



## An Epidemiological and Molecular study Evaluating The Biofilm-Related Markers of *Pseudomonas Aeruginosa* and *Klebsiella* Spp. Causing Diarrhea in Ruminants and Humans



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### Abstract

**P**SEUDOMONAS AERUGINOSA and *Klebsiella* spp. are opportunistic pathogens linked to several diseases in both humans and animals, posing a major risk to both human health and the advancement of animal husbandry. The present investigation aimed to determine the prevalence of such pathogens in sheep, cattle, and humans as well as to assess the antimicrobial sensitivity pattern in relation to the most widely prescribed antibiotics and, additionally, to use molecular assays for illustrating the genes linked to biofilm and virulence. Fecal samples were gathered from 791 cattle and 386 sheep, beside 304 human stools. Significant isolation of *Pseudomonas aeruginosa* was recorded in 16.81%, 7.51%, and 5.59% of cattle, sheep and humans, respectively, while *Klebsiella pneumoniae* was found in 1.8%, 8.9%, and 1.97% of the cattle, sheep, and human samples. Likewise, *Klebsiella oxytoca* was isolated from 2.91%, 4.4%, and 0.33% of the samples. The majority of the isolated bacteria showed inconsistent patterns of resistance to different antibiotics, however, ceftriaxone showed favorable effects. The molecular characterization of the tested isolates showed that they were virulent and revealed gene determinants for the ability to form biofilms (*pelA* and *rmpA*). It was clarified that antibiotic resistance will lengthen the course of treatment because biofilm formation in the isolates prevents antibiotics to reach the bacteria. Furthermore, there was a correlation between the emergence of multi-drug resistance and elevated biofilm production.

**Keywords:** Cattle, Sheep, Humans, Antibiotic, Resistance, Biofilm

### Introduction

Numerous pathogens cause enteric infections in calves and lambs, which are the main reason for subpar growth and newborn mortality in the infected animals [1]. In ruminants, cattle, sheep, and goats, diarrhea is one of the most common financial issues, notably in Egypt [2,3] According to estimates, acute diarrhea in the pre-weaning period accounting for 75.0% of early calf mortality in dairy herds, is still a significant factor in lost productivity and financial loss for farmers, and contributes to high morbidity and mortality rates in the global cattle industry [4,5]. Viruses (*Corona* virus and *Rota* virus) and protozoa

(*Cryptosporidium* spp.) are the common causes that can associate with infectious diarrhea [6-8]. Additionally, *Klebsiella* (*K.*) spp., *Pseudomonas aeruginosa* (*P. aeruginosa*), *E. coli* K99+ and *Salmonella* species are among the most prevalent bacterial-associated diarrhea in young ruminants [9-11].

*P. aeruginosa* is a ubiquitous opportunistic Gram-negative bacterium which has been found in a variety of environment settings, including humans. Although it rarely affects healthy people, it is frequently linked to nosocomial infections [12,13], leading to pneumonia, septicemia, and necrosis in immunocompromised individuals that is associated

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with high morbidity and mortality [14-16]. It is one of the main gastrointestinal pathogens in animals that causes calf scour and clinical and subclinical mastitis in dairy cows, sheep, and goats [9,10]. Using its powerful binding components, such as flagella, pili, and biofilms, this bacterium can survive on water, various surfaces, and medical equipment [17]. Additionally, it may adapt to environmental changes by creating a wide range of virulence factors, rapidly acquiring resistance to antibiotics and disinfectants. *P. aeruginosa* [18] as a model organism for research on biofilm development.

In addition, *Klebsiella* spp. are opportunistic bacteria in the family *Enterobacteriaceae* found as natural flora in the gastrointestinal tracts of both animals and man [19] that can cause serious infections like meningitis, bronchitis, bacteremia, pneumonia, and urinary tract illness. In man, these infections are more prevalent in those with weakened immune systems [20,21]. Although localized disease frequently occurs, other infections including endophthalmitis, liver abscess, and meningitis are reported in healthy individuals [22]. It is also, commonly linked to both nosocomial and community-acquired infections. As well, *K. pneumoniae* and *K. oxytoca* both regularly cause pneumonia, though to varying degrees [23]. The ability of *Klebsiella* spp. to elude the host's innate immune systems is one of their many virulence factors, including capsules, adhesions, lipopolysaccharides (LPSs), exo-polysaccharides, and iron absorption systems. Additionally, the *Klebsiella* strains can generate the biofilms, which have the capacity to produce a significant amount of extracellular matrix, which aids the organism in adhering to abiotic or biotic surfaces and thwarts the action of antimicrobial drugs [24].

In the past 30 years, the emergence of antimicrobial resistance (AMR) has posed a noteworthy hazard to both human and animal health. One particularly risky effect of the antibiotic-resistant strains is the potential for their transmission from animal organisms to humans and the environment through direct or indirect contact with food that comes from animals associated with the failure of the antibiotic therapy against these microorganisms [25,26].

The incidence of microbial-related diarrhea is escalating with the uncontrolled use of antimicrobial drugs for treatment in man and animals in several countries including Egypt. Therefore, the aim of this study was to spotlight on the prevalence of diarrhea caused by *P. aeruginosa* and *Klebsiella* spp. in farm animals and humans, to study the antibiogram sensitivity profile for the occurring isolates against the most commonly used antibiotics in the field and to investigate the virulence and biofilm-related markers of these isolates using the molecular assays.

## **Material and Methods**

### *Study area and period*

The current study was done in Minia Governorate (Coordinates: 28.11°N 30.11°E), Egypt from January 2022 to December 2022. Animal samples were collected from both governmental and private farms as well as small holders. Meanwhile, the human samples were gathered from individuals admitted to the outpatient clinics at Minia General Hospital, Fever Hospital, and those in contact to the screened animals.

### *Study design*

Firstly, this study was operated to determine the prevalence of *P. aeruginosa* and *Klebsiella* spp. in the fecal samples collected from cattle and sheep as well as human stool samples followed by the identification of isolates through the molecular assays using the specific 16S or 23S rDNA genes. The detection of biofilm-related genes was investigated to the isolated traits. Lastly, screening the antibiogram for the commonly used antibiotics in the field was done using the disk diffusion procedures. Animal and human sampling design and protocol was carried out based upon the International Animal Care and Use Committee (IACUC), Ref. No: (Ref. No: IRB-FVM-MU-2023-103), Minia University, Egypt. All data and results were recorded and statistically analyzed.

### *Sample collection*

Fecal samples were collected from cattle (n=791) and sheep (n=386). Also, a total of 304 human stool samples were obtained. All samples were taken aseptically according to [27] then preserved on ice box to be transported to the lab of Hygiene, Zoonoses and Epidemiology on Faculty of Veterinary Medicine, Minia University, where the bacteriological examination was done.

A detailed questionnaire for each sample was conducted for animal samples (age, sex, health status, season, breed, previous treatment, etc) while for human samples documentary proven medical history or Hospital affirmed diagnosis were collected.

### *Isolation and identification of P. aeruginosa and Klebsiella spp.*

Approximately one gram of each fecal or stool sample was pre-enriched individually into a tube containing 9 ml of the tryptic soy broth (TSB, Oxoid, Basingstoke, UK) and incubated at 37 °C for 24h, then a loopful from each tube was inoculated onto the surface of both Cetrimide and MacConkey agars. Colonies appeared as large blue-green or green-yellow with characteristic sweet-grape like aroma were chosen and biochemically identified for *Pseudomonas*, whilst, pink red colored colonies of mucoid texture were selected for identifying

*Klebsiella* [28]. The biochemical identification was operated [29, 30]. The biochemically confirmed isolates were sent to the biotechnology center in the animal health research institute, Egypt for molecular characterization. While the amplification of 16S rDNA gene specific for *P. aeruginosa* was performed, the 16S-23S ITS gene and *pehX* were specific for *K. pneumonia* and *K. oxytoca*, respectively. Furthermore, for the detection of particular virulence and biofilm, (*toxA*, *pelA*) and (*mrkA*, *rmpA*) genes were amplified, respectively. Primer sequences and target genes specific for the identified bacterial isolates were illustrated in Table 1.

#### *Molecular identification of P. aeruginosa and Klebsiella spp.*

Firstly, DNA was individually extracted from biochemically confirmed colonies using QIAamp DNA Mini kit (Qiagen, Germany, GmbH). Briefly, 200µL of the sample suspension was incubated with 10µL of proteinase K and 200µL of lysis buffer at 56 °C for 10 min. After incubation, 200µL of the absolute ethanol was added up to the lysate. Then samples were washed and centrifuged following the manufacturer's instructions. Lastly, the nucleic acid of each sample was eluted with 100µL of elution buffer supplied with the kit.

For operating the conventional-type PCR, the primers were utilized in a 25µL volume reaction containing 12.5µL of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1µL of each primer of 20pmol concentration, 4.5 µL of water as well as 6 µL of DNA template. The reaction was performed in the Applied biosystem 2720 thermal cycler. The amplification of *P. aeruginosa* (16S rDNA), *K. pneumoniae* (16S-23S ITS) and *K. Oxytoca* (*pehX*) genes as well as detection of virulence (*toxA* and *mrkA*) genes and biofilm-related genes (*pelA*, *rmpA*) were done, respectively. The parameter of thermocycling included the initial denaturation (94 °C for 5 min) and the final extension step (72 °C for 30 sec). The annealing temperatures for the targeted genes were listed in Table 1.

For gel evaluation, PCR products were disconnected by electrophoresis on 1.0% agarose gel (Applichem, Germany, GmbH) in 1×Tris/Borate/EDTA (TBE) buffer at room temperature using gradients of 5 V/cm, then 40 µl of the PCR products was loaded in each gel slot and the gel was portrayed by a gel documentation system (Alpha Innotech, Biometra) and the data were evaluated using the computer software of DigiDoc-It Imaging System.

#### *Antibiotic susceptibility profile for the isolated bacteria*

Confirmed *P. aeruginosa* and *Klebsiella* spp. isolates were subjected to antimicrobial susceptibility

testing for 10 antibiotics from different classes namely, Amoxicillin (10µg), Amoxiclav (Amoxicillin/Clavulanic acid) (50/10µg), Cefoperazone (75µg), Cefotaxime (30µg), Ceftriaxone (10µg), TMP-Sulpha (25 (1.25T+23.75S µg), Gentamicin (10µg), Metronidazole (4µg), Neomycin (30µg) and Streptomycin (25µg). The antibiotic susceptibility of the isolates was determined following the breakpoints prescribed by Clinical and Laboratory Standards Institute [37]. Isolates resistant to more than three different classes of antimicrobials were categorized as multi-drug resistant (MDR).

#### *Statistical analysis*

Data obtained were recorded and the distribution of *P. aeruginosa* and *Klebsiella* spp in the collected samples as well the Antibiotic susceptibility profile were done using non-parametric tests (Chi-Square Test) using SPSS (Inc. version 26.0, Chicago, IL, USA).

#### **Results and discussion**

The results in Table (2) revealed significant findings concerning the occurrence of *P. aeruginosa* in cattle, sheep and humans that accounted for 16.81%, 7.51% and 5.59%, respectively. Lower findings were denoted by Iatwa et al.[38 ](6.7%), Aly et al.[39 ](4.0%), and Gharieb et al. [40] (10.0%) while higher records were investigated by Chika et al [41] (35.8%) and Elshafiee et al. [42] (34.0%) in cattle. Concerning sheep, lower results were noted by Abdel-Aziz [43] (3.91%) and a nearly similar rate was reached by Ruiz-Roldán et al. [44] (6.5%). Shifting to humans, the occurrence of *P. aeruginosa* was 5.59%, which was lower than those of Shehabi et al. [45] (19.24%), Elshafiee et al. [42] (20.0%), Odoi et al. [46] (39.7%) and Martak et al. [47] (51.6%), while higher than those of Fakhkhari et al. [48] (3.4%) and similar to those found by Ruiz-Roldán et al. [49] (5%). With reference to *K. oxytoca*, it was recovered from 2.91%, 4.4% and 0.33% of cattle, sheep and human samples, respectively. A close matching of our results was introduced by Munoz et al. [50] (2.3%) in cattle. While in humans, Cheng et al. [51] and Smith et al. [6] found higher isolation rates 1.8% and 8.9%, respectively. Concerning, the results showed the occurrence of *K. pneumoniae*, it was detected in 1.39%, 3.11% and 1.97% of the examined cattle, sheep and humans, respectively. Higher records were indicated by Kisasa et al. [52] (5.8%) and Bandyopadhyay et al. [53] (14.74%), and much higher results (84.0%) were reported by Munoz et al. [50].

Virtually, it should be emphasized that the fecal carriage of the investigated pathogens in the animals as well as humans highlights the importance of such microbes as potential hazards for the public health and, further, increases the interest in collaborative efforts for preventing them from gaining access the food chain as well. In man, *K. oxytoca* infection is

frequently associated with spontaneous septic arthritis [54]. Besides, it was suggested to have a role in haemorrhagic colitis [55], necrotizing enterocolitis [56]. Also, It has been commonly associated with gastrointestinal disorders in premature infants with case mortality rate of 20.0 to 30.0% [57,58].

Concerning the age, as a risk factor associating the occurrence of the examined pathogens (Table 3), the obtained results demonstrated a significant variation in the bacteria isolation with age, which represents the single most important individual characteristic influencing disease occurrence, as most health-related factors vary with age, including susceptibility, opportunity for exposure, latency or incubation period of the disease, and physiologic response [59]. *P. aeruginosa* demonstrated a gradual elevation in cattle until it reached the peak, and then it gradually diminished. Initially, it increased progressively, starting at 7.8% in calves up to 1 month of age and then rose to 15.9% when the animals were older than 1 month till 3 months. From 3 to 6 months of age, it reached a maximum occurrence of 28.3%. However, it subsequently decreased, reaching 24.4% in the next 6 months of age, and continued to decline down to 12.0% in animals older than 12 months. These results could be comparable to those obtained by Mushin and Ziv [60] who found an alarming high fecal carriage rate among calves over 4 months old ranging from 9.0% to 78.0%. Conversely, they revealed negative results in those aged between 6 and 9 months and those under 12 months of age. The current results were higher than that of Iatwa *et al.* [38] who could isolate *P. aeruginosa* from only 4.0% of the examined calves and, moreover, did not support the results of Elenshleger *et al.* [61] who couldn't detect any positive samples. With regard to *K. oxytoca* results in cattle, no significant correlation with age was detected except for the age group (>6 to ≤12 M) where the percentage isolation declined to 1.6% in comparison to relatively higher rates in both younger and older age groups. Referring to *K. pneumoniae*, it almost had the same pattern as its relative, it failed detection at the same age group (>6 to ≤12 M), but significantly elevated at the oldest age group to reach 3.7%. That could be understood in light of the frequent occurrence of *Klebsiella* mastitis in the lactating cows which subsequently affects suckling calves while sparing the weaned calves which are far removed from this influence. The impact of *Klebsiella* mastitis on dairy herds was previously [62-64].

A different fluctuating trend was displayed by *P. aeruginosa* recovered from sheep (Table 3), beginning with 8.99% in lambs aged under 15 days, then increased to 15.4% in the next 15 days. It, latter, decreased to 3.6% in those aged over 1 month up to 3 months. In contrast to cattle, it increased to reach 6.7% at the next 6 months of age, however, it

returned to decrease to 1.6% in animals older than 6 months. In a study made by Dapgh *et al.* [65], *P. aeruginosa* was successfully recovered from 7.14 % of the surveyed lambs, which is slightly lower than the present study. For *Klebsiella* spp. the results closely resembled an inverted pyramid shape, where it started in a relatively high level and, then declined and raised again. Comparable results were formerly presented by Sharma *et al.* [66] who noted *P. aeruginosa* at 6.15% and *Klebsiella* spp. at 9.23% in lambs. Another matching result was obtained by Shabana *et al.* [67], 1.6% of *Klebsiella* spp. in adult ovine samples. The obtained distribution of *Klebsiella* spp. in sheep could be managed as that of cattle, which is supported by studies ensuring that *Klebsiella* mastitis is a critical disease in sheep also [67-69].

In Table 4 results described the relationship between the Health status and the rate of pathogen isolation in animals and humans. For cattle, *P. aeruginosa* was isolated from 18.57% of diarrheic cases but it decreased to 11.17% in those non-diarrheic which is remarkably higher than El-Tawab *et al.* [70] and Ashraf [71] who detected *P. aeruginosa* in only 4.0% and 4.9% of diarrheic calves, respectively. On the contrary, 24.8% of healthy cattle were classified as carriers of *P. aeruginosa* [49]. As for *Klebsiella* spp., results reflected a much closer values between as 4.64 % and 3.19 in diarrheic and non-diarrheic cattle samples, respectively, differing from 80.0% isolation rate noted in healthy cattle [50] Regarding diarrheal animals, nearly similar results, Okela *et al.* [72] who reported 2.6% prevalence of *Klebsiella* in fecal samples from calves less than 30 days old. Also, Herrera-Luna *et al.* [73] found that 3.3% of diarrheal calves aged 0 to 6 weeks tested positive for *Klebsiella*. In contrast, a much higher incidence (29.4%) of cattle intestine samples harboring *Klebsiella* [74]. Furthermore, *K. pneumoniae* detected in 32.0% of cattle feces [75] and *K. pneumoniae* from 15.0% of fecal samples examined [76].

Regarding the prevalence of *P. aeruginosa* and *Klebsiella* spp. in sheep (Table 4), a significant variation as 8.54% and 8.90% for diarrheic and 4.76% and 3.81% for non-diarrheic samples, respectively, was observed. Bkheet *et al.* [77] clarified that isolation of *P. aeruginosa* and *Klebsiella* spp was 8.9% and 28.3% from diarrheic and non-diarrheic samples, respectively. The obtained results may be better explained when taking into account that members of the family *Enterobacteriaceae* constitute the main causes of diarrhea in sheep as reaching up to 81.4 % among diarrheal sheep [77] Shifting to human samples, it was showed a higher incidence of *P. aeruginosa* in non-diarrheic (9.41%) than that found in diarrheic (4.11%) individuals. A similar rate was reported by

Estepa et al. [78] who recovered *P. aeruginosa* from 8.2% out of apparently healthy human beings. Though, earlier studies supposed a wide range of isolation for *P. aeruginosa* (2.6% to 24.0%) [79,80] Despite the abundant occurrence *P. aeruginosa* as normal inhabitant, it is related to many serious illness as nosocomial diarrhea in the intensive care unit and lung infections [81]. Moreover, *Klebsiella* Spp. are considered among the dangerous microbes in human GIT [82] that may be attributed to capsule, lipopolysaccharide (LPS), siderophores, and fimbriae [83] or Iron uptake [84].

The findings in Table 5 revealed that, *P. aeruginosa* showed a high degree of resistance for Amoxicillin and Amoxi-clave, which is parallel to that achieved by [85] who indicated a resistance of 90.0% for both antibiotics. Though, such result is lower than that of Gad et al.[86] who reported a resistance as high as 100.0%. It is possible from the present results to confirm the concept of Breidenstein et al. [87] that *P. aeruginosa* has a high level of intrinsic resistance to most antibiotics, particularly for  $\beta$ -lactam through restricted outer membrane permeability, efflux systems that pump antibiotics out of the bacterial cell and production of antibiotic-inactivating enzymes such as  $\beta$ -lactamases. On the other hand, a relatively low resistance was demonstrated with Ceftriaxone and Cefoperazone ranging from 9.0% up to 29.0%. Comparable results were previously announced by Ghariieb et al.40 who demonstrated that 0.0%–66.7% of *P. aeruginosa* isolates exhibited resistance to cephalosporins. Metronidazole, which is an antimicrobial used in human and veterinary practice on a large scale for treatment of diarrheic cases, showed a very high degree of resistance reaching up to 100.0%. These findings are supported by Morrill et al. [88] who observed that all *Enterobacteriaceae* appeared to be resistant to Metronidazole. Also, a moderate level of resistance for aminoglycosides (43.0% to 82.0%) was observed. That may be attributed to the genetic determinant (*rmtA*) responsible for high-level panaminoglycoside resistance in *P. aeruginosa* [89]. Likewise, TMP-Sulpha (trimethoprim+ Sulphamethoxazole) showed 45.0% to 59.0% resistance in contrast to Mizdal et al. [90] who found that TMP-Sulpha compounds presented a remarkable inhibition of *P. aeruginosa* biofilm.

Shifting to the antibiotic sensitivity pattern of *Klebsiella* spp. (Table 5), a relative resistance to Amoxicillin with or without clavulanic acid was observed ranging from 46.0% to 69.0% which is lower than that noted by Oliveira et al. [91] who revealed a high rate (>90.0%) of resistance for *Klebsiella* spp. to most  $\beta$ -lactam antibiotics. In addition, a resistance ranging from (15.0% to 50.0%) was recognized against Ceftriaxone, which is in harmony with that detected by Ghenea et al. [92]

(53.51%). Furthermore, *Klebsiella* spp. were seen to have a considerably low resistance to Cefoperazone and Cefotaxime (15.0% to 38.0%), nevertheless, it is still higher than 1.4% remarked by Abdullah et al. [93]. The obtained resistance of *Klebsiella* spp. to  $\beta$ -lactam antibiotics might be referred to the extended-spectrum  $\beta$ -lactamase (ESBL) as well as target mutation of gene-coding PBPs [94,95]. *Klebsiella* spp., additionally, appeared to have a moderate degree of resistance for TMP-Sulpha (31.0% to 67.0%), less than 86.4% and 93.9% obtained by Gad et al.[96] and Breidenstein et al. [97] respectively. It is worth noting that aminoglycoside antibiotics were associated with remarkably low resistance rates (15.0% to 33.0%) which run with that published by Landman et al. [98] (4.5% to 25.7%) but significantly lower than the report of Fernández-Martínez et al. [99] (74.8%). However, a particular gene coded as 1162281, with several additional other genes, could be encoding trimethoprim-insensitive DHFR (dihydrofolate reductase), a pentapeptide repeat protein, and sulphonamide-resistant dihydropteroate synthase beside several aminoglycoside-related resistance genes [100]. Aminoglycoside resistance might also be related to the production of AMEs (aminoglycoside-modifying enzymes) [99].

Regarding the molecular techniques illustrated in Figure 1, *P. aeruginosa* was identified by species using the *16S rDNA* gene, which was amplified 618 bp. *K. pneumonia* was identified by *16S-23S ITS* gene sequencing, which resulted in an amplified 130 bp, and *K. oxytoca* was identified using the *pehX* gene, which was amplified 343 bp. Furthermore, the ability of those pathogens to form biofilms (*pelA* and *mrkA* genes), aggregations of bacteria growing within a protective matrix of extracellular polymeric substances, is one of their most significant virulence factors. This allows the bacteria to be protected from harsh environmental conditions like chemical and physical stresses and limited nutrient availability. Because biofilms can stick to a variety of surfaces, including lung tissue and medical equipment, it can be challenging for the host immune system or medications to eradicate the infection [101,102]. Furthermore, the EPS matrix of the biofilm can promote the dissemination of antibiotic resistance genes by facilitating the transfer of genetic material between several bacteria [103,104]. Thus, methods for stopping or preventing the production of biofilms are crucial for maintaining public health, especially in hospital environments [105]. Furthermore, a number of toxins that *Pseudomonas* excretes add to the bacterium's pathogenicity [106]. Among these is exotoxin A, which prevents the creation of proteins. Additionally, the detection of *rmpA* gene markers delineates high specificity that could activate the production of capsule increasing the *Klebsiella* isolates' virulence [107,108].

## Conclusions

The current findings showed that *P. aeruginosa* and *Klebsiella* spp. shedding was common in human and animal fecal samples. Additionally, the pattern of drug resistance to the majority of commonly used antibiotics is increasing that was indicated by detection of biofilm markers, which is a major challenge and a serious concern for the one health. To slow the spread of resistant microorganisms, improvements in antibiotic stewardship programs are essential.

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## Ethical approval

Animal and human sampling design and protocol was carried out based upon the International Animal Care and Use Committee (IACUC), Ref. No: (Ref. No: IRB-FVM-MU-2023-103), Minia University, Egypt.

## Funding statement

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## Conflicts of interest

There are no conflicts to declare.

## Authors contribution

Ahmed E. Abdel-Ghany, Mohamed A. Ibrahim, Mohamed M. Abdel hakim1, Sahar Abdel Aleem Abdel Aziz2, shearing the conception and design of the study acquisition of data, analysis and interpretation of data and all the scientific writing, drafting the manuscript and grammar revision also writing revision.

**TABLE 1. Oligonucleotide and primer sequences specific for *P. aeruginosa* and *Klebsiella* spp. investigated during the study.**

Targeted bacteria	Genes	Sequences	Amplified products	Annealing	Reference
<i>K. pneumoniae</i>	<b>16S-23S</b>	ATTTGAAGAGGTTGCAAACGAT	130 bp	55°C	[30]
	<b>ITS</b>	TTCACCTCTGAAGTTTTCTTGTTGTTTC		30 sec.	
<i>K. oxytoca</i>	<b>pehX</b>	GATACGGAGTATGCCCTTACGGTG	343 bp	55°C	[31]
		TAGCCTTTATCAAGCGGATACTGG		40 sec.	
<i>Klebsiella</i>	<b>mrkA</b>	'GGTAAAGTTACCGACGTATCTTGTACT	475 bp	55°C	[32]
		GCTGTTAACCCACACCGGTGGTAAC		40 sec.	
		ACTGGGCTACCTCTGCTTCA		50°C	
	<b>rmpA</b>	CTTGCATGAGCCATCTTTC	535 bp	40 sec.	[33]
		GACAACGCCCTCAGCATCACCAGC		55°C	
		CGCTGGCCCCATTTCGCTCCAGCGCT		40 sec.	
<i>Pseudomonas</i>	<b>16S</b>	GACGGGTGAGTAATGCCTA	618 bp	50°C	[35]
		CACTGGTGTTTCCTTCCCTATA		40 sec.	
		CATACCTTCAGCCATCCGTTCTTC		60°C	
	<b>pehA</b>	CGCATTCCGCCGCACTCAG	786 bp37	40 sec.	[36]

**TABLE 2. Occurrence of *P. aeruginosa* and *Klebsiella* spp. in the examined animals and humans.**

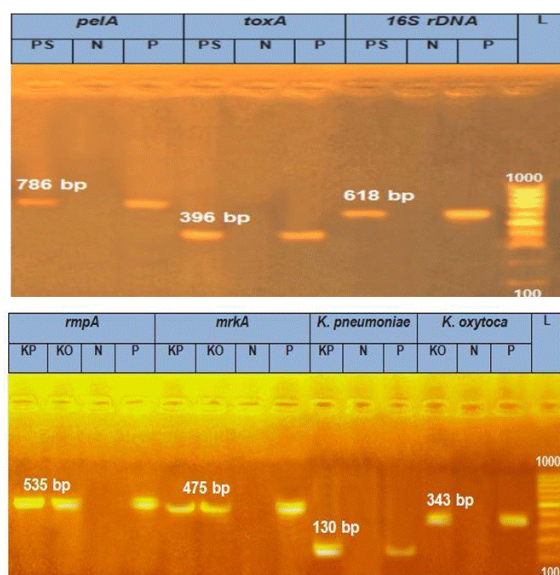
Animal species	Cattle (N=791)		Sheep (N=386)		Humans (N=304)	
	N. positive	%	N. positive	%	N. positive	%
<i>P. aeruginosa</i>	133	16.81	29	7.51	17	5.59
<i>K. oxytoca</i>	23	2.91	17	4.40	1	0.33
<i>K. pneumoniae</i>	11	1.39	12	3.11	6	1.97
<b>Total</b>	167	21.11	58	15.02	24	7.89

**P-value <0.000,  $\chi^2= 50.961$**









**Fig.1.** Agarose gel electrophoresis for PCR products of *P. aeruginosa*, *K. pneumoniae* and *K. oxytoca* isolates using identification-related genes (*16S rDNA*, *16S-23S ITS* and *pehX*), respectively as well as, virulence and biofilm markers (*toxA*, *rmpA*) and (*pelA* and *mrkA*), respectively. Lane (L): 100 bp Ladder "Marker", Lane (PS): *P. aeruginosa* tested isolates. While, KO: *K. oxytoca* and KP: *K. pneumoniae* screened isolates, Lane Pos: Positive control, Lane Neg: Negative control. human and animal fecal samples. Additionally, the pattern of drug resistance to the majority of commonly used antibiotics is increasing that was indicated by detection of biofilm markers, which is a major challenge and a serious concern for the one health. To slow the spread of resistant microorganisms, improvements in antibiotic stewardship programs are essential.

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## دراسة وبائية وجزيئية لتقييم العلامات المرتبطة بالأغشية الحيوية لبكتيريا السودومونس اريجينوزا والكليسيلا المسببة للإسهال عند الماشية والبشر

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### المستخلص

تعتبر كلا من ميكروبي السودومونس اريجينوزا والكليسيلا ذو علاقات مرتبطة بعدة أمراض في الإنسان والحيوانات، مما يشكل خطراً كبيراً على صحة الإنسان وتقدم تربية الحيوانات. تهدف الدراسة الحالية إلى تحديد مدى انتشار هذه البكتيريا في الأغنام والماشية والبشر، بالإضافة إلى تقييم حساسية المضادات الحيوية و خاصة تلك المستخدمة على نطاق واسع في مجال علاج الإنسان والحيوان. بالإضافة إلى استخدام التحاليل الجزيئية لتوضيح الجينات المرتبطة بالأغشية الحيوية وعوامل الضراوة في تلك البكتيريا. تم تجميع عينات براز من 791 من الأبقار و 386 من الأغنام، بالإضافة إلى 304 عينة من براز البشر. سُجل عزل بكتيريا السودومونس اريجينوزا في 16.81% و 7.51% و 5.59% من الماشية والأغنام والبشر على التوالي، في حين وجدت الكليسيلا نيموناي في 1.8% و 8.9% و 1.97% من عينات الماشية والأغنام والبشر على الترتيب. بالمثل، تم عزل كليسيلا أوكسينوكا من 2.91% و 4.4% و 0.33% من العينات تواليًا. أظهرت معظم البكتيريا المعزولة أنماطاً غير ثابتة من المقاومة للمضادات الحيوية المختلفة؛ ومع ذلك، فقد أظهرت السيفترياكسون تأثيراً جيداً. وقد كشف التوصيف الجزيئي للعينات المختبرة (*rmpA* و *pelA*) أنها كانت فتاكة وأظهرت المحددات الجينية قدرتها على تكوين البايوفيلم. وخلصت الدراسة أن مقاومة المضادات الحيوية ستطيل مدة العلاج لأن تكوين الأغشية الحيوية في العينات يمنع المضادات الحيوية من الوصول إلى البكتيريا. وعلاوة على ذلك، يبدو أن هناك علاقة بين ظهور المقاومة المتعددة للمضادات الحيوية وإنتاج الأغشية الحيوية.

**الكلمات الدالة:** براز، ماشية، أغنام، بشر، المقاومة، المضادات الحيوية، الأغشية الحيوية، عوامل الضراوة.