

Ethical approval

As per CPCSEA guidelines, a study involving clinical samples does not require the approval of the Institute Animal Ethics Committee.

Samples

A total of 260 different samples were collected from sheep (155) and from goat (105) selectively suffering from respiratory manifestation (40 from sheep, and 22 from goats) in triplicate as nasal, tracheal swabs, and lung tissues. Also, fecal samples were collected from diarrheic (14 sheep and 23 goats) and gathered swabs from skin lesion with abscesses (21 sheep and 16 goats). The samples were gathered from scattered local farms or owners in Great Cairo and Delta rural areas. The samples were gathered from September 2017 to April 2018. The samples were bacteriologically examined and the selected colonies were biochemically identified [14].

Antimicrobial sensitivity assay

The susceptibility of the isolates to various antibiotics was achieved using the diffusion technique [15], the following antibiotic discs were used; amikacin (30 mg), ampicillin (10 µg), bacitracin (10 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamycin (10 µg), streptomycin (10 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (2.25/7.75 µg), tobramycin (30 µg), ciprofloxacin (5 µg), and norfloxacin (10 µg) [16].

DNA extraction

DNA extraction from the samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, Gmbh) with modifications from the manufacturer’s recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer’s recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Polymerase chain reaction (PCR) amplification using oligonucleotide primers

Primers used were supplied from Metabion (Germany), as shown in Tables-1 and 2 [16,17]. Primers were utilized in a 25 µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of DNA template. The reactions were performed either uniplex (mexR) or multiplex (exoS, pclH, and toxA), as described in Tables-1 and 2 [17,18], in a T3 Biometra thermalycler.

Analysis of the PCR products

The products of PCR were electrophoresed on 1.5% agarose gel (Applichem, Germany, GmbH) in

| Table-1: Uniplex PCR: Primers sequences, target operons, amplicon sizes, and cycling conditions. |
|---------------------------------------------------------------|
| **Target operon** | **Primers sequences** | **Amplified segment (bp)** | **Primary denaturation** | **Amplification (35 cycles)** | **Secondary denaturation** | **Annealing** | **Extension** | **Final extension** | **Reference** |
|------------------|----------------------|---------------------------|-------------------------|-----------------------------|---------------------------|--------------|--------------|-------------------|--------------|
| *mexR*           | GCGCCATG GCC CCATAT TCAG GGCATC GCC AGTAAGCGGG            | 637                       | 94°C                    | 94°C                        | 45 s                      | 57°C         | 45 s          | 72°C              | 72°C         | [16]           |
| PCR = Polymerase chain reaction |

| Table-2: Multiplex PCR: Primers sequences, target genes, amplicon sizes, and cycling conditions. |
|---------------------------------------------------------------|
| **Target gene** | **Primers sequences** | **Amplified segment (bp)** | **Primary denaturation** | **Amplification (30 cycles)** | **Secondary denaturation** | **Annealing** | **Extension** | **Final extension** | **Reference** |
|-----------------|----------------------|---------------------------|-------------------------|-----------------------------|---------------------------|--------------|--------------|-------------------|--------------|
| *exoS*          | CCTTCCCT CCTTCCCCCC CGGCCATCGGGA AAAGAAATG CATCCTCA GGC TACATCCT | 270                       | 95°C                    | 95°C                        | 30 s                      | 58°C         | 30 s          | 72°C              | 72°C         | [17]           |
| *pclH*          | GAAACCAT GGCT ACGTCA AGAGTTG CAGG AGGGTGGAG | 307                       | 95°C                    | 95°C                        | 30 s                      | 58°C         | 30 s          | 72°C              | 72°C         | [17]           |
| *toxA*          | ATGGTGTAGATC GGCACAT AAGCGTTC GACC TCTGGAAAC | 433                       | 95°C                    | 95°C                        | 30 s                      | 58°C         | 30 s          | 72°C              | 72°C         | [17]           |

PCR = Polymerase chain reaction
P. aeruginosa was recovered from 2 (5%) tracheal swabs and 1 (2.5%) lung tissue belonged only to sheep samples.

These average percentage results agreed with that formerly reported [22,23] while the lower incidence was 3.6% [24] and one necropsied goat [25]. In contrast, higher isolations were determined [26]. Two P. aeruginosa isolates were recovered from diarrheic sheep 1/14 (7.1%) and goat 1/23 (4.3%), respectively. On the other hand, the animals infected with wound and abscesses, the percentage of isolated P. aeruginosa from sheep was 2/21 (9.5%) and goat 1/16 (6.25%), respectively. These obtained data close to that stated by Hears et al. [27], who isolated three strains P. aeruginosa from one of the infected sheep flocks. Abd El-Rahman [28] deduced that the incidence of P. aeruginosa recovered from diarrheic sheep was higher than that obtained among specimens of other infected animals. Furthermore, current results less than that obtained by Alkeshan [29], who isolated P. aeruginosa from abscesses with an incidence (6.18%).

The unselective use of antibiotics is potentially leading to a higher incidence of infections with resistant microorganisms such as P. aeruginosa, worse which may be transmitted from animal to human complicating the treatment of human diseases [30]. Regarding, the antimicrobial sensitivity agar test shown in (Table-4); it was noticed that the isolates were lack of susceptibility to many tested antimicrobial agents. The primary mechanism of the micro-organism’s resistance relies on its ability to shut out various agents rather than the production of antibiotic inactivating enzymes. Consequently, most of antibiotics are of limited value in the treatment of P. aeruginosa infection in animals [31].

The results indicated that the isolates were susceptible to both ciprofloxacin 13 (100%) and norfloxacin 12 (92.3%), while completely resistant to ampicillin, bacitracin, erythromycin, streptomycin, tetracycline, trimethoprim-sulfamethoxazole, and tobramycin, most of the isolates were resistant to amikacin, chloramphenicol, and gentamycin. These findings agreed with the other previous studies which illustrated that the organism is resistant to all used antibiotics except quinolones [32-35].

Assorted chromosomally encoded efflux systems and outer membrane porins have been distinguished as essential contributors to MDR phenotype.
resistance. The MexAB-OprM is the only pump which is expressed at a level enough to allow intrinsic MDR in wild type P. aeruginosa strains. Mutations in mexR cause over-expression of MexAB-OprM efflux pump [36]. It is obvious in our study as shown in Figure-1; that all isolates exhibited the amplification of 637 bp which represent the mexR operon. The emergence of MDR P. aeruginosa is turning out a challenging issue in infection control schemes.

Figure-2 shows multiplex PCR detection of virulence genes in Pseudomonas aeruginosa isolates showing: L: 100 bp DNA ladder. Lanes 1-13: P. aeruginosa isolates. Lane Pos.: Positive control; amplification of 270 bp represented mexR. Lane Neg.: Negative control.

**Conclusion**

*P. aeruginosa* could be implicated in sheep and goat infections, and the isolates showed high resistance to commonly used antibiotics as well as having numerous agents of virulence. A strict antibiotic policy and establishment of infection control programs will help to lower the incidence of resistance in *P. aeruginosa*.

**Authors’ Contributions**

ASH and AND supervised the experiment. AMA and EAE shared in the collection of the samples, AND and HAA did the isolation and identification of microorganism. EAE performed the antibiotic susceptibility assay. HAA and AMA performed PCR. ASH and AND prepared and reviewed the manuscript. All authors read and approved the final manuscript.

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**Competing Interests**

The authors declare that they have no competing interests.

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