Use Of Multiplex PCR For Detection Of Bacterial Respiratory Infections In Poultry

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ABSTRACT

Bacterial pathogens play an important role in causing respiratory diseases in poultry. Six pathogens; *Escherichia coli, Mycoplasma gallisepticum, Staphylococcus aureus, Pasteurella multocida, Mannheimia haemolytica* and *Pseudomonas aeruginosa* are known to cause respiratory infections in poultry. However, current diagnostic methods for them are not highly sensitive, laborious and time consuming. This research aimed to develop a multiplex polymerase chain reaction (m-PCR) that could detect the six species in clinical specimens from poultry with respiratory manifestations. In particular, *E. coli* alkaline phosphatase (*pho*A), *S. aureus* encoding a surface associated fibrinogen binding protein (*clf*A), *P. aeruginosa* outer membrane protein (*opr*L), *M. haemolytica* serotype specific antigen (*SSA*), *M. gallisepticum 16S* rRNA and *P. multocida* ribosomal RNA (*23S* rRNA) genes were tested. PCR Results revealed that 19 (35.8%) out of 53 examined farms were with a single infection, 31 (58.5%) associated with mixed bacterial infections, meanwhile 3 only (5.7%) were negative. The m-PCR developed in this study has been proven to be both sensitive and specific for simultaneous detection of *E. coli, M. gallisepticum, S. aureus* and *P. aeruginosa* in clinical specimens in a single reaction. However, detection of *M. haemolytica* was suboptimal due to indistinguishable fragment size and lower annealing temperature. Overall, standardized m-PCR in our research could be considered as a useful tool for diagnosis and screening of *E. coli, M. gallisepticum, S. aureus* and *P. aeruginosa* in poultry flocks as it takes almost three hours to be performed and has the potential to replace the conventional culture technique and thus can speed up the treatment process.

INTRODUCTION

The respiratory diseases are included among the very important problems in the poultry industry. The pathogenic causes of these diseases are viruses (NDV, IBV, ILTV, etc), bacteria (*Mycoplasma spp., Haemophilus paragallinarum, Escherichia coli*, Ornithobacterium, several microorganisms of the genus *Pasteurella* including *P. multocida, P. gallinarum, M. haemolylica and P. anatipestifer* as well as fungi and parasites. These factors can act either alone, leading to complete disease or in combinations, causing respiratory syndromes ***(1, 2).*** Environmental factors may augment these pathogens to produce the clinically observed signs and lesions ***(3).***

In particular, culture could recognize viable organisms only, while amplification tests are not dependent on viable or structurally intact cells and the presence of DNA was sufficient to yield a positive result. Thus, the potential for detecting non-viable microorganisms explained the discrepancies between PCR and culture results following antibiotic therapy ***(4).*** Moreover, simultaneous detection of more than one DNA sequence could be achieved using a multiplex PCR assay. It allows parallel screening of different targeted genes in the same reaction. This facilitates the

use of multiplex PCR in routine testing and reduces the time required to attain results *(5)*.

A well-adjusted multiplex PCR is of great benefit for the diagnostic laboratory. Using this highly sensitive and specific methodology, early information can be obtained about the presence of the targeted pathogens in the animal population. Furthermore, the emergence of new variants can be detected and the efficiency of immunizations and/or medications can be monitored, thereby controlling measurements can be started on time *(6)*. Annealing temperatures for each of the primer sets must be optimized to work correctly within a single reaction, and amplicon sizes, i.e., their base pair length, should be different enough to form distinct bands when visualized by gel electrophoresis *(7, 8)*.

Thereby, the aim of the study was evaluation of a multiplex PCR assay for rapid direct detection of respiratory bacterial pathogens in clinical specimens of poultry suffered from respiratory manifestations thus would allow earlier appropriate treatment as well as it may be a useful tool for screening and control of the diseases.

MATERIALS AND METHODS

Sampling

Two hundred and fifty samples (150 tracheal swabs, 50 lungs and 50 tracheas) from 53 poultry farms with a history of respiratory manifestations were used in this study. The samples were collected from ten Egyptian Governorates (Giza, Dakahlia, Fayoum, Buhaira, Assuit, Alexandria, Ismailia, Monofya, Cairo and Qalioubiya), of different types of production sectors and species [chickens (broilers, layers and broiler breeders), ostrich and duck] and of different ages during the period between September 2012 and May 2013. Each sample was transferred into 2 ml of each buffered peptone broth and PPLO broth (Difco, USA). In addition, samples from each farm were pooled into approximately 1 ml of buffered peptone broth and stored at -80°C to be examined directly by multiplex PCR.

Isolation and identification of bacteria causing respiratory diseases in poultry

PPLO agar medium was used for the specific isolation of Mycoplasma *(9)*, which was differentiated from Acholeplasma using Digitonin test *(10)*. Biochemical identification of Mycoplasma depended mainly on glucose fermentation and arginine deamination tests *(11)*.

For isolation and identification of bacteria other than Mycoplasmas, samples were cultured onto 10% sheep blood agar, trypticase soya agar (Difco, USA), Baired Parker agar (Oxoid) and MacConkey`s agar media (Oxoid), lactose fermenters were streaked onto eosin methylene blue agar media (Oxoid) to observe the characteristic greenish metallic sheen of *E. coli*. For biotyping, fresh colonies of presumptive bacterial growths were tested against IMVC tests, triple sugar iron agar medium, Christenser's urea agar medium and oxidase strips *(12)*. O-serogrouping of *E. coli* isolates was applied according to the manufacturer's instructions.

PCR for direct detection of bacteria from clinical samples

Genomic DNA was extracted using commercially available kit, QIAamp DNA Mini Kit, Catalogue no.51304 (Qiagen; Germany). Oligonucleotide primer sets that specifically amplify the target sequences of *E. coli* alkaline phosphatase (*pho*A), *M. gallisepticum* ribosomal RNA (*16S* rRNA), *S. aureus* encoding a surface-associated fibrinogen-binding protein (*clf*A), *P. multocida* ribosomal RNA (*23S* rRNA), *M. haemolytica* serotype specific antigen (*Ssa*) and *P. aeruginosa* outer membrane protein (*opr*L) genes are described in Table 1. Primers were checked for specificity in a BLAST search available through the National Centre for Biotechnology Information website www.ncbi.nlm.nih.gov.

Optimization multiplex PCR conditions

Six reference strains (control groups) of *E. coli*, *M. gallisepticum*, *S. aureus*, *P. multocida*, *M. haemolytica* and *P. aeruginosa* were obtained in collaboration with Animal Health Research Institute (AHRI), National Laboratory for Veterinary Quality Control on Poultry Production (NLQP), Central Laboratory for Evaluation of Veterinary Biologics (ClEVB) and Veterinary Serum and Vaccine Research Institute (VSVRI), Egypt. Each one was used for testing primers in a uniplex PCR before being used in the multiplex PCR assay.

Many trials were performed to obtain the best concentrations for the multiplex reaction in a single tube. The final PCR amplification reaction mixture contained 37.5 *µl* Emerald Amp GT PCR master mix (2x premix), 13.5 *µl* PCR grade water, 5 *µl* of each primer (20 pmol), and 18 *µl* template DNA in a final volume of 79 *µl*. PCR amplification was performed in T3 thermal cycler (Biometra, Germany) with the following cycling conditions: initial denaturation at 94°C for 5 min followed by 40 cycles of denaturation at 94°C for 30 s; primer annealing at 55 °C for 30 s, and extension at 72°C for 1 min followed by final extension at 72°C for 5 min. It was carried out for all previously mentioned genes except for *M. haemolytica Ssa* gene as the primer annealing temperature was lower (45°C) and its product size (500 bp) has nearly the same molecular weight of *P.aeruginosa oprL* gene (504 bp).

The amplified PCR products were electrophoresed on a 1.5% agarose gel (Applichem, Germany, GmbH) in 1x tris-boric EDTA buffer. A 100 bp DNA ladder (Qiagen, Germany, GmbH) was used as a molecular weight marker. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra, Germany) and the data were analyzed through computer software [BioDoc Analyze Digital Systems (Biometra, Germany)].

Table 1. Target genes, Oligonucleotide primers and their amplicons used in PCR

Target gene	Primer sequence (5'-3')	Amplicon (bp)	References
E. coli phoA	F: CGATTCTGGAAATGGCAAAAG R: CGTGATCAGCGGTGACTATGAC	720	*(13)*
Mycoplasma gallisepticum 16S rRNA	F:GAGCTAATCTGTAAAGTTGGTC R: GCTTCCTTGCGGTTAGCAAC	185	*(14)*
S. aureus_clfA	F: GCAAAATCCAGCACAACAGGAAACGA R: CTTGATCTCCAGCCATAATTGGTGG	638	*(15)*
P. multocida 23S rRNA	F:GGCTGGGAAGCCAAATCAAAG R: CGAGGGACTACAATTACTGTA A	1432	*(16)*
M. haemolytica Ssa	F: TTCACATCTTCATCCTC R: TTTTCATCCTCTTCGTC	500	*(17)*
Pseudomonas aeruginosa oprL	F: ATGGAAATGCTGAAATTCGGC R: CTTCTTCAGCTCGACGCGACG	504	*(18)*

RESULTS

From 53 examined poultry farms and by conventional identifications methods, *E. coli* was recorded in 25 farms (47.17%). The most prevalent serogroups were O127:K63 and O44:K74 (16% for each) followed by O119:K69, O164:K- and O118: K- (12% for each). Other infrequently encountered serogroups were included (O114:K90,

O111:K58 and O158:K-). On the other hand, *M. haemolytica* was detected in 11 farms (20.75%), followed by *P. aeruginosa* in 8 farms (15.09%), *M. gallisepticum* in 3 farms (5.66%), while *S. aureus*, *P. mirabilis* and *A. hydrophila* were detected in 2 farms for each with a percentage of 3.77 (Table 2).

Six reference strains (*E. coli, M. gallisepticum, S. aureus, P. multocida, M. haemolytica* and *P. aureginosa*) were used to test the primers in uniplex PCR amplification before application of m-PCR assay. PCR results revealed amplicons of expected sizes 720bp, 185bp, 638bp, 1432bp, 500bp and 504bp for *E. coli pho*A, *M. gallisepticum 16S* r*RNA, S. aureus clf*A, *P. multocida* 23S rRNA, *M. haemolytica Ssa* and *P. aeruginosa opr*L genes, respectively (Fig 1).

Multiplex PCR was carried out in two trials. The first trial included all 53 examined farms using five primer pairs for *E. coli, M. gallisepticum, S. aureus, P. multocida* and *P.aeruginosa*. *M. haemolytica Ssa* gene was excluded from m-PCR assay as the primer required a lower annealing temperature than other microorganisms and its product size (500 bp) is nearly the same as that of *P.aeruginosa* (504 bp). PCR results revealed only 16 positive farms, representing 30.2% of the total examined ones (Fig 2).

In the second trial, four primer pairs of *E. coli, M. gallisepticum, S. aureus* and *P.aeruginosa* were introduced into m-PCR while, primer targeting *P. multocida 23S* rRNA gene was excluded from the reaction as it was negative by culturing technique and first trial M-PCR assay. PCR results were improved and revealed positive reactions for all examined farms (Fig 3 and Table 3).

Besides, uniplex PCR was applied on clinical samples of the 53 concerned farms for detection of *M. haemolytica Ssa* gene which was recorded with a percentage of 35.85% (19/53) (Fig 4).

Overall, out of 53 examined farms, 19 (35.8%) were with single bacterial infection, 31 (58.5%) associated with mixed infections, meanwhile 3 farms only (5.7%) were negative. Possible coditions for bacterial infections associated with respiratory signs in poultry in different farms with reference to type of production are shown in Table (4).

Obviously, the diagnostic sensitivity of the PCR assay for the field samples was significantly higher than that of the culture method as shown in Table (5).

Table 2. Conventional culture consequents of bacterial pathogens recovered from poultry farms in different Egyptian Governorates

Farms/ Governorate (53)	*E. coli*	*M. haemolytica*	*P. aeruginosa*	*M. gallisepticum*	*S. aureus*	*P. mirabilis*	*A. hydrophila*
Giza (19)	2	9	7	-	2	1	-
Dakahlia (13)	9	-	1	1	-	1	-
Fayoum (6)	4	-	-	1	-	-	-
Buhaira (4)	2	1	-	-	-	-	1
Assuit (3)	2	-	-	1	-	-	-
Alexandria (2)	2	-	-	-	-	-	-
Ismailia (2)	-	-	-	-	-	-	-
Menofya (2)	2	1	-	-	-	-	1
Cairo (1)	1	-	-	-	-	-	-
Qalioubiya (1)	1	-	-	-	-	-	-
Incidence	25 (47.17%)	11 (20.75%)	8 (15.09%)	3 (5.66%)	2 (3.77%)	2 (3.77%)	2 (3.77%)

Table 3. PCR results of bacterial infections recovered from poultry farms in different Egyptian Governorates

Farms/Governorate (53)	*E. coli*	*M. haemolytica*	*P. aeruginosa*	*M. gallisepticum*	*S. aureus*
Giza (19)	2	14	16	5	3
Dakahlia (13)	12	3	5	3	-
Fayoum (6)	5	-	2	2	-
Buhaira (4)	2	1	2	-	-
Assuit (3)	2	-	2	1	1
Alexandria (2)	2	-	1	-	-
Ismailia (2)	-	-	-	-	-
Menofya (2)	2	1	-	-	-
Cairo (1)	1	-	-	-	-
Qalioubiya (1)	1	-	1	-	-
Incidence	29 (54.72%)	19 (35.85%)	29 (54.72%)	11 (20.75%)	4 (7.55%)

Table 4. Occurrence of bacterial infections in different poultry farms with reference to type of production

Bacterial infection	Positive Farms	Production type and species
E. coli	15 (28.3%)	10 broilers, 1 ostrich,4 broiler breeders
M. haemolytica	1 (1.89%)	broiler breeder
P. aeruginosa	3 (5.66%)	2 broilers, 1 broiler breeders
E. coli , MG	2 (3.77%)	1 layer, 1 broiler breeder
E. coli and *P. aerouginosa*	5 (9.43%)	2 broiler breeders, 3 broilers
S. aureus and *P. aeruginosa*	1 (1.89%)	Broiler
M. haemolytica and *P. aeruginosa*	9 (16.98%)	broiler breeders
M. gallisepticum and *P. aeruginosa*	2 (3.77%)	Broiler breeders
M. gallisepticum and *Proteus mirabilis*	1 (1.89%)	Broiler breeder
M. gallisepticum, M.haemolytica and *P.aeruginosa*	1 (1.89%)	broiler
S. aureus, M. haemolytica and *P. aeruginosa*	1 (1.89%)	Broiler breeder
E. coli, M. haemolytica and *P.aeruginosa*	2 (3.77%)	Broilers
E. coli, M. haemolytica and *A. hydrophila*	2 (3.77%)	Brolier breeders
E. coli, M. gallisepticum and *P. aeruginosa*	2 (3.77%)	Broilers
E. coli, M. gallisepticum, M. haemolytica and *P. aeuginosa*	1 (1.89%)	Broiler breeder
M. gallisepticum, S. aureus, M. haemolytica and *P. aeruginosa*	1 (1.89%)	Broiler breeder
M.gallisepticum, S.aureus, M.haemolytica, P.aeruginosa and *P.mirabilis*	1 (1.89%)	Broiler breeder
Negative	3 (5.66%)	1 broiler, 2 ducks

Table 5. Respiratory pathogens recovered from poultry from 53 different farms by conventional culture identification and PCR

	Conventional culture results	PCR results
E. coli	25	29
M. haemolytica	11	19
P. aeruginosa	8	29
M. gallisepticum	3	11
S. aureus	2	4

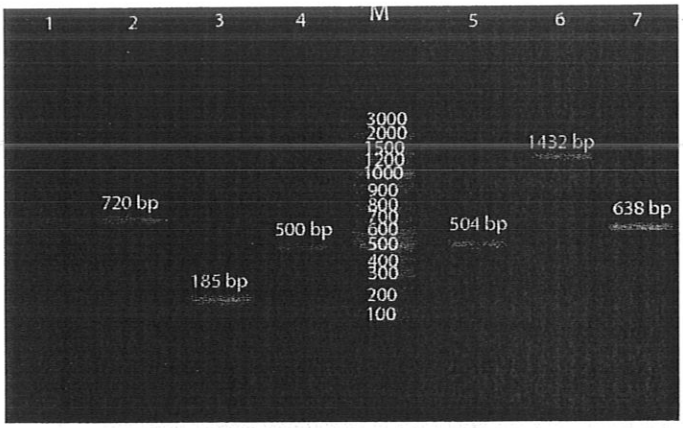

Fig 1. Agarose gel electrophoresis patterns showing typical amplification products in a uniplex PCR for the six reference strains used in the study. Lane 1: Negative control, lane 2: *E. coli pho*A, lane 3: *M. gallisepticum 16S* rRNA, lane 4: *M. haemolytica Ssa,* lane 5: *P. aeruginosa opr*L, lane 6: *P. multocida 23S* rRNA, lane 7: *S. aureus clf*A and lane M: DNA molecular size marker (100-bp). The size in base pairs (bp) of each PCR product is indicated on the bands.

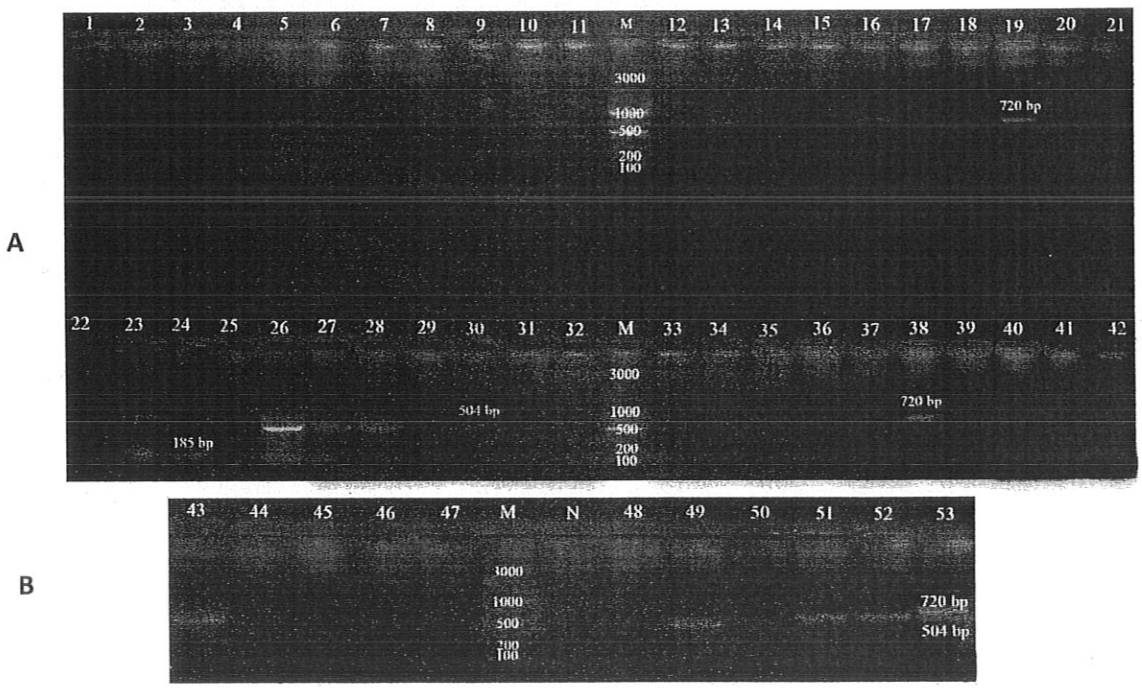

Fig 2: Electrophoretic banding patterns of the amplified genes of *E. coli, M. gallisepticum* , *S. aureus, P. multocida* and *P. aeruginosa* in clinical samples in a multiplex PCR (first trial). A: Lanes 5, 13, 16, 19, 38: positive for *E. coli*, lane 23: positive for *E. coli* (720 bp) and *M. gallisepticum* (185 bp), lane 24: positive for *M. gallisepticum*, lane 26: positive for *E. coli* (720 bp) and *P. aeruginosa* (504 bp), lanes 27, 28, 30: positive for *P. aeruginosa*. **B:** lanes 43, 49, 51, 52: positive for *P. aeruginosa*, lane 53: positive for *E. coli* (720 bp) and *P. aeruginosa* (504 bp), lane M: molecular size markers (100 bp). The size in base pairs (bp) of each PCR product is indicated on the bands.

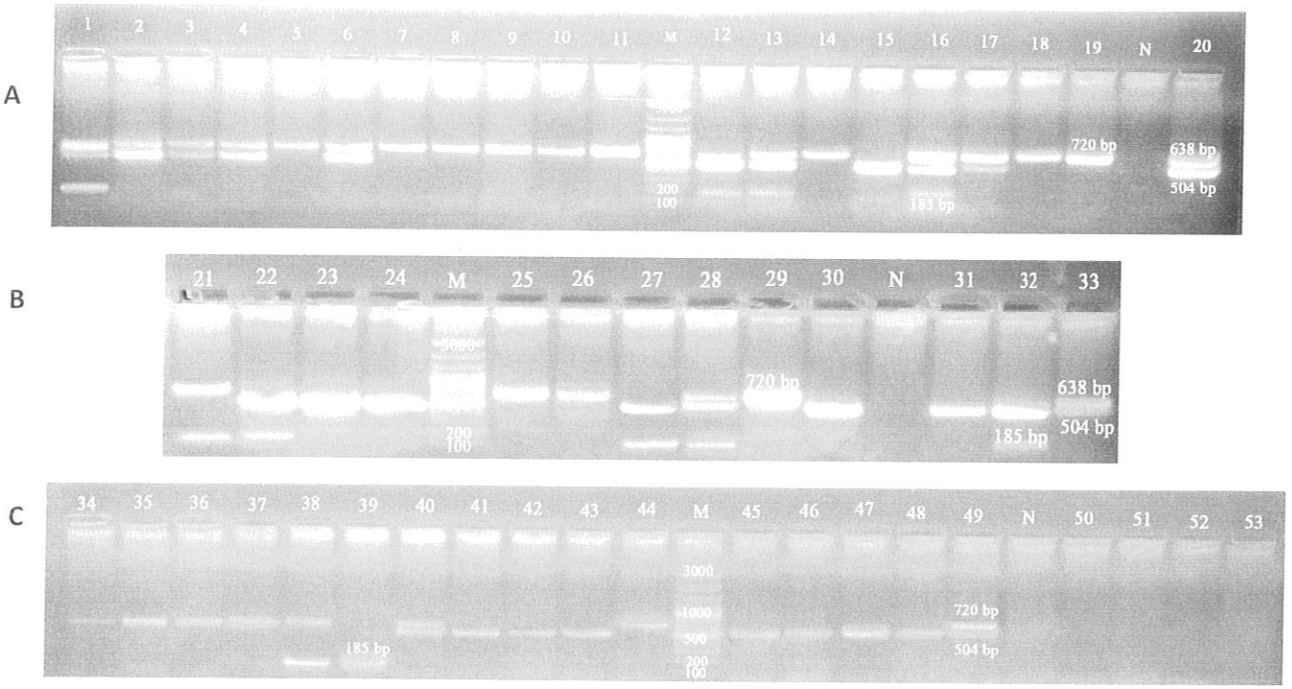

Fig 3: Detection of the amplified genes of *E. coli, M. gallisepticum S. aureus* and *P. aeruginosa* in clinical samples using a multiplex PCR system (second trial). A: lanes 1, 13, 16: *E. coli* (720 bp), *P. aeruginosa* (504 bp) and *M. gallisepticum* (185 bp), lanes 2, 3, 4, 6, 17: *E. coli* and *P. aeruginosa*, lanes 5, 7-11, 14, 18, 19: *E. coli*, lane 12: *S. aureus* (638 bp), *P. aeruginosa* and *M. gallisepticum*, lane 15: *P. aeruginosa*, lane 20: *S. aureus* and *P. aeruginosa*, lane N: negative control, lane M: molecular size markers (100 bp).

B: lane 21: *E. coli* and *M. gallisepticum*, lanes 22, 27, 32, *P. aeruginosa* and *M. gallisepticum*, lanes 23, 24, 30, 31: *P. aeruginosa*, lanes 25, 26, 29: *E. coli*, lane 28: *S. aureus*, *P. aeruginosa* and *M. gallisepticum*, lane N: negative control, lane M: molecular size markers (100 bp).

C: lanes 34 – 37, 44: *E. coli*, lane 38: *E. coli* and *M. gallisepticum*, lane 39: *M. gallisepticum*, lanes 40, 49: *E. coli*, *P. aeruginosa*, lanes 41- 43, 45- 48: *P. aeruginosa*, N: negative control

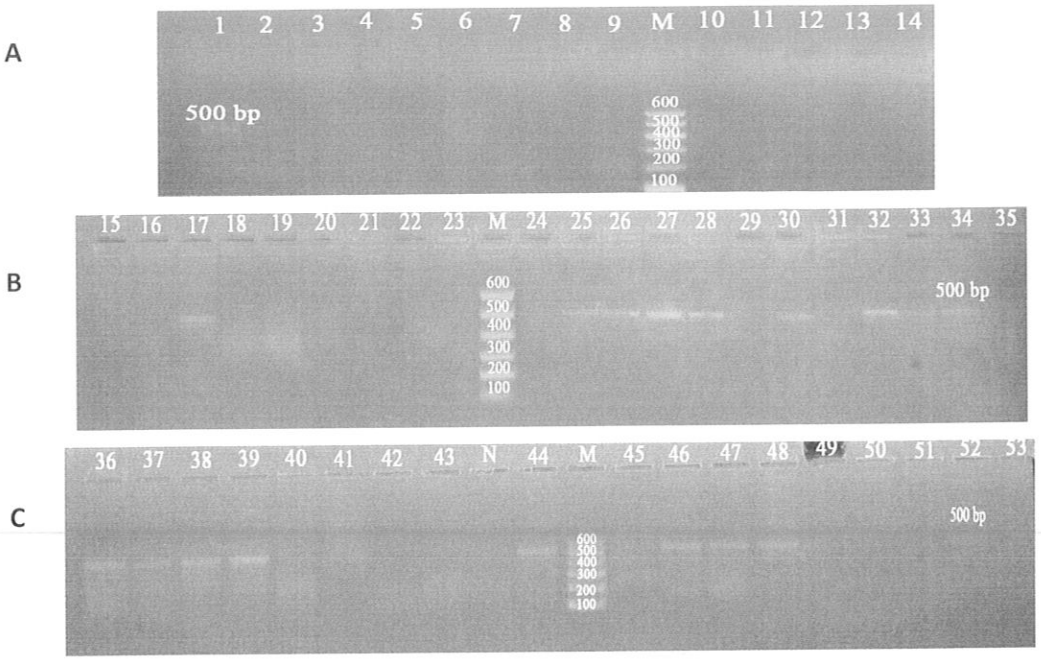

Figure 4: Agarose gel electrophoresis for the amplified product of *M. haemolytica Ssa* gene from clinical samples of poultry in a uinplex PCR.

Lanes 1, 17, 25-28, 30, 32-34, 36-39, 44, 46-48, 52: positive samples for *M. haemolytica Ssa* gene, lane N: Negative control, lane M: molecular size markers (100 bp).

DISCUSSION

Respiratory tract infections are of paramount importance in the poultry industry because high mortality may occur in poorly managed cases. The etiology of respiratory disease is complex, often involving more than one pathogen at the same time ***(19)***. Interestingly, the classical microbiological techniques currently in use for bacterial detection and identification are satisfactory in most situations and remain necessary for drug susceptibility testing but the complexity associated with them makes alternative approaches attractive ***(20)***. This is an argument for the utilization of molecular diagnostics relying on the presence of bacterial DNA and PCR methods are able to detect small amounts of pathogen even dead ones ***(21)***. The study was tailored to develop rapid multiplex PCR assay allowing simultaneous detection of respiratory bacterial pathogens in poultry flocks. From 53 examined poultry farms, *E. coli* was highly recorded (47.17%) followed by *M. haemolytica* (20.75%), *P. aeruginosa* (15.09%) and *M. gallisepticum* (5.66%), while *S. aureus*, *P. mirabilis* and *A. hydrophila* were similarly detected with a lower percentage (3.77%) by conventional identifications methods. Ironically, *P. multocida* was not recorded at any examined farm at all.

In a previous study, *M. gallisepticum* was isolated from chickens showing signs of chronic respiratory disease with a percentage of 5% ***(22)***. Additionally, *E. coli* was highly detected in respiratory organs of chickens (49%) ***(23)***. Moreover, **Pak *et al.*,** isolated *Staphylococcus aureus* from chickens with a percentage of 4.7% ***(24)***. Meanwhile, higher incidences were recorded in another investigations as some of them isolated *P. aeruginosa* from lung tissues of poultry with an incidence of 35% ***(25)*** and others recovered *M. haemolytica* from chickens with a higher frequency (42.2%) ***(26)***. On the contrary, **Muhairwa *et al.*,** isolated *P. multocida ssp.*

multocida from chickens (0.7%) and ducks (7%) *(27)*.

In the current work, multiplex PCR assay was optimized for successful detection of five respiratory pathogens including *E. coli, S. aureus, P.aeruginosa, P. multocida* and *M. gallisepticum* from clinical specimens collected from 53 diseased poultry farms. The specificity of m-PCR was determined and the results showed that m-PCR yielded a detectable DNA fragment of expected molecular weight only in the presence of their respective DNA template and gave negative results when tested with other bacteria. This observation is in harmony with another study in which a multiplex PCR was performed for six pathogens including *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumoniae, Klebsiella pneumoniae,* and *Acinetobacter spp.* and obtained results found to be sensitive and specific *(28)*.

In particular, the PCR assays demonstrated a significantly higher rate of detection of respiratory pathogens in poultry flocks than that was detected by the conventional culture methods and these findings were commonly reported by other investigators *(29)*.

In general, the use of multiplex PCR reactions for groups of organisms causing similar syndromes provides an efficient way to ask several related epidemiological questions simultaneously.

Conclusion

In this article, we have described a multiplex PCR-based diagnosis method for respiratory infections that is simple, inexpensive, and sensitive and enables the quick and precise detection of the most prevalent respiratory pathogens in clinical samples of diseased poultry flocks. Although our results are preliminary, this PCR assay would offer an effective alternative to traditional typing methods for the identification and differentiation of the most clinically relevant respiratory pathogens. Further studies should be done to design new primers specific for a wide range of pathogens whose early detection is beneficial for prognosis.

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الملخص العربى

أستخدام تفاعل البلمرة المتسلسل المتعدد للكشف عن العدوى البكتيرية المسببة للأمراض التنفسية فى الدواجن

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تلعب مسببات الأمراض البكتيرية دورا هاما في الاصابة بأمراض الجهاز التنفسي في الدواجن. هناك ستة مسببات شهيرة للأمراض التنفسية فى الدواجن وهم الميكروب القولونى، الميكوبلازما جاليسبتكم، المكورات العنقودية الذهبية، الباستريلا مالتوسيدا، المانهيميا هيموليتيكا والزائفة الزنجارية. و بالرغم من ذلك، طرق التشخيص الحالية بالنسبة لهم ليست حساسة للغاية، شاقة وتستغرق وقتا طويلا. يهدف هذا البحث إلى تصميم تفاعل البلمرة المتسلسل المتعدد (m-PCR) والذى لديه القدرة على الكشف عن الميكروبات الستة في العينات السريرية من الدواجن المصابة بالأمراض التنفسية. وكشفت النتائج أن ١٩(٣٥,٨%) مزرعة من أصل ٥٣ مزرعة التى تم فحصهم كانت ذات عدوى أحادية، ٣١ (٥٨,٥٪) بها اصابات بكتيرية مختلطة، وفي الوقت نفسه ٣ فقط (٥,٧٪) سلبي . وقد أثبتت الدراسة ان تصميم تفاعل البلمرة المتسلسل المتعدد المستخدم في هذه الدراسة حساس ومحدد للكشف عن الميكروب القولونى، الميكوبلازما جاليسبتكم، المكورات العنقودية الذهبية، والزائفة الزنجارية في العينات السريرية في نفس التفاعل الواحد بينما لم يمكن الكشف عن المانهيميا هيموليتيكا نظرا لانخفاض درجة حرارة annealing عن باقى الميكروبات علاوة على ان حجم المنتج نفس حجم منتج الزائفة الزنجارية والتى يصعب تمييزها عند تمريرها فى الجل الكهربائى كما لم يمكن أيضا الكشف عن الباستريلا مالتوسيدا حيث ان جميع العينات جاءت سلبية للميكروب. اجمالا يمكن اعتبار تصميم تفاعل البلمرة المتسلسل المتعدد المستخدم فى البحث أداة مفيدة لتشخيص ورصد كل من الميكروب القولونى، الميكوبلازما جاليسبتكم، المكورات العنقودية الذهبية، والزائفة الزنجارية في قطعان الدواجن حيث أن هذه التقنية تأخذ ما يقرب من ثلاث ساعات ليتم تنفيذها ولديها القدرة لتحل محل طرق العزل التقليدية، وبالتالي يمكن من إسراع عملية العلاج.