Characterization of *E. coli* and *P. aeruginosa* associated with diarrhea and pneumonia in calves and lambs

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1. Abstract

This study aimed to investigate the prevalence, antimicrobial resistance, biofilm forming ability and certain virulence-associated genes of *E. coli* and *P. aeruginosa* causing diarrhea and pneumonia in calves and lambs. A total of 101 samples including; 71 deep nasal swabs from pneumonic animals (57 calves and 14 lambs) and 30 fecal swabs from diarrheic animals (19 calves and 11 lambs) were investigated bacteriologically. Regarding pneumonic calves and lambs examined samples, 33 (57.9%) and 7 (50%) *E. coli* as well as 2 (3.5%) and 6 (42.9%) *P. aeruginosa* isolates were recovered, respectively. Meanwhile the enteric samples revealed that 17 (89.5%) and 5 (45.5%) *E. coli* were recovered, respectively while only one *P. aeruginosa* isolate (5.2%) was recovered from calves. The *in vitro* antimicrobial susceptibility testing revealed that all *E. coli* isolates either from pneumonic or diarrheic calves or lambs showed complete resistance against amoxicillin, amoxicillin-clavulanic acid, and ceftriaxone while they showed complete or high susceptibility to amikacin, gentamicin and apramycin. Concerning *P. aeruginosa* isolates, isolates either from pneumonic calves or lambs revealed complete or even high resistance against amoxicillin, amoxicillin-clavulanic acid, ceftriaxone, cephalexin, kanamycin, and sulfamethoxazole-trimethoprim, while they were completely or highly susceptible to amikacin, apramycin, gentamicin, and colistin. The biofilm formation assay of the tested *E. coli* and *P. aeruginosa* isolates from pneumonic samples revealed that a total of 4/9 tested *E. coli* (44.4%) and 3/6 tested *P. aeruginosa* (50%) were positive biofilm formers while in enteric isolates, a total of 3/5 *E. coli* (60%) and one *P. aeruginosa* from calves (100%) were positive. Results of PCR showed that Five *E. coli* isolated from diarrheic calves harbored *eae*A virulence gene while 60% of the tested isolates harbored *hly* and *chu*A genes and 40% harbored *yja*A and *tsp*E4C2 genes. The three examined *P. aeruginosa* isolated from pneumonic lambs harbored *fli*C, *exo*S, and *tox*A virulence genes. In conclusion, the synergism between phenotypic and genotypic methods is effective for characterizing *E. coli* and *P. aeruginosa* associated with diarrhea and pneumonia.

Keywords: Antimicrobial susceptibility; Calves; Lambs; *E. coli*; *P. aeruginosa*

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2. Introduction

Diarrhea and pneumonia are frequent causes of morbidity and mortality in calves that affect frequently on livestock propagation [1]. Neonatal diarrhea is still the driving force behind the cause of small ruminants and calf mortality in Egypt that ranges between 27.4-55% in newly born calves and 75% in newly born calves in the dairy sector worldwide [2, 3, 4].

Pneumonia is a disorder has multiple contributing factors in calves and lambs causing more than 10% and 1% morbidity and mortality of young calves, respectively, worldwide that it may be associated with bacteria involving; *E. coli, P. aeruginosa* [5, 6].

E. coli (k99 and f41) are triggering very severe dehydration and metabolic imbalance within a few hours of the beginning of the disease in calves that less than one week old. *E. coli* is harboring specific virulence factors including; production of colicins, resistance of phagocytosis, resistance of killing by serum, utilization of iron acquisition system, and adherence, colonization, and invading host cells [7].

P. aeruginosa is an opportunistic pathogen that may cause the lower respiratory tract infection and enteritis contributing to its pathogenicity through the production of enterotoxins that causing gastroenteritis (diarrhea and enteritis) and several protein exotoxins [8].

The indiscriminate usage of the antimicrobial agents triggered a rises in multidrug-resistant in both human populations and veterinary medicine that became more widespread worldwide [9].

Biofilm is considered a significant problem for the public health that regulated by the mechanism of quorum sensing (QS) through the up and down regulation of the associated genes in which the biofilm formation is a group of bacterial cells that surrounded in a polysaccharide matrix and bonded on biotic or abiotic surfaces that leading to an increase in the resistance of the micro-organisms to antibiotics through prevention of them from penetrating the bacterial cells in the biofilm by altering the plasmids which responsible for antibiotic resistance [10, 11].

This study aimed to determine the antimicrobial susceptibility and biofilm formation of *E. coli* and *P. aeruginosa* isolated from different samples from calves and lambs.

3. Materials and Methods

3.1. Ethical statement

Animal care and experimental protocols were approved by the Institutional Animal Ethics Committee of Beni-Suef University (BSU-IACUC), Egypt (Number 022-488).

3.2. Animals

A total of 101 samples including; 71 deep nasal swabs from pneumonic

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animals (57 calves and 14 lambs) suffering from signs of pneumonia (coughing, nasal discharging, abnormal lung sounds and fever) and 30 fecal swabs from diarrheic animals (19 calves and 11 lambs). Samples were collected during the period from September to December 2022 from different farms located in El-Fayoum Governorate.

3.3. Bacteriological examination of the collected samples

Bacterial isolation was performed according to Collee et al. [12]. Nasal and fecal swabs were collected by sterile bacteriological swabs from the diseased calves and lambs. These swabs were transported within 2-3 hrs in ice box to the laboratory of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Beni-Suef University.

The samples were inoculated into tryptone soya broth (TSB) and MacConkey's broth and incubated for18-24 hrs at 37˚C. A loopful of each broth cultures was streaked onto tryptone soya agar (TSA), MacConkey's agar, eosin methylene blue (EMB), and *Pseudomonas* cetrimide agar and incubated for 24 hrs at 37˚C. The colonies were observed for their cultural characters and morphological appearance. All suspected isolated bacteria were subjected to Gram's staining and then identified biochemically by oxidase, catalase, triple sugar iron (TSI) and IMViC tests according to Collee et al. [12] and Quinn et al. [13].

3.4. Antimicrobial susceptibility test

The antimicrobial susceptibility of *E. coli* and *P. aeruginosa* isolates to 10 antimicrobial discs; including amoxicillin (25 µg), amoxicillin-clavulanic acid (30 µg), ceftriaxone (30 µg), cephalexin (30 µg), amikacin (30 µg), gentamicin (10 µg), apramycin (15 µg), kanamycin (30 µg), colistin (10 µg), and sulfamethoxazole-trimethoprim (25 µg) (Himedia, India), was performed using the agar disc diffusion test on Mueller Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) [14].

3.5. Detection of biofilm formation

The ability of *E. coli* and *P. aeruginosa* isolates to form biofilm was evaluated using Congo red (CR) method to evaluate curli production [15]. The pure colonies of 9 *E. coli* and 6 *P. aeruginosa* isolates from pneumonic samples and 5 *E. coli* and one *P. aeruginosa* isolated from enteric samples were cultivated on Luria bertani (LB) agar plates and incubated at 37˚C for 48 hrs. Single colonies were picked up and cultivated on YESCA CR agar plates and incubated at 25 ˚C for 48-72 hrs. The red bacterial colonies were positive for biofilm formation while the white colonies were negative for biofilm formation.

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3.6. Molecular characterization of virulence genes in E. coli and P. aeruginosa isolates

DNA from 5 *E. coli* and 3 *P. aeruginosa* isolated from diarrheic calves and pneumonic lambs, respectively was extracted using the QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer instructions. The polymerase chain reaction (PCR) amplification of *E. coli* and *P. aeruginosa* isolates using specific primers (Table 1). Twenty five microlitter Volume/reaction mixture including 12.5 µl Emerald Amp GT PCR master mix (2x premix) (Takara, Japan), 5.5µl PCR grade water, 1µl forward primer (20pmol), 1µl reverse primer (20pmol), and 5µl template DNA). 35 cycles of the PCR protocol for each (primary denaturation for 5min at 94˚C, secondary denaturation for 30 sec at 94˚C, annealing for 30-40 sec at 51-60˚C (depending on the primer), extension for 30sec-1min at 72˚C and finally final extension for 7-10 min at 72˚C. 1.5% gel was prepared and electrophoresed then photographed using a gel documentation system.

4. Results

4.1. Prevalence and identification of E. coli and P. aeruginosa isolated from calves and lambs

Out of 57 and 14 pneumonic calves and lambs examined samples, 33 (57.9%) and 7 (50%) *E. coli* as well as 2 (3.5%) and 6 (42.9%) *P. aeruginosa* isolates were recovered, respectively. On the other hand, out of 19 and 11 enteric examined samples, 17 (89.5%) and 5 (45.5%) *E. coli* were recovered, respectively while only one *P. aeruginosa* isolate (5.2%) was recovered from calves (Table 2).

4.2. Antimicrobial Susceptibility Test

The *in vitro* antimicrobial susceptibility assay revealed that the *E. coli* isolates detected from diarrheic calves showed complete resistance (100%) against amoxicillin, amoxicillin-clavulanic acid, and ceftriaxone, followed by colistin (80%). while they revealed complete susceptibility (100%) to amikacin, gentamicin, and apramycin, followed by cephalexin, kanamycin, and sulfamethoxazole-trimethoprim (80% for each). Concerning *E. coli* isolates from pneumonic samples of calves, they also were completely resistant to amoxicillin, amoxicillin-clavulanic acid, and ceftriaxone while they were completely susceptible to amikacin and gentamicin, followed by apramycin (86.7%), sulfamethoxazole-trimethoprim (73.3%), kanamycin (66.7%), and colistin (60%) (Table 3).

Meanwhile, *E. coli* isolates detected from diarrheic lambs showed complete resistance (100%) against amoxicillin, amoxicillin-clavulanic acid, and ceftriaxone, followed by colistin (80%). while they revealed complete susceptibility (100%) to amikacin and gentamicin, followed by apramycin (80%), then cephalexin, kanamycin and

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sulfamethoxazole-trimethoprim (60% for each). Concerning *E. coli* isolates from pneumonic samples of lambs, they also were completely resistant to amoxicillin, amoxicillin-clavulanic acid, and ceftriaxone while they were completely susceptible to amikacin and gentamicin, followed by apramycin (85.7%), then sulfamethoxazole-trimethoprim and kanamycin (71.4% for each), and colistin (57.1%)(Table 4).

Regarding *P. aeruginosa* isolates, isolates from pneumonic calves revealed complete resistance against amoxicillin, amoxicillin-clavulanic acid, ceftriaxone, cephalexin, kanamycin, and sulfamethoxazole-trimethoprim, while they were completely susceptible to amikacin, apramycin, gentamicin, and colistin. Also, *P. aeruginosa* isolates from pneumonic lambs revealed complete resistance against amoxicillin, amoxicillin-clavulanic acid, ceftriaxone, and cephalexin, followed by sulfamethoxazole-trimethoprim (66.7%) while they were completely susceptible to amikacin, gentamicin, and colistin , followed by apramycin (66.7%)(Table 5). One *P. aeruginosa* isolate recovered from diarrheic calves showed resistance against amoxicillin, amoxicillin-clavulanic acid, ceftriaxone, cephalexin, kanamycin, and sulfamethoxazole-trimethoprim while was susceptible to amikacin, gentamicin, apramycin, and colistin.

4.3. Biofilm formation

As shown in (Table 6) the biofilm formation of the tested *E. coli* and *P. aeruginosa* isolates on YESCA CR agar revealed that a total of 7/15 tested isolates from pneumonic samples were positive biofilm formers including 4/9 (44.4%) *E. coli* isolates (3/5; 60% from calves and 1/4; 25% from lambs) and 3/6 (50%) *P. aeruginosa* isolates (1/2; 50% from calves and 2/4; 50% from lambs). Regarding the tested enteric *E. coli* and *P. aeruginosa* isolates, a total of 5/6 tested isolates were positive biofilm formers including 3/5 (60%) *E. coli* isolates (2/3; 66.7% from calves and 1/2; 50% from lambs) and one *P. aeruginosa* from calves (100%).

4.4. Molecular characterization of virulence genes in E. coli and P. aeruginosa

Five *E. coli* isolated from diarrheic calves were screened by PCR to monitor 5 virulence genes (*eae*A, *hly*, *yja*A, *tsp*E4C2, and *chu*A). It is clear that *eae*A gene was the most prevalent gene found in all isolates (100%), followed by both *hly* and *chu*A genes (60% for each) and finally both *yja*A, and *tsp*E4C2 genes (40% for each). Moreover, 3 *P. aeruginosa* isolates from pneumonic lambs were screened for 3 virulence genes including *fli*C, *exo*S, and *tox*A genes which were recorded in all the tested isolates (100%) (Tables 7& 8 and Figs. 1-3).

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5. Discussion

Diarrhea and pneumonia are frequent causes of morbidity and mortality in calves [16]. Calf illnesses including pneumonia and diarrhea are multifactorial disorders caused by complex interactions of the environment and management techniques, infectious agents [17].

In the current study, out of 71 deep nasal swabs from pneumonic calves and lambs, 57.9% (33/57) calves and 50% (7/14) lambs' samples were *E. coli* positive. While 3.5% (2/57) calves and 42.9% (6/14) lambs' isolates were *P. aeruginosa* positive among pneumonic cases. The lowest results were reported as 23.5% and 15.7% *E. coli* isolated from pneumonic samples of calves [6, 18]. Concerning *P. aeruginosa* a lower prevalence from pneumonic samples of lambs recorded with a percentage of 20% [8]. A higher prevalence of *P. aeruginosa* from pneumonic samples of calves recorded as 4.5% and 13.3% [19, 18].

Out of 30 fecal swab samples collected from diarrheic calves and lambs, 89.5% (17/19) of calves and 45.5% (5/11) of lambs' samples were positive for *E. coli* isolation. However, a lower result of *E. coli* was reported with an incidence of 47% [20] and a higher result was recorded with an incidence of 100% [21]. In the present study, 5.2% (1/19) of calves' samples were positive for *P. aeruginosa*. This result was nearly similar to that recorded by Abd El-Tawab et al. [20] who isolated *P. aeruginosa* with a percentage of 4%.

Antibiotics are commonly used in animal medicine to treat a variety of bacterial infections. However, the indiscriminate antibiotic usage is related with the emergence of antimicrobial resistance in bacterial pathogens. Antimicrobial resistance is a major problem not just to animals but perhaps to humans [22].

The current study revealed that all enteric or pneumonic *E. coli* isolates recovered from calves were completely resistant to amoxicillin, amoxicillin-clavulanic acid, and ceftriaxone while they were completely or highly susceptible to amikacin, gentamicin, and apramycin. These results were in agreement with Elhady et al. [23] who reported that all *E. coli* isolates recovered from diarrheic calves were resistant to amoxicillin and sensitive to amikacin. The lowest resistance against colistin was reported with a rate of 30% [24].The highest resistance of *E. coli* isolates recovered from pneumonic calves was recorded against gentamicin with a percentage of 90% [6].

Also, all enteric or pneumonic *E. coli* isolates recovered from lambs were completely resistant to amoxicillin, amoxicillin-clavulanic acid, and ceftriaxone while they were completely susceptible to amikacin and gentamicin. These results were disagreed with Bkheet et al. [25] who recorded the lowest susceptibility of *E. coli* isolates recovered

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from diarrheic lambs to gentamicin with a rate 30%.

The antimicrobial susceptibility of *P. aeruginosa* isolates either from pneumonic calves or lambs revealed complete or even high resistance against amoxicillin, amoxicillin-clavulanic acid, ceftriaxone, cephalexin, kanamycin, and sulfamethoxazole-trimethoprim, while they were completely or highly susceptible to amikacin, apramycin, gentamicin, and colistin. These findings were agreed with what reported by Algammal et al. [6] who recorded a complete resistance of *P. aeruginosa* isolated from pneumonic calves to amoxicillin and complete susceptibility to gentamicin.

The biofilm formation is important for bacterial pathogens as it assists the long-term colonization and shields the bacterial cell against antibiotics [26, 27]. The current results showed that a total of 4/9 tested *E. coli* (44.4%) and 3/6 tested *P. aeruginosa* (50%) were positive biofilm formers while in enteric isolates, a total of 3/5 *E. coli* (60%) and one *P. aeruginosa* from calves (100%) were positive. A previous study reported that 32.9% and 17.1% of *E. coli* and *P. aeruginosa* isolates, respectively from cattle were able to produce biofilm on Congo red agar [28]. Moreover, *E. coli* isolates from diarrheic sheep were able to produce biofilm with a percentage of 81.2% as 6.6% and 74.6% moderate and weak biofilm production, respectively [29].

In the present study, All *E. coli* isolates recovered from diarrheic calves carried *eae*A virulence-associated gene while 60% of the tested isolates harbored *hly* and *chu*A genes and 40% harbored *yja*A and *tsp*E4C2 genes. The prevalences of *eae*A and *hly* genes among *E. coli* isolates were similar to previous findings reported the presence of *eae*A (100%) and *hly* (60%) genes in *E. coli* isolated from diarrheic calves [30, 23]. *eae*A gene encodes intimin protein that causing characteristic attaching and effacing intestinal lesions of entero-pathogenic *E. coli* (EPEC), while entero-hemorrhagic *E. coli* (EHEC), a virulent strain of STEC, has a plasmid coding for hemolysin (*hly*A) [21]. It has been suggested that the *chu*A gene is responsible for the heme-transport in entero-hemorrhagic *E. coli* (EHEC) [31]. *yja*A gene encodes an uncharacterized protein, while *tsp*E4C2, a DNA fragment that recently found as part of lipase esterase gene, Phylogenetic classification of *E. coli* strains based on the combination of *chu*A, *yja*A, and *tsp*E4C2 genes [32]. Concerning *P. aeruginosa* isolates of pneumonic lambs, all the tested isolates harbored *fli*C, *exo*S, and *tox*A virulence genes (100%). In which *fli*C, *tox*A, and *exo*S genes have a crucial role in tissue penetration, cell death, and anti-internalization activities, respectively [33, 34].

6. Conclusion

Diarrhea and pneumonia are multifactorial disorders. *E. coli* and *P.*

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aeruginosa are main bacterial causes. Recovered isolates' pathogenecity contributing to their virulence genes, as *eae*A, *hly* and *chu*A for *E. coli* while, *fli*C, *exo*S and *tox*A for *P. aeruginosa*. In addition to their ability to form a biofilm and resist most of the commercial antimicrobials.

Conflict of interest

The authors declare no conflict of interest.

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Table (1): Oligonucleotide primer sequences for some virulence genes used in this study

Bacteria	Gene	Primer Sequence 5'-3'	Amplified product (bp)	Reference
P. aeruginosa	*fli*C	*TGAACGTGGCTACCAAGAACG*	180	[35]
		TCTGCAGTTGCTTCACTTCGC		
	*tox*A	*GACAACGCCCTCAGCATCACCAGC*	396	[36]
		CGCTGGCCCATTCGCTCCAGCGCT		
	*exo*S	*GCGAGGTCAGCAGAGTATCG*	118	[37]
		TTCGGCGTCACTGTGGATGC		
E. coli	*eae*A	*ATGCTTAGTGCTGGTTTAGG*	248	[38]
		GCCTTCATCATTTCGCTTTC		
	hly	*AACAAGGATAAGCACTGTTCTGGCT*	1177	[39]
		ACCATATAAGCGGTCATTCCCGTCA		
	*chu*A	*GACGAACCAACGGTCAGGAT*	279	[40]
		TGCCGCCAGTACCAAAGACA		
	*yja*A	*TGAAGTGTCAGGAGAYGCTG*	211	
		ATGAAGAATGCGTTCCTCAAC		
	*tsp*E4C2	*GAGTAATGTCGGGGCATTCA*	152	
		CGCGCCAACAAAGTATTACG		

Table (2): The prevalence rate of *E. coli* and *P. aeruginosa* isolates from the pneumonic and enteric samples of diseased calves and lambs

Species / Isolates	Calves		Lambs	
	No.*	%	No.*	%
Pneumonic samples				
E. coli	33/57	57.9	7/14	50
P. aeruginosa	2/57	3.5	6/14	42.9
Enteric samples				
E. coli	17/19	89.5	5/11	45.5
P. aeruginosa	1/19	5.2	0/11	0

*** Positive number / number of examined samples**

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Table (3): The antimicrobial susceptibility profile of *E. coli* isolated from enteritis and pneumonia in calves

Antimicrobial agent	Disc content (µg)	*E. coli* isolates (n=25)							
		Enteric calves samples (n=10)				Pneumonic calves samples (n=15)			
		Resistance		Susceptible		Resistance		Susceptible	
		NO.	%	NO.	%	NO.	%	NO.	%
Amoxicillin (AX)	30	10	100	0	0	15	100	0	0
Amoxicillin-Clavulanic acid (AMC)	30	10	100	0	0	15	100	0	0
Ceftriaxone (CTR)	30	10	100	0	0	15	100	0	0
Cephalexin (CL)	30	2	20	8	80	7	46.7	8	53.3
Amikacin (AK)	30	0	0	10	100	0	0	15	100
Apramycin (APR)	30	0	0	10	100	2	13.3	13	86.7
Gentamicin (GEN)	10	0	0	10	100	0	0	15	100
Kanamycin (K)	30	2	20	8	80	5	33.3	10	66.7
Colistin (CL)	10	8	80	2	20	6	40	9	60
Sulfamethoxazole-Trimethoprim (SXT)	25	2	20	8	80	4	26.7	11	73.3

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Table (4): The antimicrobial susceptibility profile of *E. coli* isolated from enteritis and pneumonia in lambs

Antimicrobial agent	Disc content (µg)	*E. coli* isolates (n=12)							
		Enteric lambs samples (n=5)				Pneumonic lambs samples (n=7)			
		Resistance		Susceptible		Resistance		Susceptible	
		NO.	%	NO.	%	NO.	%	NO.	%
Amoxicillin (AX)	30	5	100	0	0	7	100	0	0
Amoxicillin-Clavulanic acid (AMC)	30	5	100	0	0	7	100	0	0
Ceftriaxone (CTR)	30	5	100	0	0	7	100	0	0
Cephalexin (CL)	30	2	40	3	60	4	57.1	3	42.9
Amikacin (AK)	30	0	0	5	100	0	0	7	100
Apramycin (APR)	30	1	20	4	80	2	28.6	5	71.4
Gentamicin (GEN)	10	0	0	5	100	0	0	7	100
Kanamycin (K)	30	2	40	3	60	0	0	7	100
Colistin (CL)	10	4	80	1	20	4	57.1	3	42.9
Sulfamethoxazole-Trimethoprim (SXT)	25	1	20	4	80	3	42.9	4	57.1

Table (5): The antimicrobial susceptibility profile of *P. aeruginosa* isolated from pneumonic lambs and calves

Antimicrobial agent	Disc content (µg)	*P. aeruginosa* isolates (n=8)							
		Pneumonic calves samples (n=2)				Pneumonic lambs samples (n=6)			
		Resistance		Susceptible		Resistance		Susceptible	
		NO.	%	NO.	%	NO.	%	NO.	%
Amoxicillin (AX)	30	2	100	0	0	6	100	0	0
Amoxicillin-Clavulanic acid (AMC)	30	2	100	0	0	6	100	0	0
Ceftriaxone (CTR)	30	2	100	0	0	6	100	0	0
Cephalexin (CL)	30	2	100	0	0	6	100	0	0
Amikacin (AK)	30	0	0	2	100	0	0	6	100
Apramycin (APR)	30	0	0	2	100	2	33.3	4	66.7
Gentamicin (GEN)	10	0	0	2	100	0	0	6	100
Kanamycin (K)	30	2	100	0	0	3	50	3	50
Colistin (CL)	10	0	0	2	100	0	0	6	100
Sulfamethoxazole-Trimethoprim (SXT)	25	2	100	0	0	4	66.7	2	33.3

Table (6): Results of biofilm formation of *E. coli* and *P. aeruginosa* isolated from pneumonic lambs and calves

Isolates	Positive biofilm producers					
	No.*	%	No.*	%	Total	
Pneumonic samples					No.*	%
	Calves isolates (n=7)		Lambs isolates (n=8)			
***E. coli* (n=9)**	3/5	60	1/4	25	4/9	44.4
***P. aeruginosa* (n=6)**	1/2	50	2/4	50	3/6	50
Enteric samples					No.*	%
	Calves isolates (n=4)		Lambs isolates (n=2)			
***E. coli* (n=5)**	2/3	66.7	1/2	50	3/5	60
***P. aeruginosa* (n=1)**	1/1	100	-	-	1/1	100

*** Positive number / number of examined samples**

Table (7): Virulence-associated genes of *E. coli* isolated from enteric samples of calves

Isolates	Gene	Positive		Negative	
		No.	%	No.	%
***E. coli* (n=5)**	***eae*A**	5	100	0	0.0
	hly	3	60	2	40
	***chu*A**	3	60	2	40
	***yja*A**	2	40	3	60
	***tsp*E4C2**	2	40	3	60

Table (8): Virulence-associated genes of *P. aeruginosa* isolated from pneumonic samples of lambs

Isolates	Gene	Positive		Negative	
		No.	%	No.	%
***P. aeruginosa* (n=3)**	***fli*C**	3	100	0	0.0
	***exo*S**	3	100	0	0.0
	***tox*A**	3	100	0	0.0

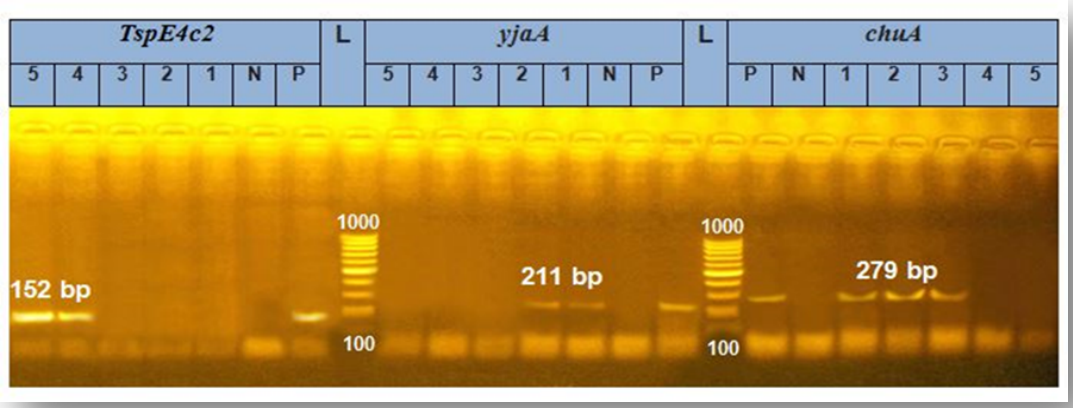

Fig (1): Agarose gel electrophoresis showing positive amplification of *tsp*E4C2, *yja*A,and *chu*A genes of *E. coli* isolated from diarrehic calves at 152, 211 and 279 bp, respectively using specific PCR primer. N: Negative control, P: Positive control, and L: (100-1000 bp) DNA ladder.

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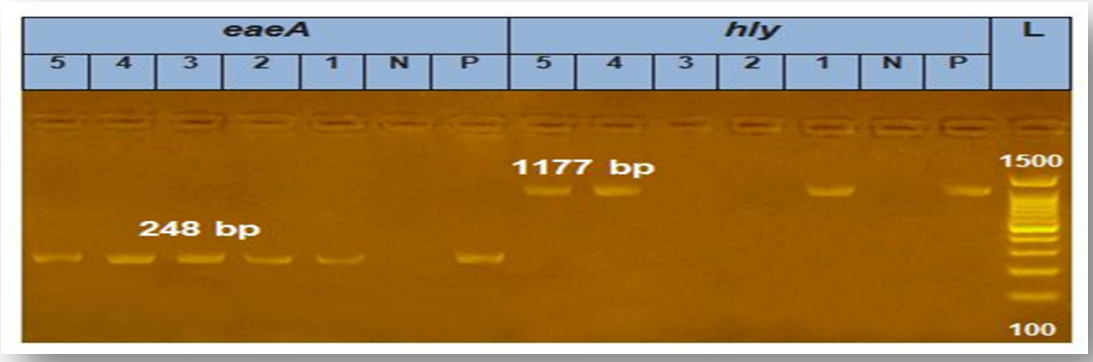

Fig (2): Agarose gel electrophoresis showing positive amplification of *eae*A and *hly* genes of *E. coli* isolated from diarrehic calves at 248 and 1177 bp, respectively using specific PCR primer. N: Negative control, P: Positive control, and L: (100-1000 bp) DNA ladder

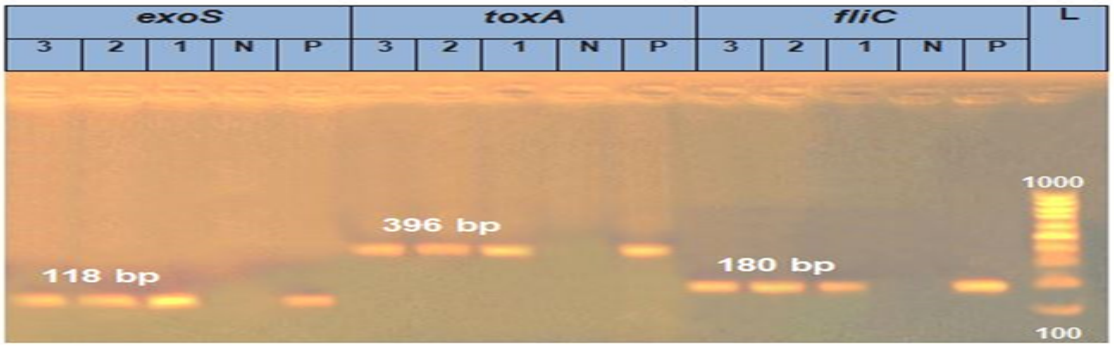

Fig (3): Agarose gel electrophoresis showing positive amplification of *exo*S, *tox*A and *fli*C genes of *P. aeruginosa* isolated from pneumonic lambs at 118, 396 and 180 bp, respectively using specific PCR primer. N: Negative control, P: Positive control, and L: (100-1000 bp) DNA ladder.