Comparison of phenotypic and virulence genes characteristics in human and chicken isolates of *Proteus mirabilis*

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The objective of this work is to compare the phenotypic and virulence genes characteristics in human and chicken isolates of *Proteus mirabilis*. The bacterial examination of 50 livers of individual broilers, marketed by four major outlets, revealed a high recovery of *P. mirabilis* (66%), and a low recovery frequency of *Salmonella* spp. (4%), *Serratia odorifera* (2%), *Citrobacter brakii* (2%), and *Providencia stuartii* (2%). The phenotypic biochemical characterization of the recovered 33 chicken isolates of *P. mirabilis* were compared to 30 human isolates (23 urinary and six respiratory isolates). The comparison revealed significant differences in the presence of gelatinase enzyme (100% presence in chicken isolates versus 91.3 and 83.3% presence in human urinary and respiratory isolates, respectively, $P < 0.05$). The H_2S production occurred in 100% of chicken isolates versus 95.6 and 66.7% presence in human urinary and respiratory isolates, respectively, $P < 0.05$). The other 17 biochemical characteristics did not differ significantly among the three groups of isolates ($P > 0.05$). Two virulence genes, the *mrpA* and *FliL*, were having a typical 100% presence in randomly selected isolates of *P. mirabilis* recovered from chicken livers ($N=10$) versus isolates recovered from urinary ($N=5$) and respiratory specimens of humans ($N=5$) ($P > 0.05$). The average percentage similarity of *mrpA* gene nucleotide sequence of poultry isolates to human urinary and respiratory isolates was 93.2 and 97.5%, respectively. The high similarity in phenotypic characteristics, associated with typical frequency of presence of two virulence genes, and high similarity in sequences of *mrpA* gene among poultry versus human *P. mirabilis* isolates justifies future investigations targeting the evaluation of adaptable pathogenicity of avian *Proteus mirabilis* isolates to mammalian hosts.

Keywords: Characteristics, Chicken, Genotype, Humans, Phenotype, *Proteus mirabilis*, Relatedness

Introduction

Proteus species are part of the Enterobacteriaceae family of Gram-negative bacilli. Many organisms infecting chickens were able to infect the mammalian hosts.^{1–5} The most important poultry organisms causing zoonoses are *Salmonella* spp.,⁵ *Campylobacter* spp.,⁶ and avian influenza.^{3,4}

Proteus mirabilis has been reported as one of the causative agents of human pneumonia^{7,8} and other lung infection conditions.⁹ *Proteus mirabilis* causes 90% of *Proteus* infections and is mostly found in people with compromised immune system.¹⁰ This infection can develop into endotoxin-induced sepsis, resulting in systemic inflammatory response syndrome, associated with a mortality rate of up to 50%. In addition, *Proteus mirabilis* is proven to cause central nervous system infection¹¹ and urolithiasis.¹² The development of urolithiasis is due to the urea hydrolysis caused by the urease enzyme present in this

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bacterium. Actually, the hydrolysis induces a rise in pH that causes a precipitation of magnesium ammonium phosphate (struvite) and calcium phosphate (apatite) crystals, resulting in urinary tract obstruction.¹³ *P. mirabilis* is also involved in reproductive failure of avian species.¹⁴ In addition, the role of *P. mirabilis* in urinary tract and kidney infection of animals has been previously documented.^{15–20}

The literature is rich in studies of the biochemical reactions of human isolates of *P. mirabilis* and the magnitude of variability in their characteristics.^{21,22} Unfortunately, the reporting of biochemical characteristics of *P. mirabilis* of animal origin is very sparse.¹⁵ The determination of similarities in biochemical characteristics of organisms involved in human and animal infections is important, especially when the biochemical characteristic is itself a virulence factor, such as the presence of urease enzyme in *P. mirabilis* that is involved in kidney stone formation.^{12,17,18}

Besides the similarities in phenotypic-biochemical characteristics of the compared animal and human isolates, the occurrence of the same genotypic virulence genes in the compared isolates will augment the importance of their role in diseases. The *mrpA* virulence gene in human isolates of *P. mirabilis* is the most studied fimbrial genetic material. Mice immunized against such fimbriae were completely protected against challenges with *P. mirabilis*;²³ many studies have correlated the expression of *mrpA* with the development of acute pyelonephritis in humans.^{15,24}

The *FliL* virulence gene of human isolates of *P. mirabilis* includes also the motor/switch (*fli* MN) and export genes (*fli*OPQR) of the flagella responsible for swarming.^{25,26} The *FliL* gene encodes a small protein (18 kDa) composed of 160 aminoacids, located in the vicinity of the Mot proteins and interacts with the MS ring that is situated within and above the cytoplasmic membrane.²⁷ Mutation in *FliL* gene results in non-motile *P. mirabilis* cells, decreased cell division, and abnormal swarming.²⁸ To our knowledge, no studies documented the presence of *mrpA* and *FliL* virulence genes in poultry isolates of *P. mirabilis*.

This is the first work that compares the phenotypic and virulence genes characteristics in *P. mirabilis* isolated from chicken livers and clinical human samples. This research is a prerequisite to a future work that will study the adaptability of avian *P. mirabilis* isolates to mammalian hosts, and their role in infectivity and pathogenesis.

Materials and Methods

Bacterial isolates in broiler livers

Fifty livers from broiler chicken were collected from four major markets in Lebanon, and were subjected to

culturing onto Brilliant Green Agar medium for the purpose of isolation of non lactose fermenters.^{21,29} Non-lactose fermenting colonies were subcultured onto Tryptose Broth, incubated at 37°C for 24 hours, and stored in their growth medium (Tryptose Broth) at –80°C.

Clinical human isolates

Twenty nine isolates of suspected *P. mirabilis* recovered from individual patients (23 urinary and 6 respiratory isolates) were provided to us by the Pathology and Clinical Laboratories of the American University of Beirut Medical Center in Beirut, Lebanon. Each human isolate was grown and stored by the same protocol used for the poultry isolates.

Phenotypic-biochemical characterization

The stored human ($n=29$) and chicken ($n=50$) isolates were subjected to phenotypic characterization. Each purified colony from a Nutrient Agar culture was subjected to 19 biochemical tests present in the API20E strip (BioMerieux, Marcy L'Etoile, France) for identification and phenotypic characterization.

mrpA and *FliL* genes in confirmed *P. mirabilis* isolates

A random representative sample of the biochemically confirmed *P. mirabilis* isolates, recovered from chicken and clinical human cases, was subjected to PCR amplification of their *mrpA*³⁰ and *FliL*²⁸ genes. One *Salmonella* isolate and one *P. mirabilis* isolate from the American Type Culture Collection were included to study cross-reactivity and positivity, respectively.

Briefly, the PCR for the *mrpA* gene targeted the amplification of 550 bp present in *P. mirabilis*. The PCR mixture contained 2 µl of 45 µg/µl of extracted DNA of *P. mirabilis* and 4 µl of 5 pmol/µl of each of the forward MRP-F (5'-ACACCTGCCCCATATGG-AAGATACTGGTACA-3') and the reverse primer MRP-R (5'-AAGTGATGAAGCTTAGTGATGGT-GATGGTGATGAGAGTAAGTCACC-3'). In addition, the mix had 25 µl REDTaq[®], containing 20 mM TrisHCl, 100 mM KCl, 3 mM MgCl₂, 0.0002% gelatine, 0.4 mM of each DNTP, and 0.06 unit/µl of Taq DNA polymerase (Sigma-Aldrich, Saint Louis, MO, USA). The thermal cycling conditions were 94°C for 3 minutes, 30 cycles of 94°C for 1 minute, 40°C for 1 minute, and 72°C for 1 minute, and a final cycle of 72°C for 5 minutes using the iCycler model (BioRad Laboratories, Hercules, CA, USA). Amplicons were stained with Ethidium Bromide for 30 minutes, and visualized under UV light by the GelDoc system (BioRad Laboratories).

The PCR for the *FliL* gene amplified a 770-bp part, and followed the same protocol used for *mrpA* amplification. The sequence of the *FliL* forward primer *FliL*-F was 5'-CTCTGCTCGTGGTGGTGTGCG-3'

Table 1 Nucleotide sequences of *mrpA* amplicons of *P. mirabilis* recovered from chicken livers, human respiratory system, and human urinary system

<i>P. mirabilis</i> isolate source	Isolate no.	Nucleotide sequence
Chicken liver	1	CCCATTACCAACAGAGAAGTTACAGTTAATGGTGGTACTATTAAC TTTGAAGGTTCTGTCTTAATGCTGCTTGCTGGTGTGATAGTAGT TCAAGTAACCAAACTGTTTCGTTTGGGTCAATTCCGTGTCGCTGA ATTCATAAAAAAGGTGATGAAACAGGACGTATTCTTTTGAGC ATTAAATTAAATAACTGCGATATTACTGTTTC
	2	CCCTATCAGCAACAGAGAAGTTACAGTTAATGGTGGTACTATTAAC TTTGAAGGTTCTGTCTTAATGCTGCTTGCTGGTGTGATAGTAGT TCAAGTAACCAAACTGTTTCGTTTGGGTCAATTCCGTGTCGCTGA ATTCATAAAAAAGGTGATGAAACAGGACGTATTCTTTTGAGC ATTAAATTAAATAACTGCGATATTCCGCCTCC
	3	CCCTTTCAGCAACAGAGAAGTTACAGTTAATGGTGGTACTATTAAC TTTGAAGGTTCTGTCTTAATGCTGCTTGCTGGTGTGATAGTAGT TCAAGTAACCAAACTGTTTCGTTTGGGTCAATTCCGTGTCGCTGA ATTCATAAAAAAGGTGATGAAACAGGACGTATTCTTTTGAGC ATTAAATTAAATAACTGCGATATTCCGCCTCC
	4	AAAAAAGACTTTCAGAGAAGTTACAGTTAATGGTGGTACTATTAAC TTTGAAGGTTCTGTCTTAATGCTGCTTGCTGGTGTGATAGTAG TTCAAGTAACCAAACTGTTTCGTTTGGGTCAATTCCGTGTCGCT GAATTCATAAAAAAGGTGATGAAACAGGACGTATTCTTTTG AGCATTAAATTAAATAACTGCGATATAAGGGTATC
	5	CAGAAGTTACAGTTAATGGTGGTACTATTAACTTGAAGGTTCT GTCTTAATGCTGCTTGCTGGTGTGATAGTAGTTCAAGTAACCA AACTGTTTCGTTTGGGTCAATTCCGTGTCGCTGAATTCATAAAA AAGGTGATGAAACAGGACGTATTCTTTTGAGCATTAAATTAA TAAGTGCATAT
Human respiratory system	1	CCAACAGAGAAGTTACAGTTAATGGTGGTACTATTAACTTGAAGG TTCTGTCTTAATGCTGCTTGCTGGTGTGATAGTAGTTCAAGTAA CCAAACTGTTTCGTTTGGGTCAATTCCGTGTCGCTGAATTCATA AAAAAGGTGATGAAACAGGACGTATTCTTTTGAGCATTAAATT AAATAACTGCGATAT
	2	CAGAAGTTACAGTTAATGGTGGTACTATTAACTTGAAGGTTCTG TCTTAATGCTGCTTGCTGGTGTGATAGTAGTTCAAGTAACCAAA CTGTTTCGTTTGGGTCAATTCCGTGTCGCTGAATTCATAAAAAAG GTGATGAAACAGGACGTATTCTTTTGAGCATTAAATTAAATAAC TGCGATATTAC
	3	GGGAAAGACTTTCAGAGAAGTTACAGTTAATGGTGGTACTATTAAC TTTGAAGGTTCTGTCTTAATGCTGCTTGCTGGTGTGATAGTAGT TCAAGTAACCAAACTGTTTCGTTTGGGTCAATTCCGTGTCGCTGA ATTCATAAAAAAGGTGATGAAACAGGACGTATTCTTTTGAGC ATTAAATTAAATAACTGCGATATAAGGGTATC
	4	GGGTAAGACTTTCAGAGAAGTTACAGTTAATGGTGGTACTATTA CTTTGAAGGTTCTGTCTTAATGCTGCTTGCTGGTGTGATAGTA GTTCAAGTAACCAAACTGTTTCGTTTGGGTCAATTCCGTGTCGC TGAATTCATAAAAAAGGTGATGAAACAGGACGTATTCTTTT GAGCATTAAATTAAATAACTGCGATATAAGGGTTCC
Human urinary system	1	AAAATAGTCAGCCAGAAGTTACAGTTAATGGTGGTACTATTAAC TTTGAAGGTTCTGTCTTAATGCTGCTTGCTGGTGTTCATAGTAGT TCAAGTTACCA
	2	GCCTTTCGGCGCAGAAGTTACAGTTAATGGTGGTACTATTAAC TTTGAAGGTTCTGTCTTAATGCTGCTTGCTGGTGTGATAGTAGT TCAAGTTACCAAACTGTTTCGTTTGGGTCAATTCCGTGTCGCTGA ATTCATAAAAAAGGTGATGAAACAGGACGTATTCTTTTAGCA TTAAATTAAATAACTGCGATATTACTG
	3	TAAAAAGACTTTCAGAGAAGTTACAGTTAATGGTGGTACTATTAAC TTGAAGGTTCTGTCTTAATGCTGCTTGCTGGTGTGATAGTAGT CAAGTAACCAACCTGTTTCGTTTGGGTCAATTCCGTGTCGCTGAA TTCACTAACAAAGGTGATGAAACCGGACGTATTCTTTTCGAGCAT TAAATTAAATAACTGCGATAT
	4	AAATATCAGCAACAGAGAAGTTACAGTTAATGGTGGTACTATTAAC TTGAAGGTTCTGTCTTAATGCTGCTTGCTGGTGTGATAGTAGT AGTAACCAAACTGTTTCGTTTGGGTCAATTCCGTGTCGCTGAA CTAAAAAAGGTGATGAAACAGGACGTATTCTTTTGAGCATTAAAT TAAATAACTGCGATATAAGCGTATG

Table 2 Number of chicken liver samples positive for *Proteus mirabilis* and other non-lactose fermenters

Number of tested livers	Number of livers with identified organisms by API-20E					
	<i>Proteus mirabilis</i>	<i>Salmonella</i> spp.	<i>Serratia odorifera</i>	<i>Citrobacter brakii</i>	<i>Providencia stuartii</i>	Unidentified Profile
50	33	2	1	1	1	12

and that of the reverse primer FliL-R was 5'-GCGTCG-TCACCTGATGTGTC-3'.

Sequencing of *mrpA* amplicons in *P. mirabilis* isolates

A representative 50% random selection of the amplified *mrpA* gene of poultry isolates, and 80% of the amplified *mrpA* gene of isolates recovered from respiratory and urinary samples of clinical human cases were subjected to nucleotide sequencing of their amplicons. The sequencing was performed at the American University of Beirut, and the sequence data is presented in Table 1. Briefly, the amplicons were purified by the QIAquick Gel Extraction Kit (QIAGEN Sciences, Valencia, CA, USA). A volume of 2 µl of the purified DNA amplicon was mixed with 1 µl of 1.6 pmol/µl of the *mrpA* reverse primer and 2 µl Big Dye Terminator version 1.1 for amplification (Applied Biosystem, Carlsbad, CA, USA). The nucleotides of the resulting amplicons were sequenced in a ABI-Prism 3130 type sequencer (Applied Biosystem).

The nucleotide sequences of the *mrpA* gene of chicken and human *P. mirabilis* isolates were compared using the program of the NCBI (Basic Local Alignment Search Tool, BLAST v. 2.2.15, www.ncbi.nlm.nih.gov).³¹

Statistics

The comparison of proportions of presence of a phenotypic (biochemical) characteristic in chicken versus urinary and respiratory *P. mirabilis* isolates of humans is conducted by using the Chi-square test, at a 95% confidence level (SPSS Inc., Chicago, IL, USA).

The similarity percentage among the nucleotide sequences of the *mrpA* amplicons of the *P. mirabilis* of chicken versus clinical isolates of humans (respiratory

and urinary) were also compared by Chi-square test, at a confidence level of 95%.

Results

The fifty chicken livers, collected from four major markets, revealed by the API-20E system the following frequency of recovered non-lactose fermenters, in decreasing order: *Proteus mirabilis* (66%), *Salmonella* spp. (4%), *Citrobacter brakii* (2%), *Providencia stuartii* (2%), and *Serratia ordorifera* (2%) (Table 2).

The frequency of the presence of each of 19 biochemical characteristics of the 33 chicken isolates of *P. mirabilis* were compared with that of 29 human isolates recovered from 23 urinary and 6 respiratory clinical cases (Table 3). The majority of the biochemical characteristics (17 out of 19) (89.5%) were similar in chicken versus human isolates of *P. mirabilis* ($P>0.05$). There was a significant difference in two biochemical characteristics of *P. mirabilis* namely, a 100% presence of gelatinase in the chicken isolates versus a 91.3% and a 83.3% presence in human urinary and respiratory isolates respectively, $P<0.05$; in addition, there was a 100% production of H₂S by chicken isolates versus a 95.6% and 66.7% production by urinary and respiratory isolates, respectively ($P<0.05$).

All *P. mirabilis* isolates from chickens and humans had a similar prevalence of *mrpA* (550 bp amplicon) and FliL (770 bp amplicon) virulence genes (Fig. 1). There was a significantly higher mean similarity percent in nucleotide sequences of *mrpA* amplicons of chicken versus clinical human respiratory isolates (97.5%); however, a lower similarity of 93.2% was detected between the chicken and human clinical urinary isolates (Table 4) ($P<0.05$).

Table 3 Comparison of frequency of biochemical characteristics of *P. mirabilis* recovered from chicken livers ($N=33$) versus isolates recovered from the urinary ($N=23$) and respiratory systems ($N=6$) of humans

Sample type	Percent of isolates with positive test* results																		
	ONPG	ADH	LDC	ODC	CIT	H ₂ S	URE	TDA	IND	VP	GEL	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA
Chicken liver	0	6.1	3	97	100	100†	100	100	3	0	100†	0	3	0	0	0	0	0	0
Human urinary system	0	0	0	100	100	95.6†	100	100	0	0	91.3†	0	0	0	0	0	0	0	0
Human respiratory system	0	0	0	100	100	66.7‡	100	100	0	0	83.3‡	0	0	0	0	0	0	0	0

Note: *Test codes: ONPG=α-nitrophenyl-β-galactosidase, ADH=Arginine dehydrolase, LDC=Lysine decarboxylase, ODC=Ornithine decarboxylase, CIT=Citrate, H₂S= hydrogen disulphide, URE=Urease, TDA=Tryptophane deaminase, IND=Indole, VP=Voges-Proskauer reaction, GEL=Gelatinase, MAN=Mannitol fermentation, INO=Inositol fermentation, SOR=Sorbitol fermentation, RHA=Rhamnose fermentation, SAC=Saccharose fermentation, MEL=Melibiose fermentation, AMY=Amygdalin fermentation, ARA=Arabinose fermentation.

†‡Percentages in a column followed by different symbols are significantly different ($P<0.05$).

Figure 1 *mrpA* and *FliL* amplicons detection by PCR in chicken liver isolates (lanes 5–14), urinary human isolates (lanes 16–20), and respiratory human isolates (lanes 21–25). Another lanes are for the negative control (lane 2), *Salmonella spp.* (Lane 3), *P. mirabilis* reference isolate (American Type Culture Collection) (lane 4), and genetic base-pair ladder (lanes 1 and 15).

Discussion

The high frequency of *P. mirabilis* recovery from chicken livers (Table 2) is alarming, due to the documented literature of the involvement of this organism in different human conditions including pneumonia,^{8,10} septicemia, central nervous system infection,¹¹ food poisoning,^{32,33} and urolithiasis.¹² In addition, *P. mirabilis* is a proteolytic bacterium that is proven lately to alter the pathogenicity of avian influenza viruses, due to its ability to cleave the hemagglutinin protein of this virus.³⁴

The data presented in Tables 3 and 4, and in Fig. 1 show the high relatedness of the poultry to human isolates of *P. mirabilis*. The high similarity of poultry and human isolates in the dependence on utilization of amino acids (ornithine and tryptophane) and on citrate as the sole source of carbon ($P>0.05$), instead of the seven tested sugars (amygdalin, arabinose, mannitol, melibiose, rhamnose, saccharose, and sorbitol), might result in a similar potential of survival of these isolates in both hosts. Moreover, the similar utilization of ornithine by the chicken and human isolates of *P. mirabilis* is essential for formation of putrescine. This formation of putrescine

is used in cell–cell signaling, and in initiation of swarming, a defined virulence factor in this organism.¹² In addition, the 100% presence of urease enzyme in chicken and human *P. mirabilis* is indicative of a similar potential of these isolates in inducing urolithiasis in both hosts.^{12,13} Moreover, the higher presence of H₂S production and gelatinase activity in chicken compared to human isolates ($P<0.05$) will be the subject of a future investigation, targeting the relatedness of these two biochemical characterization to pathogenicity.

To our knowledge, this study is the first to document the presence of the two studied virulence genes in chicken isolates of *P. mirabilis* (Fig. 1). We believe that the presence of such genes increases their potential in inducing similar pathogenicity in the two studied hosts. Future investigation will target the isolation of the two virulence genes from chicken isolates of *P. mirabilis* for testing their cytopathic effects in cell culture.

The high similarity in *mrpA* nucleotide sequence of chicken compared to that of human respiratory isolates of *P. mirabilis* (Table 4), could indicate a higher potential in the avian isolates to cause

Table 4 Average similarity percent in sequences of *mrpA* amplicons of *P. mirabilis* recovered from chicken livers ($N=5$) in comparison to human isolates from clinical respiratory ($N=4$) and urinary ($N=4$) conditions

Mean % similarity in <i>mrpA</i> amplicon sequences of chicken to human isolates										
Chicken liver <i>P. mirabilis</i> isolates	Human respiratory isolates					Human urinary isolates				
	1	2	3	4	Mean	1	2	3	4	Mean
1	100	100	94	94	97.0	87	92	94	89	90.5
2	100	100	92	91	95.7	84	91	96	100	92.7
3	100	100	95	95	97.5	87	94	95	88	91.0
4	92	100	100	98	97.5	93	100	98	100	97.7
5	100	100	100	100	100.0	98	86	98	94	94.0
Mean	98.4	100.0	96.2	95.6		89.8	92.6	96.2	94.2	
Mean of Means					97.5†					93.2‡

†‡Mean of means in a row followed by different symbols are significantly different ($P<0.05$).

respiratory, rather than urinary conditions in humans. The farmers and slaughter house workers that are in direct contact with carrier chickens are expected to be mostly affected by this bacterium.³⁵ Actually, most of the documented literature about the impact of Gram negative organisms, harboring the chicken host on the farms and in slaughter houses, were those describing their injurious effect on the respiratory system of poultry handlers.³⁶

In conclusion, the high prevalence of *P. mirabilis* isolates in marketed broiler livers, and their similar phenotypic and virulence genes characteristics to that of the human isolates, alert us to the need of expanding future research on their adaptability and pathogenesis in different host species. This investigation will include wider number of virulence genes, such as the 'Proteus toxic agglutinin', hpmA and others.³⁷ Future epidemiological studies can also shed a light on success or failure of cross-species transmission of *P. mirabilis*, especially from avian to mammalian hosts.

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