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Short Communication

Distribution of Antibiotic Resistance Genes among the Phylogroups of *Escherichia coli* in Diarrheic Calves and Chickens Affected by Colibacillosis in Tehran, Iran

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ABSTRACT

Antibiotic resistance occurs in the endogenous flora of exposed population in addition to pathogenic bacteria. This study was conducted to evaluate the distribution of antibiotic resistance genes among 63 isolates of Escherichia coli of *Escherichia coli* (*E. coli*) in diarrheic calves and poultry. According to the results, B_1 and B_2 were the most prevalent phylogroups of *E. coli* in calves and poultry carcasses, respectively. Antimicrobial resistance was observed in 76% of the isolates, and 62% of the strains were multi-drug resistant. Antibiotic resistance in *E. coli* strains obtained from calves strains was significantly higher than those obtained from poultries. Additionally, the strains of B_1 and D phylogroups had the highest and lowest antimicrobial resistance, respectively. At least one encoding gene for integrone was detected in 23 strains (36.5%) and Class I integron had the highest prevalence. Accordingly, this study gave baseline information on the magnitude of the resistance problem and its genetic background in *E. coli* from domesticated animals of the Tehran, Iran. Moreover, the power of oligonucleotide array technology in the discrimination of different genotypes during a short time was confirmed in this study.

Keywords: *Escherichia coli*, phylogroups, Integrons, Resistance genes

Distribution des gènes de résistance aux antibiotiques parmi les phylogroupes d'*Escherichia coli* chez les veaux diarrhéiques et les poulets touchés par la colibacillose à Téhéran, Iran

Résumé: La consommation d'antibiotiques peut induire une résistance non seulement dans la flore endogène mais également chez les bactérie pathogène. Ce travail de recherche a été mené pour évaluer la distribution des gènes de résistance parmi 63 phylogroupes d'*Escherichia coli* (*E. coli*) chez les veaux diarrhéiques et la volaille. Selon les résultats, B_1 et B_2 étaient respectivement les phylogroupes les plus répandus d'*E. coli* chez les veaux et les carcasses de volaille. Unerésistance antimicrobienne a été observée chez 76% des isolatset 62% des souches étaient multi-résistantes aux médicaments. La résistance aux antibiotiques chez les souches d'*E. coli* isoléesà partir des échantillons provenant des veaux était significativement plus élevée que celles obtenues à partir des volailles. De plus, les souches des phylogroupes B1 et D présentaient la résistance antimicrobienne la plus élevée et la plus faible. Au moins, un gène codant pour l'intégrone a été détecté en 23 souches (36.5%) et l'intégrone de Classe I montraitla prévalence la plus élevée. En conclusion, ce travail de recherche a fourni des informations de base sur l'ampleur du problème de résistance et son contexte génétique chez les souches d'*E.*

coli isolées chez des animaux domestiques de Téhéran, Iran. De plus, l'efficacité de la technologie de réseau d'oligonucléotides dans la discrimination de différents génotypes surune courte durée a été confirmée dans cette recherche.
Mots-clés: *Escherichia coli, Intégrone, Gènes de résistance*

INTRODUCTION

Escherichia coli (*E. coli*) is a species of the family Enterobacteriaceae, which could be find in the intestine of warm-blooded hosts as normal flora. The virulence factors of several *E. coli* strains cause different intestinal and extra-intestinal diseases by the disruption of cellular and physiological processes (Kaper et al., 2004). *E. coli* strains are divided into four phylogroups of A, B_1, B_2, and D based on harboring *chuA*, *yjaA*, and *TspE4.C2* genes (Gordon et al., 2008). B_2 and D groups are the main parts of extra-intestinal pathogenic *E. coli* strains. However, A and B_1 groups are the lowest pathogenic *E. coli* strains and are defined as non-human enteropathogenic strains (Clermont et al., 2002). The prevalence of antimicrobial resistance in the *E. coli* strains obtained from domesticated animals tends to grow because more than half of the antimicrobial agents in several regions are administered in farm animals resulting in an increase of antibiotic resistance determinants in bacteria (Guerra et al., 2003). Antibiotic resistance genes within bacterial communities are plasmid-mediated, transposons and integrons, so rising the population of antimicrobial multi resistant bacteria against several drugs in environment and various hosts is considered as a public health concern (Schwarz and Chaslus-Dancla, 2001). Recently, the prevalence of animal antimicrobial resistant (AMR) bacterial pathogens is growing among humans (Ewers et al., 2012). The increase of the population of AMR *E. coli* strains within animal hosts suggested that domesticated animals can play an important role as sources or reservoirs of such bacterial agents for human and environment (Carattoli, 2008; Smet et al., 2010; Ewers et al., 2012). Microarray analysis is one of the newest modalities used in various biological studies like gene detection and expression in huge numbers and very short time. This technology enables researchers to characterize the virulence and genotype of the microorganisms (Bumgarner, 2013). DNA microarrays rely on the hybridization properties of nucleic acids to measure the abundance levels of DNA or RNA on a genomic scale in different types of cells such as bacteria. The hybridization refers to the annealing of two nucleic acid strands following the base-pairing rules. In this molecular technique, which is used for genotyping and gene detecting, DNA probes of the known genes (DNA oligonucleotide sequences spotted on the slide array and immobile substrate) are printed on the microscopic slides. After the genomic extraction of samples like bacterial genome and fluorescent labelling of whole extracted genome, the labelled nucleic acids are applied on the printed slide and incubated in a hybridization chamber. Finally, the slides are washed by related buffers and scanned by microarray scanners to detect hybridized spots on the slide and identify the presence of related genes of each printed spot in the extracted sample. In addition, microarrays analysis is used to measure transcript expression, obtain DNA copy number, calculate identity-by-descent, measure mRNA decay rates, identify protein binding sites, and determine subcellular localization of gene products (Bilitewski, 2009; Call et al., 2003; Wang et al., 2003; Wu et al., 2001). This study was conducted to evaluate the prevalence of several AMR determinants and their relation to *E. coli* phylogroups using DNA microarray based on oligonucleotides hybridization on microscopic slides.

MATERIALS AND METHODS

In this study, *E. coli* strains were the part of the culture collections of the Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. These strians were previously collected from domesticated cattle and poultry farms around Tehran for genotyping purposes. All of them have been described in previous studies (Salehi et al., 2013; Staji et al., 2012; Staji et al., 2015). AccuPrep® Genomic DNA Extraction Kit (Bioneer, Korea) was used to extract the genomic DNA samples from the isolated strains according to the manufacturer's protocol. Then, the genomic DNA samples were purified and labelled based on the method developed by Zahraei Salehi et al., 2011. The microarray slides used in the present study were composed of DNA probes targeting phylogenetic markers, AMR genes, and all known virulence factors of *E. coli*, originally developed by Bruant et al. Thereafter, hybridization and data acquisition steps were carried out as described previously (Bruant et al., 2006; Staji et al., 2012; Staji et al., 2015).

RESULTS AND DISCUSSION

The selected strains were divided into several groups based on the presence of phylogenetic markers. According to the results, the phylotypes were distributed as A (13%), B_1 (56%), B_2 (15.5%), and D (15.5%). In addition, the B_1 was the most distributed phylogroup in this study, which caused diarrhea in calves, and those strains belonged to this phylogroup were the most distributed strains in several poultry farms (Table 1). Regarding the results, out of 63 *E. coli* isolates, 15 isolates (24%) did not have antimicrobial resistance genes. However, 76% of all strains were resistant to at least one of the antimicrobial agents tested, and 14% and 62% of them were mono- and multi-drug resistant (2-13 resistance determinants), respectively. Additionally, ereA, dhfrXII, catII, aac3vi, aacC2, and ampC (1.5%) antimicrobial resistance genes had the least distribution within all *E. coli* isolates, consequently. At least one gene encoding for an integron was detected in 23 strains. All integron classes were detected; nevertheless, the class I integron was detected as a single integron in 15 strains and together with classes II and III integrons were found in 4 strains. A few strains showed the class II or III integron as single integrin, and 13 strains (31% of examined strains) showed an association between class I integron and TnpM gene. All the observed TnpM genes were present in calf strains and none of the poultry strains were positive for this resistance marker. There were 63 antimicrobial resistance genotypes and none of the genetic profiles were repeated within the isolates. The antibiotic resistance in calf strains (77.14%) was significantly higher than poultry strains (22.86%) ($\chi^2$6.635, P<0.01). Moreover, there was a significant difference between the phylogroups in terms of the prevalence of antibiotic resistance. Although the B_1 phylogroup (71.90%) significantly showed the most resistance comparing to other phylogroups, there was no significant difference in the resistance prevalence between A (14.29%) and B_2 (11.43%) phylogroups. Furthermore, a significant difference between the D strain and other strains was observed considering the prevalence of antibiotic resistance (2.38%; $\chi^2$6.635, P< 0.01). In the present study, the DNA oligonucleotide array was used to identify the phylogenetic and antibiotic resistance genotypes of pathogenic *E. coli* strains collected in the Department of Microbiology, Faculty of Veterinary Medicine, Tehran University, Tehran, Iran, isolated from diarrheic calves and poultry in short time. The relationship between *E. coli* phylogroups and various host species are demonstrated in the literature. Gordon and Cowling in 2003 indicated that the relative abundance of phylogenetic groups among mammals was dependent on the host diet and body mass and climate (Gordon and Cowling, 2003). Escobar-Páramo et al. analyzed fecal samples obtained from different hosts and showed that B_1 and D, B_1 and A, and A and B_2 phylogroups were mainly distributed among avians, non-human mammals, and humans, respectively (Escobar-Páramo et al., 2006). Moreover, they concluded that domestication was one of the main

forces that shape the genetic structure of *E. coli* populations in habitats. Baldy Chudzik et al. in 2008 analyzed *E. coli* obtained from wild animals kept in zoo and understood that group B_1 was related to herbivorous and group A was prevalent in carnivorous and omnivorous hosts. In this study, we described the distribution of different *E. coli* phylogroups in several cattle farms of Tehran, Iran. Considering the results, B_1 phylogroup was the main cause of diarrhea in newborn calves followed by D, A, and B_2 phylogroups, respectively. Furthermore, the distribution of phylogroups in *E. coli* strains from poultry colibacillosis showed that B_2 group was the dominant one within these hosts followed by group D. The results about the calves were in line with the results of other investigations conducted by Escobar-Páramo et al., in 2006, Baldy-Chudzik et al., and Staji et al., in 2015. In addition, the results were consistent with the results of the study carried out by Shahaboddin and Staji in Semnan, Iran, about poultry strains, while they were partially resembling to the results of other investigations (Escobar-Páramo et al., 2006; Shahaboddin and Staji 2016). The high prevalence of multi-drug resistant *E. coli* strains (62%) as infectious agents in poultry and cattle farms of the region is due to immethodical and irregular use of antibiotics in feeding regimes and high prevalence of resistance in the pathogenic *E. coli* strains. Additionally, diverse resistance types between various *E. coli* strains obtained from calves and poultries could be related to the different antibiotic regimes used for different agents and livestock species (Schwarz and Chaslus-Dancla, 2001; Guerra et al., 2003). In the present study, the comparison of the resistance prevalence between different phylogroups demonstrated that the members of B_1 groups were harboring the highest genetic resistance (71.90%) to antimicrobial agents and those belonging to D phylogroup showed the lowest resistance (2.38%). Gordon et al. in 2008 revealed that the majority of the *E. coli* strains that were able to persist in the environment belong to the B_1 phylogenetic group. It is worth mentioning that the B_1 phylogroup are commensal bacteria. Regarding the results of this study, the circulation of antimicrobial resistance genes between the members of this group was at the highest level comparing to other phylogroups, and they were able to transmit these factors vertically and horizontally (Staji et al., 2015). Accordingly, it seems that these agents may play an important role in the distribution of virulence and antimicrobial genetic elements to other strains causing the high prevalence of resistance within pathogenic and nonpathogenic *E. coli* strains. The AMR genes are located on a genetic unit called gene cassettes, and a cassette consists of a gene and a recombination site (*attC*). They can exist as cell-free circular DNA; however, they cannot be replicated or transcribed in this form. A recombination occurs between the attachment sites of Integrons (*attI*) and cassette (*attC*), which integrates the cassette into the integrin. Then, the gene on the cassette is subcloned into plasmid (Mammeri et al., 2005). The genes carried by mobile genetic elements are used by Gram-negative bacteria to escape antimicrobial effects. The integration of AMR into gene cassette and plasmid is operated by integrons, which are not transmissible thelmselves. Therefore, the transfer of DNA from one microorganism to another happens via transposons, plasmids, and bacteriophages (Davies, 2007). In the current study, the analysis of the prevalence of integrons and AMR genes within *E. coli* strains determined that the gene cassettes, *aac(3)-VI*, *aadA1*, and/or the set of *tet* and *sul* genes were diffusely associated with Class I integron. These results demonstrated the importance of this mobile elements in the genetic recombination of plasmid material. About 20% of examined strains possessing the Class I integron and *TnpM* genes showed a multi-drug resistance genetic profile related to the Class A and C β-lactamases and fluoroquinolone resistance protein together with aminoglycosides, tetracycline, and sulfonamide-resistance encoding genes. Accordingly, these strains had high potential genetic transferability.

Table 1. Distribution of Antimicrobial Resistance genes in *E. coli* isolates & their prevalence in different phylogroups.

	Frequency		Origin (calf)		Origin(poultry)		A		B1		B2		D	
	No/Total	Percentage	No/Total	Percentage	No/Total	Percentage	No	%	No	%	No	%	No	%
Transposon	**12**		**12/12**	**100**	**0/12**	**0**	**2**	**16.67**	**10**	**83.33**	**0**	**0**	**0**	**0**
tnpM	12/64	18.75	12/12	100	0/12	0	2	16.67	10	83.33	0	0	0	0
Beta Lactamase	**32**		**25/32**	**78.13**	**7/32**	**21.87**	**6**	**18.75**	**18**	**56.25**	**3**	**9.38**	**5**	**15.62**
ampC	1/64	1.56	1/1	100	0/1	0	0	0	0	0	0	0	1	100
tem	10/64	15.63	8/10	80	2/10	20	4	40.00	4	40.00	1	10.00	1	10.00
blaCMY-2	21/64	32.81	16/21	76.19	5/21	23.81	2	9.52	14	66.67	2	9.52	3	14.29
Phenicols	**11**		**8/11**	**72.73**	**3/11**	**27.27**	**1**	**9.09**	**7**	**63.64**	**3**	**27.27**	**0**	**0**
cml	7/64	10.94	4/7	57.14	3/7	42.86	0	0	4	57.14	3	42.86	0	0
cat	3/64	4.69	3/3	100	0/3	0	1	33.33	2	66.67	0	0	0	0
catII	1/64	1.56	1/1	100	0/1	0	0	0	1	100	0	0	0	0
Quinolones	**4**		**4/4**	**100**	**0/4**	**0**	**0**	**0**	**4**	**100**	**0**	**0**	**0**	**0**
qnrS1	4/64	6.25	4/4	100	0/4	0	0	0	4	100	0	0	0	0
Macrolides	**1**		**1/1**	**100**	**0/1**	**0**	**0**	**0**	**1**	**100**	**0**	**0**	**0**	**0**
ereA2	1/64	1.56	1/1	100	0/1	0	0	0	1	100	0	0	0	0
Sulfanamides	**21**		**14/21**	**66.67**	**7/21**	**33.33**	**2**	**9.53**	**13**	**61.90**	**6**	**28.57**	**0**	**0**
sulII	11/64	17.19	9/11	81.82	2/11	18.18	2	18.18	7	63.64	2	18.18	0	0
sulIII	10/64	15.63	5/10	50	5/10	50	0	0	6	60	4	40	0	0
Aminoglycosides	**64**		**49/64**	**76.56**	**15/64**	**23.44**	**10**	**15.63**	**51**	**79.69**	**3**	**4.68**	**0**	**0**
aphA	17/64	26.56	16/17	94.12	1/17	5.88	2	11.76	15	88.24	0	0	0	0
strA	6/64	9.38	5/6	83.33	1/6	16.67	1	16.67	5	83.33	0	0	0	0
aph6/strB	20/64	31.25	16/20	80	4/20	20	5	25.00	15	75.00	0	0	0	0
aadA1	19/64	29.69	10/19	52.63	9/19	47.37	2	10.53	14	73.68	3	15.79	0	0
aacC2	1/64	1.56	1/1	100	0/1	0	0	0	1	100	0	0	0	0
aac3vi	1/64	1.56	1/1	100	0/1	0	0	0	1	100	0	0	0	0
Tetracyclines	**49**		**39/49**	**79.59**	**10/49**	**20.41**	**6**	**12.24**	**35**	**71.43**	**8**	**16.33**	**0**	**0**
tet(A)	13/64	20.31	9/13	69.23	4/13	30.77	1	7.69	9	69.23	3	23.08	0	0
tet(B)	10/64	15.63	8/10	80	2/10	20	2	20.00	8	80.00	0	0	0	0
tet(R)	16.64	25.00	12/16	75	4/16	25	2	12.50	10	62.50	4	25.00	0	0
CM_tet30	10/64	15.63	10/10	100	0/10	0	1	10.00	8	80.00	1	10.00	0	0
Trimethoprim	**13**		**9/13**	**69.23**	**4/13**	**30.77**	**3**	**23.08**	**9**	**69.23**	**1**	**7.69**	**0**	**0**
dhfrI	7/64	10.94	4/7	57.14	3/7	42.86	1	14.29	5	71.43	1	14.29	0	0
dhfrV	5/64	7.81	4/5	80	1/5	20	2	40.00	3	60.00	0	0	0	0
dhfrXII	1/64	1.56	1/1	100	0/1	0	0	0	1	100	0	0	0	0
Quads	**3**		**1/3**	**33.33**	**2/3**	**66.67**	**0**	**0**	**3**	**100**	**0**	**0**	**0**	**0**
qac	3/64	4.69	1/3	33.33	2/3	66.67	0	0	3	100	0	0	0	0
Total	**210**		**162/210**	**77.14**	**48/210**	**22.86**	**30**	**14.29**	**151**	**71.90**	**24**	**11.43**	**5**	**2.38**

The DNA microarray technology is a powerful tool for genetic characterization of the *E. coli* AMR and suggested the probable circulation of genetic elements related to a multi-drug resistance in cattle and poultry populations in Tehran, Iran. In this study, the *E. coli* strains were collected 3-4 years ago, and microbial populations genotypes may change during time. As a result, it is recommended to assess antimicrobial resistance among *E. coli* strains circulating in cattle and poultry farms in the present time to assess real and exact statues. Moreover, the data presented may support the exposure assessment within the scientific risk analysis of antimicrobial resistance and may highlight the need for the prudent use of antibiotics in animal husbandry. Furthermore, commensal bacteria can act as an important spreaders of antimicrobial resistance genes to pathogenic and nonpathogenic strains.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

Baldy-Chudzik, K., Mackiewicz, P. and Stosik, M., 2008. Phylogenetic background, virulence gene profiles, and genomic diversity in commensal Escherichia coli isolated from ten mammal species living in one zoo. Vet microbiol 131(1), 173-184.

Bilitewski, U., 2009. DNA microarrays: an introduction to the technology. Methods Mol Biol 509, 1-14.

Bruant, G., Maynard, C., Bekal, S., Gaucher, I., Masson, L., Brousseau, R. and Harel, J., 2006. Development and validation of an oligonucleotide microarray for detection of multiple virulence and antimicrobial resistance genes in Escherichia coli. Applied and environ microbiol 72(5), 3780-3784.

Bumgarner, R., 2013. DNA microarrays: Types, Applications and their future. Curr Protoc Mol Biol 0 22: Unit–22.1.

Call, D. R., Bakko, M. K., Krug, M. J., Roberts, M. C., 2003. Identifying antimicrobial resistance genes with DNA microarrays. Antimicrob Agents Chemother 47, 3290-3295.

Carattoli, A., 2008. Animal reservoirs for extended spectrum β-lactamase producers. Clin Microbiol and Infect 14(s1), 117-123.

Clermont, O., Bonacorsi, S. and Bingen, E., 2000. Rapid and simple determination of the Escherichia coli phylogenetic group. Appl environ microbiol 66(10), 4555-4558.

Davies, J., 2007. Microbes have the last word. EMBO Rep 8, 616 - 21.

Escobar-Páramo, P., Menac'h, L., Le Gall, T., Amorin, C., Gouriou, S., Picard, B., Skurnik, D. and Denamur, E., 2006. Identification of forces shaping the commensal Escherichia coli genetic structure by comparing animal and human isolates. Environ Microbiol 8(11), 1975-1984.

Ewers, C., Bethe, A., Semmler, T., Guenther, S. and Wieler, L.H., 2012. Extended-spectrum β-lactamase-producing and AmpC-producing Escherichia coli from livestock and companion animals, and their putative impact on public health: a global perspective. Clin Microbiol and Infec 18(7), 646-655.

Gordon, D.M. and Cowling, A., 2003. The distribution and genetic structure of Escherichia coli in Australian vertebrates: host and geographic effects. Microbiology 149(12), 3575-3586.

Gordon, D.M., Clermont, O., Tolley, H. and Denamur, E., 2008. Assigning Escherichia coli strains to phylogenetic groups: multi-locus sequence typing versus the PCR triplex method. Environ microbiol 10(10), 2484-2496.

Guerra, B., Junker, E., Schroeter, A., Malorny, B., Lehmann, S. and Helmuth, R., 2003. Phenotypic and genotypic characterization of antimicrobial resistance in German Escherichia coli isolates from cattle, swine and poultry. J Antimicrobial Chemotherapy 52(3), 489-492.

Kaper, J.B., Nataro, J.P. and Mobley, H.L., 2004. Pathogenic escherichia coli. Nat Rev Microbiol 2(2), 123-140.

Mammeri, H., Van De Loo, M., Poirel, L., Martinez-Martinez, L. and Nordmann, P., 2005. Emergence of plasmid-mediated quinolone resistance in Escherichia coli in Europe. Antimicrobial Agents and Chemotherapy 49(1), 71-76.

Salehi, T.Z., Derakhshandeh, A., Tadjbakhsh, H. and Karimi, V., 2013. Comparison and phylogenetic analysis of the ISS gene in two predominant avian pathogenic E. coli serogroups isolated from avian colibacillosis in Iran. Research in veterinary science, 94(1), 5-8.

Salehi, T.Z., Tonelli, A., Mazza, A., Staji, H., Badagliacca, P., Tamai, I.A., Jamshidi, R., Harel, J., Lelli, R. and Masson, L., 2012. Genetic characterization of Escherichia coli O157: H7 strains isolated from the one-humped camel (Camelus dromedarius) by using microarray DNA technology. Mol biotech 51(3), 283-288.

Schwarz, S. and Chaslus-Dancla, E., 2001. Use of antimicrobials in veterinary medicine and mechanisms of resistance. Vet Res 32(3-4), 201-225.

Schwarz, S. and Chaslus-Dancla, E., 2001. Use of antimicrobials in veterinary medicine and mechanisms of resistance. Vet res 32(3-4), 201-225.

Shahaboddin, E., Staji, H., 2015. Determination and comparison of phylogenetic groups of E. coli strains isolated from wild birds and human with urinary infections in Semnan city by using multiplex PCR technique [master's thesis]. Faculty of Veterinary Medicine, Semnan University.

Smet, A., Martel, A., Persoons, D., Dewulf, J., Heyndrickx, M., Herman, L., Haesebrouck, F. and Butaye, P., 2010. Broad-spectrum β-lactamases among Enterobacteriaceae of animal origin: molecular aspects, mobility and impact on public health. FEMS microbiol rev 34(3), 295-316.

Staji, H., Salehi, T.Z., Dastjerdi, A.M. and Tonelli, A., 2012. Pathotyping of isolated Escherichia coli from domesticated

calves and poultry using modern DNA microarray technique. J Vet Res 67(1), 1-9.

Staji, H., Tonelli, A., Javaheri-Vayeghan, A., Changizi, E. and Salimi-Bejestani, M.R., 2015. Distribution of Shiga toxin genes subtypes in B1 phylotypes of Escherichia coli isolated from calves suffering from diarrhea in Tehran suburb using DNA oligonucleotide arrays. Iran J Microbiol 7(4), 191.

Wang, H. Y., Malek, R. L., Kwitek, A. E., Greene, A. S., Luu, T. V., Behbahani, B. et. al., 2003. Assessing unmodified 70-mer oligonucleotide probe performance on glass-slide microarray. Genome Biol 4, 5.

Wu, L., Thompson, D. K., Li, G., Hurt, R. A., Tiedje, J. M., Zhou, J., 2001. Development and evaluation of functional gene arrays for detection of selected genes in the environment. Appl Environ Microbiol 67, 5780-5790.

Youssefi, M., Rahimi, M., 2010. Haemoproteus Columbae in Columba livia domestica of Three Areas in Iran in 2010. Glob Vet 7, 593-5.