

Research Article

Antibiotic resistance and plasmid profiling of *Pseudomonas aeruginosa* isolated from some ruminants in Basrah, Iraq

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Abstract

This work aimed to determine the antibiotic-resistant patterns of *Pseudomonas aeruginosa* isolates obtained from clinical, healthy, and environmental samples from some ruminants (cows, and sheep). A total of 200 *P. aeruginosa* were obtained, and 52 isolates resisted all antibiotics used in the antibiotic sensitivity test. The antibiotic-resistant pattern showed that *P. aeruginosa* had high resistance (100%) to ampicillin, ceftazidime, gentamycin, ciprofloxacin, piperacillin, tobramycin, imipenem, amikacin, streptomycin, levofloxacin, rifampin, tetracycline, trimethoprim, ofloxacin, carbenicillin, penicillin, and nalidixic acid and had low resistant to colistin and fosfomycin. The plasmid profile was carried out on 12 selected multidrug-resistant (MDR) isolates that were resistant to more classes of antibiotics. All strains were found to possess plasmid bands. Five of the strains had 3 plasmid bands, 4 strains 2 plasmid bands and 3 strains possessed a single band. The sizes of the plasmids among *P. aeruginosa* isolates were 735, 1400 and 3000bp. All the strains that had plasmids were resistant to gentamycin, ciprofloxacin, piperacillin, tobramycin, imipenem, carbenicillin and tetracycline.

Keywords: *Pseudomonas aeruginosa*, Antibiotic resistance gene, Ruminant, DNA.

Citation: Al-Tememe, T.M.K. & Abbas, B.A. 2022 Antibiotic resistance and plasmid profiling of *Pseudomonas aeruginosa* isolated from some ruminants in Basrah, Iraq. Iranian Journal of Ichthyology 9(Special Issue 1, 2022): 334-339.

Introduction

Multidrug-resistant pathogenic bacteria are a major health problem in animals resulting from the misuse of antibiotics. It is used in animals to treat, prevent, and control diseases and growth promoters (Abdalhamed et al. 2021). These pathogens are abundant in various environments and can colonize and infect livestock (Haenni et al. 2015). *Pseudomonas aeruginosa* can cause mastitis in dairy cows and multiple diseases in sheep and goats, including ovine mastitis, respiratory manifestations, urogenital disorders, gastrointestinal illness, sinusitis and osteomyelitis (Rasooli et al. 2018). It is naturally resistant to a wide spectrum of antibiotics and many antimicrobial agents (Connie & Donald 2019).

Major mechanisms of *P. aeruginosa* used to counter antibiotic attacks can be classified into

intrinsic, acquired, and adaptive resistance. The intrinsic resistance includes low outer membrane permeability, expression of efflux pumps that expel antibiotics out of the cell, and the production of antibiotic-inactivating enzymes. The acquired resistance can be achieved by either horizontal transfer of resistance genes or mutational changes. In addition, adaptive antibiotic resistance is a recently characterized mechanism, which includes biofilm-mediated resistance and formation of multidrug-tolerant persisted cells, and is responsible for recalcitrance and relapse of infections (Panga et al. 2019; Hassell et al. 2019).

Pseudomonas aeruginosa has a large genome with 5.5-7Mb, characterized by pronounced genomic plasticity (Suenaga et al. 2017). A high rate of spread of resistant genes has been suspected as the cause of

Table 1. Resistance patterns of *Pseudomonas aeruginosa* isolated in this study and MDR index.

Source	No. of isolates	Resistance profile	MDR
Infected cow	19	CAZ,FEP,CIP,OFX,AM, TOP,CAR,ATM,CLM,R,T,TMP,NA,S,GM AK, IPM, MEM	0.9
Infected sheep	12	CAZ,FEP,CIP,OFX,AM, TOP,CAR,ATM,CLM,R,T,TMP,NA,S,GM AK, IPM, MEM	0.9
Healthy cow and sheep	3	CAZ,FEP,CIP,OFX,AM, TOP,CAR,ATM,CLM,R,T,TMP,NA,S,GM AK, IPM, MEM	0.9
Environmental isolates	18	CAZ,FEP,CIP,OFX,AM, TOP,CAR,ATM,CLM,R,T,TMP,NA,S,GM AK, IPM, MEM	0.9

increased antibiotic resistance. Plasmids carry genes that could be spread by conjugation and transduction, while the genome-based resistant genes are spread by replication and plasmid-mediated (Livermore 2004; Botelho et al. 2019; Munita & Arias 2016; Zalewska et al. 2021). However, antimicrobial resistance and genotyping of *P. aeruginosa* in companion animals remain largely unknown. Therefore, this study was carried out to determine the antibiotic-resistant patterns of *P. aeruginosa* isolates obtained from clinical, healthy, and environmental samples from some Ruminants (cows, and sheep) in Basrah against 21 commonly antibiotics and determine the plasmid profile of the multiple antibiotic-resistant strains.

Materials and Methods

Sample collection and isolation of *P. aeruginosa*:

Samples were collected, isolated, and identified phenotypically and genetically in our previous study (Al-Tememe et al. 2022). Fifty-two isolated clinical, healthy, and environmental samples were collected from some ruminants of cows and sheep in Basrah.

Antimicrobial susceptibility testing: Antimicrobial susceptibility test has been performed according to the Kirby-Bauer disc diffusion method on Mueller–Hinton agar (Oxoid, UK) (CLSI 2020). The standard antibiotic discs of piperacillin, gentamicin, amikacin, tobramycin, imipenem, meropenem, ceftazidime, levofloxacin, colistin, ampicillin, ciprofloxacin, ofloxacin, carbenicillin, penicillin, aztreonam, rifampin, tetracycline, trimethoprim, fosfomycin and nalidixic acid (Candalab) were used. *Pseudomonas aeruginosa* was designated as susceptible (S) or

resistant (R) according to CLSI guidelines.

Multidrug-resistant (MAR): The multiple antibiotic resistance (MAR) index for each isolate was determined for 21 antimicrobial agents according to the formula of MAR index = Number of antibiotics resistant / Total number of antibiotics tested.

Plasmid Isolation and Profiling: The plasmid DNA was extracted and purified from 12 isolates, 6 from clinical, 2 from healthy, and 4 environmental isolates using a commercial kit Column-pure plasmid Mini-Prep Kit (Abm, Korea) according to the manufacturer instructions. This technique was done at room temperature. Gel electrophoresis of the plasmid DNA was carried out on a 1% agarose gel according to Bikandi et al. (2004).

Results

The results indicated that all strains had extensively multi-drug resistance to different antibiotics (Table 1). The antibiotic susceptibility test results of the isolates revealed that all isolates were resistant to all antibiotics (100%) except colistin and fosfomycin, which had low resistance (Table 2).

Plasmid distribution and profiling: All 12 strains possess plasmid bands. Five of the strains had 3 plasmid bands, 4 strains had 2, while 3 strains possessed a single band. The sizes of the plasmids were 735, 1400 and 3000bp (Fig.1, Table 3). All the plasmids strains were resistant to gentamycin, ciprofloxacin piperacillin, tobramycin, imipenem, carbenicillin and tetracycline.

Table 2. Antibiotic resistance of the 52 *Pseudomonas aeruginosa* isolates.

Class of Antibiotic	Antibiotic	Con. mcg	Number and Percentage of High* Resistant		Number and Percentage of Low* Resistant	
			No	%	No.	%
Penicillin	Ampicillin (AM)	30 µg	52	100	0	0
	Penicillin (P)	10 µg				
	Piperacillin (PRL)	100 µg				
Aminoglycoside	Amikacin (AK)	30 µg	52	100	0	0
	Gentamicin(GM)	10 µg				
	Tobramycin(TOB)	30 µg				
	Streptomycin (S)	10 µg				
Cephalosporin	Ceftazidime(CAZ)	30 µg	52	100	0	0
	Imipenem (IPM)	10 µg				
Carbapenems	Meropenem(MEM)	10 µg	52	100	0	0
	Carbenicillin(CAR)	100 µg				
Fluoroquinolones	ciprofloxacin(CIP)	10 µg	52	100	0	0
	Levofloxacin (LEV)	5 µg				
	Ofloxacin (OFX)	5 µg				
Monobactams	Aztreonam (ATM)	30 µg	52	100	0	0
Polymyxins	<i>Colistin</i> (CLM)	10 µg	0	0	52	100
	Rifampin (R)	5 µg	52	100		
Rifampcins	Tetracycline (T)	30 µg				
Tetracyclines	Trimethoprim(TMP)	10 µg	52	100		
Trimethoprim	Fosfomycin (FOS)	100 µg				
Phosphonics	Nalidixic acid (NA)	30 µg	52	100		

High*=high-level resistant; Low* = low-level resistant

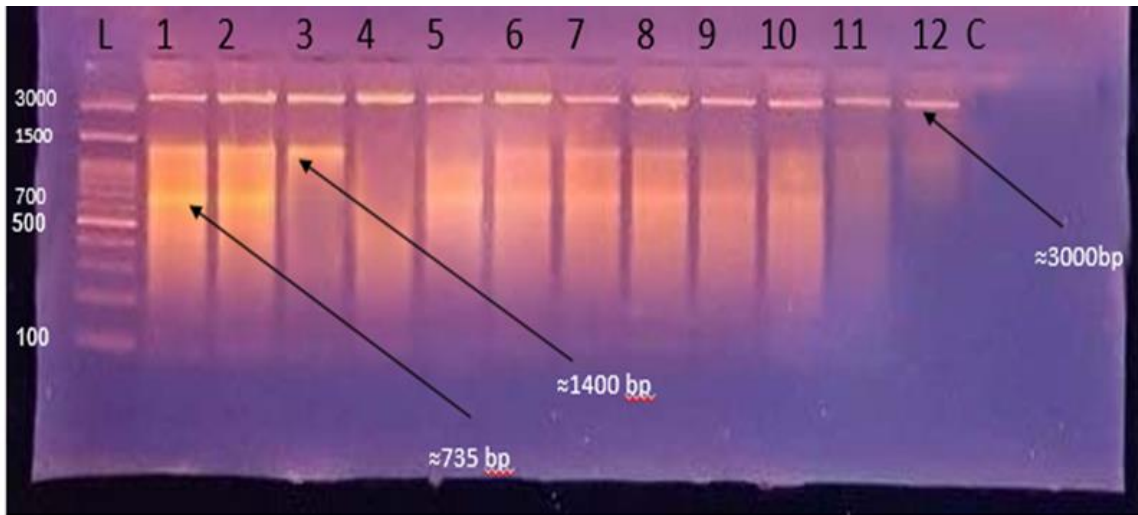


Fig.1. Plasmid profile of resistance *Pseudomonas aeruginosa* isolates: lane 1-2: (3000, 1400, and 735bp), Lane 3: (3000, and 1400bp), Lane 4: (3000bp), Lane 5: (3000, and 735bp), Lane 6-8: (3000, 1400, and 735bp), 9-10 (3000, and 735 bp), 11-12 (3000bp). C: Negative control, Lane L: 100-3000bp ladder.

Discussion

This study investigates a severe fact not done in previous local studies in Basrah (Shamkhi & Khudaier 2020; Jaber et al. 2020) in which *P. aeruginosa* was isolated from ruminants, especially infected and healthy cows and sheep, and from their environment. All isolates of this study

were resistant to 21 locally available antibiotics of various classes. The antibiotic-resistant pattern showed that *P. aeruginosa* had high resistance (100%) to ampicillin, ceftazidime, gentamicin, ciprofloxacin, piperacillin, tobramycin, imipenem, amikacin, streptomycin, levofloxacin, rifampin, tetracycline, trimethoprim, ofloxacin, carbenicillin,

Table 3. Distribution of plasmids in *P. aeruginosa* isolates used in this study (number, size).

No. of isolates	Sources of isolates	No. of plasmids	Plasmid size (bp)
1	clinical	3	3000, 1400, 735
2	clinical	3	3000,1400,735
3	clinical	2	3000,1400
4	clinical	1	3000
5	clinical	2	3000, 735
6	clinical	3	3000, 1400, 735
7	environmental	3	3000, 1400,735
8	environmental	3	3000bp, 1400,735
9	environmental	2	3000, 735
10	environmental	2	3000, 735
11	healthy	1	3000
12	healthy	1	3000

penicillin, and nalidixic acid and low resistant to Colistin and fosfomycin. The resistance of isolates to ampicillin, ceftazidime, penicillin, and tetracycline has been reported in previous studies (Hossain et al. 2013; Ghazy et al. 2015), and also to streptomycin, tobramycin, ampicillin, and tetracycline (Dapgh et al. 2019), but the resistance of isolates of this bacterium to ciprofloxacin and gentamycin not confirmed by a previous study (Saha et al. 2020).

A study on healthy cows and sheep (Ruiz-Roldán et al. 2020) found the isolates were susceptible to all antipseudomonal agents tested, while in this study, healthy cows and sheep resist all antibiotics as same as those from their environment. Humans, animals, and the environment are reservoirs of bacteria carriers of antimicrobial resistance and virulence genes that could be mobilized to human pathogens like *P. aeruginosa*. Animals are important reservoirs and potential disseminators of bacteria and their antimicrobial resistance genes because of their proximity to humans (Argudín et al. 2017). Resistant bacteria can be transmitted from ruminants to humans through food consumption (meat and milk) or direct or indirect contact with animals or their waste (Hassell et al. 2019). MDR index (0.9) results confirmed all isolates MDR and XDR. All antimicrobials classes include sulfonamides, penicillin, tetracycline, ampicillin, aminoglycosides, and cephalosporin (cephalexin) used in veterinary

and human medicine rise drug resistance. This exerts selective pressure on bacteria in livestock digestive systems to acquire and maintain antibiotic resistance genes (ARGs) and fosters an increase in the relative abundance of resistant populations (Gullberg et al. 2011).

The plasmid DNA extraction and profiling results indicated that the 12 examined isolates of *P. aeruginosa* have plasmid DNA with a molecular weight of 735, 1.4K, and 3Kpb having resistance to gentamycin ciprofloxacin, piperacillin, tobramycin, imipenem, carbenicillin, and tetracycline. In veterinary, plasmid-mediated resistance to various infections is caused by antibiotic-resistant *P. aeruginosa* and their environment demonstrated by several workers (Ghazy et al. 2015; Admosu et al. 2016; Talukder et al. 2021). There is no study on plasmid-mediated resistance by antibiotic-resistant *P. aeruginosa* isolated from healthy ruminants, but there are extensive studies on antibiotic resistance in other bacterial species (Khudor et al. 2012; Al-Ghanim & Abbas 2021; Khalid & Abbas 2021). *Pseudomonas aeruginosa* was resistant to 21 locally available antibiotics from infected and healthy ruminants and their environment, which seriously threatens our society from the health and economic aspects.

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