Investigation on Some Bacterial and Fungal Causes Respiratory Infections in Egyptian Buffalloes

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Abstract:

Respiratory microbial infections were a major health problems of Egyptian buffaloes and had economic looses in meat and milk production. That caused pneumonia leading to morbidity and mortality in intensive rearing system. The aim was to study some investigations on some bacterial and fungal causes respiratory infections in Egyptian buffalloes. That were by isolation and identification to detect molecularity and antibiogram sensitivities. The methodology were included samples collection, microbial isolation and identification, antibiogram technique, DNA extraction and PCR amplification protocol for microorganisms. in the present study was conducted to examine 100 nasal swabs of buffalos (1_5 years) reared on private farms at El Dakahlia Governorate showing symptoms of depression, reduced the feed intake, respiratory problems_ dyspnea with or without nasal discharge and pneumonia also 50 mouth swabs collected from mucous discharge of buffalos suffering from cough and sneezing. Pasteurella multocida (p. multocida), Escherichia coli (E.coli) and Staphylococcus aureus(staph aureus) where isolated in percentage of (12%_7%_5%) from nasal swabs respectively and (8%_4%_4%) from mouth swabs respectively. The main isolated fungi were Aspergills fumigatus (A. fumigatus) and Aspergillus flavous (A.flavous) that isolated percentage (11% and 5%) from nasal swabs respectively and (8% and 4%) from mouth swabs respectively . p. multocida were sensitive to enrofloxacin, spectinomycin, and trimethoprim-sulfamethoxazole, Multi-Drug Resistance (MDR) to amoxicillin, ciprofloxacin, gentamicin, streptomycin, and chloramphenicol. E. coli were sensitive to ciprofloxacin, enrofloxacin, spectinomycin, and chloramphenicol, had MDR to amoxicillin,

gentamicin, streptomycin, and trimethoprim-sulfamethoxazole. Staph. aureus were sensitive to enrofloxacin, and spectinomycin, had MDR to amoxicillin, ciprofloxacin, gentamicin, streptomycin, trimethoprim- sulfamethoxazole, and chloramphenicol. A. fumigatus was sensitive to fluconazole, ketoconazole, nystatin, and amphotericin B, had MDR to clotrimazole. A. flavus were sensitive to fluconazole, ketoconazole, and nystatin, had MDR to clotrimazole, and amphotericin B., The PCR using the KMT1 gene confirmed P. multocida isolates, developed specific 457 bp molecular size bands. The 16s rRNA gene confirmed E. coli isolates, developed specific 662 bp molecular size bands. The Coagulase gene confirmed Staph. aureus isolates, developed specific 600-1000 bp molecular size bands. The Asphs gene confirmed A. fumigatus isolates, developed specific 180 bp molecular size bands. The aflR1 gene confirmed A. flavus isolates, developed specific 798 bp molecular size bands. It was concluded that the microbial respiratory infection affected farm health. It was recommended for taken animal health precaution for protection from respiratory microbial infection for safe farm health.

Key words: Egyptian Buffalloes, Pasteurella multocida, Escherichia coli, Staphylococcus aureus, Asprigullus fumigatus, Asprigullus flavus .

INTRODUCTION

Respiratory infections were a major health problems of Egyptian buffaloes and economic looses in meat and milk production and in sever cases may cause pneumonia leads to morbidity and mortality in buff-calves especially in intensive rearing system **(Ismail *et al* ., 1993).**

P. multocida was isolated from respiratory diseases. The most active antibacterial enrofloxacina and gentamycin, Random

Amphified Polymymerase DNA (RAPD) diagnosed *P. multocida* **(Zakaria *et al.*, 2013).** El-Menoufea Ruminant Nasal swabs had pneumonia resulted *P. multocida* 18% and 14% in nasal swabs and lung tissues. Polymerase chain reaction (PCR) diagnosed *P. multocida* sensitive to florfenicol, gentamicine, amoxcilline, enrofloxacine and trimethoprim **(Sayed *et al.*, 2014).** The five selective *P. multocida* had similar size of PCR products having *KMT1 gene*. The phylogenetic tree and similarity of the five selective from GenBank shared 94.08% homology with buffalo isolates **(Hassan *et al.*, 2017).** *P. multocida* were confirmed by PCR. They were sensitive to nitrofurantoin, florfenicol, ciprofloxacin, enrofloxacin, trimethoprim-sulfamethoxazole, oxytetracycline, and ceftriaxone. It was resistance to tylosin and oxacillin **(Gharibi *et al.*, 2017).** Isolates were *P. multocida* either morphological and biochemical, multiplex PCR identified using universal primers **(Abbas *et al.*, 2018).** The etiological of buffaloes respiratory disease were *P. multocida*, was identified by PCR **(Reddy and Subramanyam, 2018).** Buffaloes samples had *P. multocida*, had multi-drug resistance (MDR)to penicillin, cephalosporins and fluoroquinolones **(Choudhary *et al.*, 2019).** *P. multocida* isolated from pneumonia ruminant in Beni-Suef and El-Fayoum. PCR *kmt1* showed 87.9% positive for corresponding universal *gene* **(Abed et al. 2020).** Nasal swabs from El-Menoufea and El-Qalyubia, Egypt, were confirmed by *kmt1* existence. They resistances chloramphenicol, ciprofloxacin, amoxicillin/clavulanic acid, and levofloxacin **(Elsayed *et al.*, 2021).** *P. multocida* 16.22% were isolated from Egyptian buffaloes had respiratory manifestations. It had MDR against ampicillin, amoxicillin, penicillin-G, tetracycline, streptomycin, cefotaxime and chloramphenicol **(Bahr *et al.*, 2021).**

The nasal discharges from clinical cases revealed *E. coli* 8.33% **(Reddy and Subramanyam, 2018).** Buffaloes Nasal

swabs had bacterial isolates by *16S rRNA* sequence analysis. The predominant were *E. coli*, MDR was 90% **(Choudhary *et al.*, 2019).** Calves samples had *E. coli* 16.4%, had MDR to gentamicin, erythromycin, streptomycin and trimethoprim-sulphamethoxazol **(Algammal *et al.*, 2020).**

Broncho-pneumonia, in buffalo, were isolated *Staph.* spp. **(Sayed and Zaitoun, 2009).** Isolated *Staph. aureus* 33.8% from nasal swabs of buffalo-calves in Turkey **(Esra and Hakan, 2010).** The nasal swabs, and pharyngeal swabs from Egyptian buffaloes had respiratory symptoms, revealed *Staph.* spp. The nasal swabs were 24%, and pharyngeal swabs were 8%. The most isolates were *Staph.* spp. 43% **(Hassan *et al.*, 2014).** Buffalloes' nasal swabs, had *Staph. aureus* 40% **(Hassan *et al.*, 2017).** *Staph. aureus* had cefoxitin and methicillin resistant, had MDR to macrolides, fluoroquinolones, aminoglycosides, tetracyclines, and lincosamides **(Gajdács, 2019).** The predominant samples isolates were *Staph. aureus* **(Choudhary *et al.*, 2019).** The pneumonic calves had *Staph. aureus* 11.8%, examined *coa genes*, showed MDR to amoxicillin, ampicillin and tetracycline **(Algammal *et al.*, 2020).**

The *alfR* gene were detected in *A. flavus* by PCR and different results of DNA bands occurred **(Cruz and Buttner, 2008).** Primers *aflR1-F/aflRS1-R* were utilized *A. flavus* **(Levin, 2012).** Egyptian dairy buffaloes at El-Sharkia and Giza had respiratory symptoms. Nasal swabs, pharyngeal swabs revealed *Aspergillus* 53%, *A. flavus* from nasal swab was 40%, and from pharyngeal swabs 52%, *A. fumigatus* from nasal swab was 32% and from pharyngeal swabs 40% **(Hassan *et al.*, 2014).** Nasal swabs had *A. fumigatus* and *A. flavus* 16% and 8% also 8% and 8% in nasal swabs and lung tissues. PCR was rapid diagnosis of *A. fumigatus* and *A. flavus* **(Sayed *et al.*, 2014).** Buffalloes' nasal swabs, had *Aspergillus* spp. 40% **(Hassan *et al.*, 2017).** The

molecular detection of *virulent genes* of *A. flavus* (*AflR*) by PCR and the Real-Time PCR (RT-PCR) rapidly of than genetic methods **(Hassan *et al.*, 2020).**

The aim was to study some investigations on some bacterial and fungal causes respiratory infections in Egyptian buffalloes. That were by Microbial isolation and identification, detect molecularity and antibiogram sensitivity of microbial isolates causative agents which affected the animal health and money losing from ruminant farms.

MATERIALS AND METHODS

Samples collection: The samples were 100 nasal swabs of buffalloes aged (1-5 years) reared on private farms at El-Dakahlia Governorate. They had symptoms of depression, reduced the intake feed, respiratory problems, dyspnea with or without nasal discharge and pneumonia. The other samples 50 mouth swabs were collected from mucous discharge of Buffalloes suffering from cough and sneezing. The collected samples were kept in ice box then were transfer to laboratory **(Reddy and Subramanyam, 2018)**.

Microbial isolation and identification: The samples were diluted by Peptone Water then were incubated at 37ºC for 24 hour. The tubes were centrifuged, the sediment was firstly streaked on Mannitol salt agar, Blood agar, MacConkey agar and Eduards media. The plates were incubated at 37ºC for 24-48 hour. The bacterial growth were identified by biochemical tests and API **(Abdullah, 2010).** The sediment was secondly streaked on modified Rose Bengal agar (MRBA). The plates were incubated for 3 days at 31˚C. *Aspergillus* colonies were identified by colony morphology. Then were sub cultured onto *A. flavus* parasiticus agar and incubated at 28˚C for 42-72 hours to confirm *Apergillus* section Flavi by colony reverse colour **(Nyongesa *et al.*, 2015).**

Antibiogram technique: The isolated bacteria and fungi were

tested against the most commonly antibacterial and antifungal agents using in livestock farms. It was used the standard disc technique **(Page and Gautier, 2012).**

DNA extraction: That using PathoGene-Spin TM DNA/RNA Extraction kit iNtRON Cat. No. 17154, Korea according manufacturer's instructions. That was following the DNA concentration was determined spectrophotometrically at 260/230 nm. DNA used as template. DNA extraction from *Asprigullus* DNeasy plant Mini kit Qiagen Genomic described by manufacturer manual of Qiagen, Germany. Cat. No. 69104. The PCR primers were used corresponded to sequences were synthesized by metabion international AG, (Germany); (Table 1); **(Uerlings *et al.*, 2021).**

Table 1: Oligonucleotide primer sequences of virulence genes of bacterial isolates (Uerlings et al., 2021)

Microorganisms	***Gene***	**Primer name**	**Primer sequence (5_–3_)**	**amplified size (bp)**	**Reference**
****P. multocida***	*KMT1*	KMT1T7 KMT1SP6	ATCCGCTATTTACCCAGTGG GCTGTAAACGAACTCGCCAC	457	**(Townsend *et al.*, 2001)**
****E. coli***	*16s rRNA*	F R	GCTTGACACTGAACATTG GCACTTATCTCTTCCGCATT AG	662	**(Riffon *et al.*, 2001)**
****Staph. aureus***	*Coagulase*	F-Eco 2083 R-Eco 2745	ACCACAAGGTACTGAATCAACG TGCTTTCGATTGTTCGATGC	600-1000	**(Aarestrup *et al.*, 1995)**
****A. fumigatus***	*Asphs*	F-Asphs R-Asphs	TGGTACAAGGACGGTGACAA GTCCCAGTGGACTCTTCCAA	180	**(Dennis and Allen, 2006)**
****A. flavus***	*aflR1*	F-aflR1	AACCGCATCCACA	798	(Farber ***et***

		R-aflR1	ATCTCAT AGTGCAGTTCGCT CAGAACA		***al.*, 1997)**
**P. multocida: Pasteurella multocida, *E. coli: Escherichia coli, *Staph. aureus: Staphylococcus aureus, *A. fumigatus: Asprigullus fumigatus, *A. flavus: Asprigullus flavus*					

The positive control with DNA of *A. fumigatus* Af293, and a no-template control (NTC). PCR reaction was performed in an Gradient Thermal cycler (1000 S Thermal cycler Bio-RAD USA). The reaction mixture (total volume of 50 µl) was 25 µl Thermoscientific PCR Mix (Green PCR Master Mix (2X) thermoscientific Company,cat., No.K1080, USA.), 3 µl target DNA, 1 µl of each primers (containing 10 p mole/ µl) and the mixture was completed by sterile D. W. to 50 µl. Amplification condition for all bacteria done; (Table 2); **(Johnson, 2014).**

Table 2: Cycling conditions and predicted sizes of PCR products for virulence genes (Johnson, 2014)

Target gene	**Initial denaturation °C/min**	**Actual cycles °C/sec**				**Final extension °C/min**
		Denaturation	**Annealing**	**Extension**	**No. of cycle**	
KMT1	95/5	94/60	55/60	72/60	30	72/9
16S-rRNA	94/2	94/45	57/60	72/120	35	72/10
Coagulase	94/45 sec	94/20	57/15	72/15	30	72/10
Asphs	95/10	95/60	60/60	72/60	32	72/10
Afl	95/10	94/30	55/45	72/75	30	72/10

PCR amplification protocol for microorganisms: It was for *P. multocida* by an initial denaturation at 95°C for 5 min followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and final step at 72°C for 9 min. It was for *E. coli* by an initial denaturation at 94 °C for 2 min, 35 cycles of 45 sec denaturation at 94°C, 1 min annealing at 60°C, 2 min extension at 72°C, and a final 10 min extension at 72°C. It was for *coagulase gene* of *Staph. aureus by* one cycle of 5 min at 94°C,

30 cycles of 20 sec at 94°C (decentralization), 15 s at 57°C (annealing), 15 sec at 72°C (extension) and finally 1 cycle of 2 min at 72°C. It was for *A. fumigatus virulence gene* by an initial denaturation at 95°C for 10 min followed by 32 cycles of 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min, and a final step at 72°C for 10 min. It was for *A. flavus gene* by an initial denaturation at 95°C for 10 min followed by 30 cycles of 94°C for 30 sec, 55°C for 45 sec., and 72°C for 1.15 min, and a final step at 72°C for 10 min. The PCR was amplified products were electrophoresis in Agarose Gels (2% w/v) (Agarose, Sigma ,USA) was used for running of DNA stained with ethidium bromide Using GeneRuler 100bp DNA Ladder: Thermoscientific Company,Cat.No.SM0243,US **(Devi *et al.*, 2018).**

Following the bio-safety procedures: It was in the specialized laboratory while conducting the experiments **(Nieuwenweg *et al.*, 2021).**

Data analysis: The data were treated by "Simple Excel Program" **(Gündüz and Asan, 2021).**

RESULTS AND DISCUSSION

Incidence of isolated bacteria causes respiratory infections in Egyptian buffalloes: Table 3 showed isolated bacteria were *P. multocida, E. coli,* and *Staph. aureus* as 12%, 7% and 5% from nasal swabs. As well were 8%, 4% and 4% from mouth swabs. Through the result, *P. multocida,* was main bacterial causes than others bacteria. It was isolated from samples **(Abed *et al.*, 2020)**. Then were *E. coli,* this revealed *E. coli as* 8.33% **(Reddy and Subramanyam, 2018).** *Staph. aureus* was predominant **(Sayed, and Zaitoun, 2009).** Isolated *Staph. aureus* 33.8% from nasal swabs **(Esra and Hakan, 2010).** Nasal swabs, pharyngeal swabs revealed *Staph.* spp., nasal swabs 24%, pharyngeal swabs 8%. The most was *Staph.* spp 43%, **(Hassan *et al.*, 2014).**

Buffalloes' nasal swabs, had *Staph. aureus* 40% **(Hassan *et al.*, 2017).**

Table 3 : Incidence of isolated bacteria causes respiratory infections in Egyptian buffalloes

Isolated bacteria	*Nasal swabs (n=100)*		*Mouth swabs (n=50)*	
	Positive samples	*Percent*	*Positive samples*	*Percent*
**P. multocida*	*12\100*	*12%*	*4/50*	*8%*
**E. coli*	*7/100*	*7%*	*2/50*	*4%*
**Staph. aureus*	*5/100*	*5%*	*2/50*	*4%*
**P. multocida: Pasteurella multocida, *E. coli: Escherichia coli, *Staph. aureus: Staphylococcus aureus.*				

Incidence of isolated fungi causes respiratory infections in Egyptian buffalloes: Table 4 showed the isolated fungi *A. fumigatus* was 11% and *A. flavus* was 5% from nasal swabs, were 8% and 4% from mouth swabs. The pharyngeal swabs revealed *A. flavus* from nasal swab was 40%, and from pharyngeal swabs 52%, *A. fumigatus* from nasal swab was 32% and from pharyngeal swabs 40% **(Hassan *et al.*, 2014).** Nasal swabs had isolated *A. fumigatus* and *A. flavus were* 16% and 8% also 8% and 8% in both nasal swabs and lung tissues **(Sayed *et al.*, 2014).** Buffalloes' nasal swabs, had *Aspergillus* spp. 40% **(Hassan *et al.*, 2017).** It was found from the results of the mixed microbial isolation of the presence of three bacteria and two fungi. That causes infection of the respiratory system of Egyptian buffaloes. This indicated the presence of microbial infection that affects the respiratory system and spreads to the animal. In the terms of infection spread leading to loss animals and the corresponding material value, which affects the livestock materially.

Table 4: Incidence of isolated fungi causes respiratory infections in Egyptian buffaloes

Isolated fungi	*Nasal swabs (n=100)*		*Mouth swabs (n=50)*	
	Positive samples	*Percent*	*Positive samples*	*Percent*
**A. fumigatus*	*11/100*	*11%*	*4/50*	*8%*
**A. flavus*	*5/100*	*5%*	*2/50*	*4%*
**A. fumigatus: Asprigullus fumigatus, *A. flavus: Asprigullus flavus*				

Antibiotics susceptibility of isolated bacteria causes respiratory infections in Egyptian buffaloes: Table 5 showed the isolated *P. multocida* were sensitive to enrofloxacin, spectinomycin, and trimethoprim-sulfamethoxazole only. It had MDR to amoxicillin, ciprofloxacin, gentamicin, streptomycin, and chloramphenicol. It was resistance to tylosin 90.9% and oxacillin 54.54% **(Gharibi *et al.*, 2017).** The total buffaloes samples had *P. multocida*, it was MDR to penicillin, cephalosporins and fluoroquinolones **(Choudhary *et al.*, 2019).** They had resistances against chloramphenicol, ciprofloxacin, amoxicillin/clavulanic acid, and levofloxacin **(Elsayed *et al.*, 2021).** Also against ampicillin, amoxicillin, penicillin-G, tetracycline, streptomycin, cefotaxime and chloramphenicol **(Bahr *et al.*, 2021).** *E. coli* were sensitive to ciprofloxacin, enrofloxacin, spectinomycin, and chloramphenicol. It had MDR to amoxicillin, gentamicin, streptomycin, and trimethoprim-sulfamethoxazole. The predominant were *E. coli*, MDR in 90% **(Choudhary *et al.*, 2019).** It had MDR to gentamicin, erythromycin, streptomycin and trimethoprim-sulphamethoxazol **(Algammal *et al.*, 2020).** *Staph. aureus* were sensitive to enrofloxacin, and spectinomycin. It had MDR to amoxicillin, ciprofloxacin, gentamicin, streptomycin, trimethoprim-sulfamethoxazole, and chloramphenicol. They had resistant to

cefoxitin and methicillin, and MDR to macrolides, fluoroquinolones, aminoglycosides, tetracyclines, and lincosamides **(Gajdács, 2019).** The pneumonic showed MDR to amoxicillin, ampicillin and tetracycline **(Algammal *et al.*, 2020).**

Table 5 : Antibiotics susceptibility of isolated bacteria causes respiratory infections in Egyptian buffalloes

Antibiotics agent	*Isolated bacteria*					
	**P. multocida n=16*		**E.. coli n=9*		**Staph. Aureus n=7*	
	Sensitive	*Resistant*	*Sensitive*	*Resistant*	*Sensitive*	*Resistant*
Ampicillin 10 µg	00	16	7	2	0	7
Amoxicillin 20 µg	00	16	6	3	0	7
Erythromycin 15 µg	00	16	2	7	6	1
Enrofloxacin 5 µg	16	00	9	0	7	0
Gentamicin 10 µg	16	00	1	8	6	1
Norfloxacin 10 µg	16	00	9	0	6	1
Streptomycin 10 µg	00	16	2	7	4	3
Tetracycline 30 µg	14	2	7	2	2	5
Trimethoprim-sulfamethoxazole 25 µg	6	10	3	6	7	2

**P. multocida: Pasteurella multocida, *E. coli: Escherichia coli, *Staph. aureus: Staphylococcus aureus*

Antifungal susceptibility of isolated fungi causes respiratory infections in Egyptian buffaloes: Table 6 showed antifungal susceptibility, it was found through the fungi carry MDR. The isolates *A. fumigatus* was sensitive to fluconazole, ketoconazole, nystatin, and amphotericin B, while had MDR to clotrimazole. *A. flavus* were sensitive to fluconazole, ketoconazole, and nystatin, while had MDR to clotrimazole, and amphotericin B. *A. flavus* was resistant to amphotericin B **(Kiakojur *et al.*, 2021).** The most effective antimycotic agents were nystatin followed by terbinafine, ketoconazole, miconazole, fluconazole and povidine

iodine. Griseofulvin, on the other hand, was not effective against any of the fungi tested **(Farrag *et al.*, 2012).**

Table 6 : Antifungal susceptibility of isolated fungi causes respiratory infections in Egyptian buffaloes

Antifungal agent	*Isolated fungi*			
	**A. fumigatus n=15*		**A. flavus N=7*	
	Sensitive	*Resistant*	*Sensitive*	*Resistant*
Fluconazole	*15*	*0*	*7*	*0*
Clotrimazole	*0*	*15*	*0*	*7*
Ketoconazole	*15*	*0*	*7*	*0*
Nystatin	*15*	*0*	*7*	*0*
Amphotericin B	*15*	*0*	*0*	*7*

**A. fumigatus: Asprigullus fumigatus, *A. flavus: Asprigullus flavus*

Agarose gel electrophoresis of isolated bacteria causes respiratory infections in Egyptian buffaloes: The PCR using the *KMT1 gene* confirmed the culture positive *P. multocida* isolates, developed specific 457 bp molecular size bands (Table 1 and Photo 1). The five selective isolates of *P. multocida* had similar size of PCR products having *KMT1 gene* **(Hassan *et al.*, 2017).** The multiplex PCR identified only 22 isolates as *Pasteurella* species using universal primers **(Abbas *et al.*, 2018).** PCR *kmt1* showed 87.9% were positive for the corresponding universal *gene* **(Abed *et al.*, 2020).** The PCR using the *16s rRNA gene* confirmed the culture positive *E. coli* isolates, developed specific 662 bp molecular size bands (Table 1 and Photo 2). Nasal swabs buffaloes recovered bacterial isolates by the *16S rRNA* sequence analysis **(Choudhary *et al.*, 2019).** The PCR using the *Coagulase gene* confirmed the culture positive *Staph. aureus* isolates, developed specific 600-1000 bp molecular size bands (Table 1 and Photo 3). The pneumonic calves had *Staph. aureus* examined *coa genes*, showed MDR **(Algammal *et al.*, 2020).**

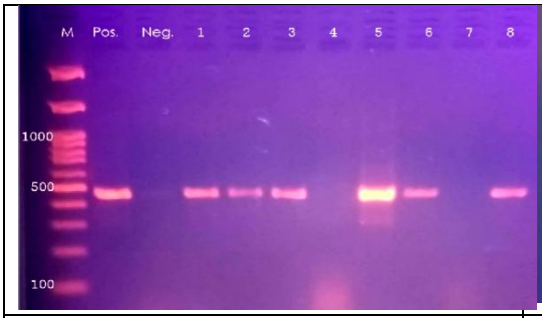

Photo 1: Agarose gel electrophoresis of *P. multocida PCR products using primer KMT1
Lane M: 100 bp DNA ladder, Lane +ve: C +ve, Lane -ve: C -ve, Lane1-9: Isolates

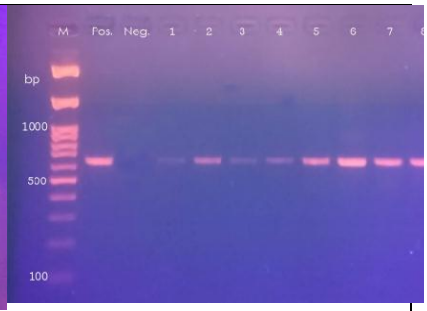

Photo 2: Agarose gel electrophoresis of *E. coli PCR products using 16SrRNA gene primer Lane M: 100 bp molecular weight standard, Lane +ve: C +ve, Lane -ve: C -ve, Lane1-9: Isolates

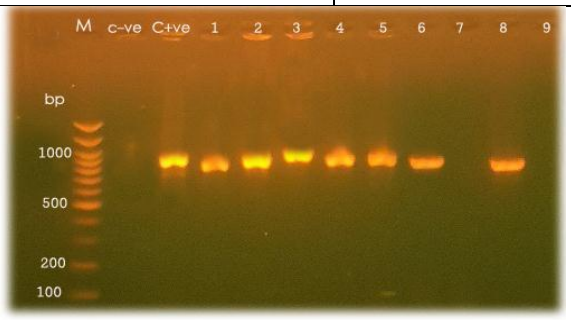

Photo 3: Agarose gel electrophoresis of *Staph. aureus PCR products using primer Coagulase gene
Lane M: 100 bp DNA ladder, Lane C+ve: C +ve, Lane C-ve: C -ve, Lane1-9: Isolates

P. multocida: Pasteurella multocida, *E. coli: Escherichia coli, *Staph. aureus: Staphylococcus aureus

Agarose gel electrophoresis of isolated fungi causes respiratory infections in Egyptian buffaloes: The PCR using

the *Asphs gene* confirmed the culture positive *A. fumigatus* isolates, which developed specific 180 bp molecular size bands (Table 1 and Photo 4). The PCR using the *aflR1 gene* confirmed the culture positive *A. flavus* isolates, which developed specific 798 bp molecular size bands (Table 1 and Photo 5). The *alfR* gene were detected in *A. flavus* by PCR **(Cruz and Buttner, 2008).** Primers *aflR1-F/aflRS1-R* were utilized *A. flavus* **(Levin, 2012).** PCR rapid diagnosis of *A. fumigatus* and *A. flavus* **(Sayed *et al.*, 2014).** Buffalloes' nasal swabs, had *Aspergillus* spp. 40% **(Hassan *et al.*, 2017).** The molecular detection of *virulent genes* of *A. flavus* (*AflR*) by PCR **(Hassan *et al.*, 2020).**

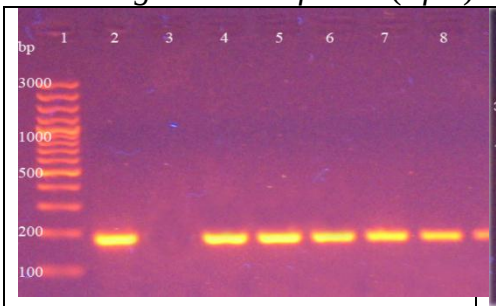

Photo 4: Agarose gel electrophoresis of *A. fumigatus PCR products using Asphs gene primer Lane 1:100 bp molecular weight standard, Lane 2: C +ve, Lane 3: C -ve, Lane: 4-10: Isolates

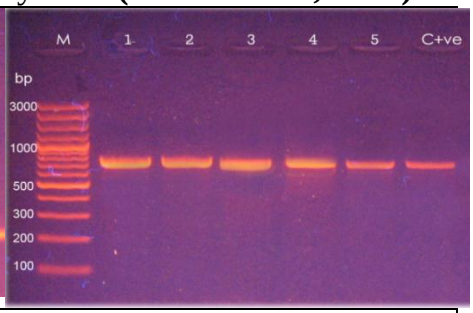

Photo 5: Agarose gel electrophoresis of *A. flavus PCR products using aflR1 toxin gene primer Lane M: 100 bp DNA ladder, Lane C +ve: C +ve, Lane C -ve: C -ve, Lane: 1-5: Isolates

A. fumigatus: Asprigullus fumigatus, *A. flavus: Asprigullus flavus

Conclusion

It was concluded that according to the results of present study, *P. multocida* and *A. Fumigatus we*re main cause of respiratory infections in buffaloes. The bacterial and fungal isolates had MDR.

Recommendation

It was recommended that particularly calves of low immunity must be treated by suitable antibiotics and antifungal. Must used strict hygienic measures in farms as periodical application of disinfectants for protection from respiratory diseases causing microbes. Sun exposure of animals specially in closed system to avoid over crowded and infections speeding. Periodical clinical examination of animals to easy detect any infections. Antibiotics misuse should be avoided and only used according to the laboratory results also must be annual vaccinations according vaccination schedule.

Acknowledgments

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