



Genetic Characterization of Methicillin Resistance *Staphylococcus aureus* Isolates of Poultry and Human Origin

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OUR study applied on 160 collected samples, from poultry and human patients. Infection percent of human skin, poultry bumble foot and human nasal swab and Diabetic foot swab were 66%, 10%, 67.5%, 37.5% respectively. Methicillin resistant *Staph aureus* (MRSA) were 83.3% and 50% from poultry and human patient respectively, *mec A* gene is detected by 100% on samples of poultry and human origin. Whereas *blaZ* and *spa* gene and also, 83.3% and 50% respectively either from human or poultry samples respectively. Methicillin Resistance *S. aureus* (MRSA) strains isolated from human nasal swabs was 80%. PCR technique was used for *mec A*, *blaZ*, and *Spa* gene detection. PCR results were identical to disc diffusion method in 11 isolates out of 15 (73%) isolates from poultry and human samples.

Keywords: Methicillin resistant, *S.aureus*, Human, Poultry, *mec A* gene, *blaZ* gene, *spa* gene.

Introduction

MRSA has been found to colonize livestock including pigs, cattle and poultry. Since many of the MRSA clone lineages identified in livestock were uncommon for methicillin-resistant staphylococcus aureus (MRSA) isolates found until then in human hosts, the term “livestock-associated MRSA” (LA-MRSA) has been introduced to distinguish these MRSA from classical human hospital-acquired (HA-MRSA) or community-associated MRSA (CA-MRSA) [1].

In poultry, *S. aureus* is associated with many clinical syndromes including tenosynovitis, omphalitis, femoral head necrosis, infected hock and stifle joints secondary to coccidiosis and “bumble foot” [2]. Further *S. aureus* is Gram positive producing smooth, circular colonies, convex and lustrous; size of the colony may be 0.5-1.5 µm in diameter. Under microscope, it appears

like irregular three dimensional bunches of grapes like cluster of cells. The colony pigmentation may vary from grey, grey white, grey white with yellowish to orange shades and in blood agar typical β-hemolysis may be produced; depending on the growth condition [3 & 4]. Identification of bacteria by VITEK2 system has revealed prominent inter laboratory reproducibility and is quickly being included as a routine method for animal and human laboratory microbiology [5].

Here, we report the performance of Vitek 2 for staphylococci for testing contemporary isolates with currently used antimicrobial agents and the most up-to-date software and AST cards available from bioMérieux. [6 & 7].

The aim of this study directed to isolate, identify and characterize staph aureus isolates from human and poultry species which causing poultry bumble foot and (nosocomial infections, skin and diabetic foot infections) for human at

Giza and Cairo Governorate beside detection antimicrobial susceptibility pattern for these isolates .

Material and Methods

Samples collection

A total of 160 poultry and human were examined in Cairo and Giza Governorate for bacteriological examination. Samples were collected on 5 ml nutrient broth in screw capped tubes in an ice box and transferred to laboratory for bacteriological examination.

Bacteriological examination

Human and poultry samples were inoculated to pre-enriched non selective medium (buffered peptone water) then incubated at 37°C for 24 hours under aerobic condition. A loopful from incubated nutrient broth was streaked into: 7% salted nutrient agar; Baird parker agar; Mannitol salt agar and Blood agar. All plates were incubated for 24-48 hours at 37°C. The developed colonies were picked up and subcultured for purification. The purified colonies were morphologically identified by Gram stain and biochemical tests [8] .

Anti-microbial sensitivity test

Isolated *S. aureus* strains were subjected to the sensitivity test against different antibiotics, using the disc and agar diffusion method [9] & vitek2 .

Detection of resistance genes of isolated S. aureus strain

MecA, *blaZ*, *Spa* gene PCR was applied by using 8 sets of primers for detection of 8 resistance genes that may play a role in resistance of *S. aureus*. These genes were protein (*spa*) , beta lactamase (*blaZ*) , mecithicillin (*mecA*), It was applied on 8 random isolated *S.aureus* following QIAamp® DNA Mini Kit instructions (Catalogue no. M501DP100) {10}.

Results

Total incidence of S. aureus from poultry and human

Staph.aureus isolated from poultry skin, poultry bumble foot, human nasal swab and human diabetic foot were, 66%, 10% , 67.5% and 37.5% respectively as shown in Table (1)

Staph. aureus conformation by using VITEK2 technique

Out of 78 *Staph. aureus* isolated (human and poultry origin) there were 30 isolates (15 isolates from poultry and 15 isolates from human) confirmed by VITEK2 technique (very good identification) in photo (1).

Antibiotic sensitivity test

Data showed in Table (3) stated that oxacillin, vancomycin , tetracycline , clindamycin , Doxycycline , Rifampicin , Erythromycin , Gentamicin , Ciprofloxacin and Moxifloxacin were the most resistance antibiotics against the isolated staphylococcus aureus from human sample, on the other hand , Trimethoprim / Sulfamethoxazole and Nitrofurantoin antibiotics were the most sensitive whereas oxacillin, vancomycin ,tetracycline ,clindamycin , Doxycycline , Rifampicin and Erythromycin , were the most resistance antibiotics against the isolated staphylococcus aureus from poultry sample , on the other hand , Trimethoprim / Sulfamethoxazole , Moxifloxacin , Levofloxacin, Ciprofloxacin ,Gentamicin ,Nitrofurantoin,Tigecycline and Linezolid were the most sensitive .

Incidence of *MRSA* among *S.aureus* isolated from human and poultry samples .

In Table (2) *MRSA* isolates were higher in poultry samples than in human samples . 2/3 samples (66.6%) of total 3 *S.aureus* from Poultry Bumble foot whereas 9/27 (33.3%) *S.aureus* from human nasal swabs

TABLE 1. Incidence of *S. aureus* from Poultry and human sample .

Origin	Type of sample	Total no.of samples	Suspected <i>S. aureus</i>	
			NO.	%
Poultry	Skin swab	50	33	66%
	Pumple foot	30	3	10%
	Nasal swab	40	27	67.5%
Human	Diabetic foot swab	40	15	37.5%
TOTAL		160	78	48.75%

Biochemical Details																	
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	+	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	(-)	39	ILATk	+	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Photo 1. Biochemical of vitek 2 for *S. aureus* isolates (human and poultry origin) .

TABLE 2. Incidence of *MRSA* of *S. aureus* isolated from poultry and human samples .

Type of Sample	Total number of <i>S. aureus</i> isolates	<i>MRSA</i> NO.	%
Poultry Bumble foot	3	2	66.6%
Human nasal swabs	27	9	33.3%
Total	30	11	36.6%

Incidence of mecA and blaZ gene from MRSA isolates of poultry and human samples by PCR.

In Table (3) shows that *mec A* gene of *MRSA* isolates either from human sample were 5 positive , 1 negative and poultry sample were 1 positive , 1 negative by PCR. the highest percent of (*mecA*) gene PCR positive results were represented in *MRSA* isolates of poultry origin 83.3%. and *MRSA* isolates of human origin positive (*mecA*) gene were 83.3% while, the percent of *MRSA* isolates of poultry was 50% .

In Table (4) shows that *blaZ* gene of *MRSA* isolates either from all human sample were positive and all poultry sample were positive by PCR. and *MRSA* isolates of human origin positive (*blaZ*) gene were 100% while, the percent of *MRSA* isolates of poultry was 100% .

Detection of (mecA)and (blaZ) gene in MRSA isolates from poultry and Human S.aureus

Photo (2): illustrated a molecular weight 310bp for (*mecA*) gene and 173bp for (*blaZ*) gene in positive samples from human and poultry Sample. In lane 4 , 7 was negative for (*mecA*) gene by PCR. Lane 1-3, 5-6 (human *MRSA* isolates) were positive for (*mecA*) gene. Lane 8 (poultry *MRSA* isolate) were positive for (*mecA*) gene. lane 4 (human *MRSA* isolate) was negative for (*mecA*) gene .lane 7 (poultry *MRSA* isolate) was negative for (*mecA*) gene . and In all lane was positive for (*blaZ*) gene by PCR. Lane 1-6 (human *MRSA* isolates) were positive for (*blaZ*)

gene. Lane 7-8 (poultry *MRSA* isolate) was positive for (*blaZ*) gene.

Agarose gel electrophoresis of PCR products after amplification of *mecA* gene at 310bp amplified product. Lane (L): 100-600bp DNA Ladder “Marker” (100 Pharmacia). lanes (1:3 , 5:6 , 8) positive isolates at 310 bp. and lanes (4 , 7): negative isolates at 310 bp. Lane Pos: Positive control . Lane Neg: Negative control and amplification of *blaZ* gene at 173bp amplified product. Lane (L): 100-600bp DNA Ladder “Marker” lanes (1: 8) positive isolates.

Incidence of (spa) gene in MRSA isolates among poultry and human samples by PCR.

Table (5) shows that *Spa* gene of *MRSA* isolates either from human sample were 5 positive, 1 negative and poultry sample were 1 positive, 1 negative by PCR. and Table (6) showed that *MRSA* isolates of human origin positive (*spa*) gene was 83.3% while, the percent of isolates of poultry was 50 % .

Detection of (spa) gene in MRSA isolates from animal and human samples.

Photo (3) illustrates a 226bp for (*spa*) gene in *MRSA* strains from poultry and human. Positive samples for (*spa*) gene were from *MRSA* isolated from human lane 1-5 and from poultry lane 8 and Negative sample for (*spa*) gene were from *MRSA* isolated from human lane 6 and from poultry lane 7

TABLE 3. The result obtained by using VITEK2 system for detection antibiotic sensitivity of *Staph aureus* .

Antimicrobial	Poultry		Human	
	MIC	Interpretation	MIC	Interpretation
Oxacillin	≥ 4	R	≥ 4	R
Gentamicin	≤ 0.5	S	≥ 16	R
Ciprofloxacin	1	S	4	R
Levofloxacin	2	S	4	I
Moxifloxacin	≤ 0.25	S	≥ 8	R
Erythromycin	≥ 8	R	≥ 8	R
Clindamycin	≥ 4	R	≥ 4	R
Vancomycin	≥ 32	R	≥ 32	R
Doxycycline	≥ 16	R	≥ 16	R
Tetracycline	≥ 16	R	≥ 16	R
Nitrofurantoin	≥ 16	S	32	S
Rifampicin	≥ 32	R	≥ 32	R
Trimethoprim /Sulfamethoxazole	≤ 10	S	≤ 10	S

TABLE 4. Incidence of *mecA* gene from *MRSA* isolates of poultry and human isolates by PCR.

Type of isolate	Total No. of examined isolates.	Positive <i>mecA</i> No. %		Negative PCR No. %	
Human nasal Swabs	6	5	83.3	1.0	16
Poultry Bumble foot	2	1	50	1.0	50

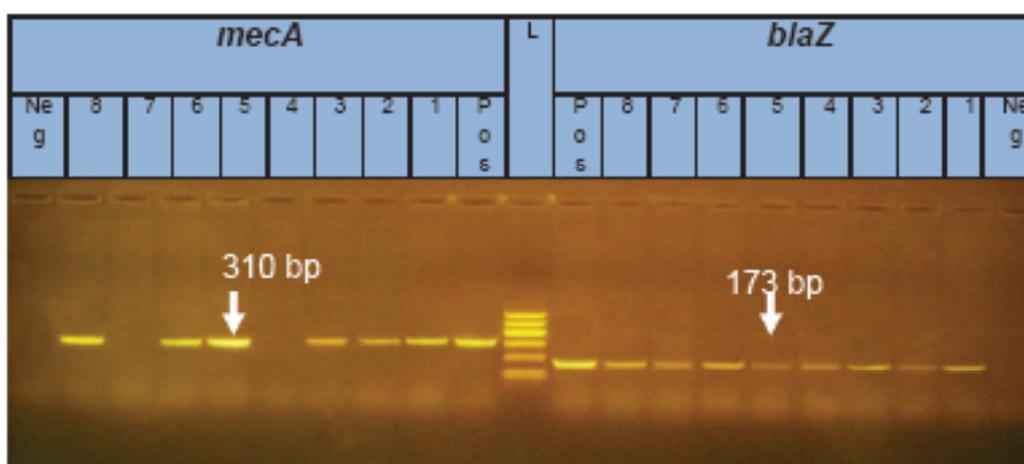
**Photo 2.** In the Electrophoretic pattern of (*mecA*) and (*blaZ*) gene in *MRSA* strains isolated from human and poultry samples.

TABLE 5. Incidence of blaZ gene from MRSA isolates of poultry and human isolates by PCR.

Type of isolate	Total No. of examined isolates.	Positive blaZ No.	%	Negative PCR No.	%
Human nasal Swabs	6	6	100	0.0	0
Poultry Bumble foot	2	2	100	0.0	0

TABLE 6. Incidence of (spa) gene in MRSA isolates among animal and human samples by PCR.

Type of isolate	Total No. of examined isolates.	Positive Spa No.	%	Negative PCR No.	%
Human nasal swabs	6	5	83.3	1.0	16
Poultry Bumble foot	2	1	50	1.0	50

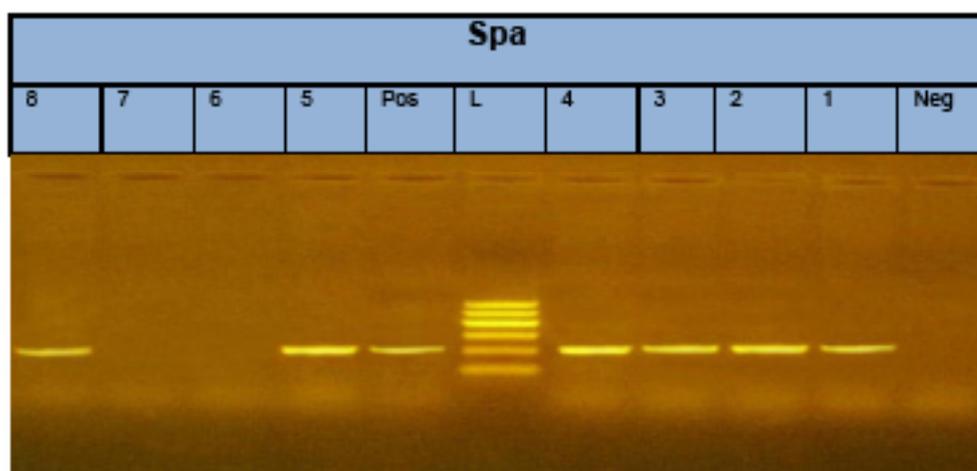


Photo 3. Agarose gel electrophoresis of PCR products after amplification of spa (IgG- binding protein) gene at 226 bp amplified product. Lane (L): 100-600bp DNA Ladder “Marker” (100 Pharmacia). All lanes (1-5): positive isolates at 226 bp. Lane Pos: Positive control (reference strain deposited to gene bank with accession no. P38507). Lane Neg : Negative control and Lane 6-7: Negative isolate.

Discussion

S. aureus infection has become an increasingly grave problem in industrialized poultry farming and of zoonotic importance. Staphylococcal infections including, synovitis with arthritis, osteomyelitis, dermatitis, endocarditis, septicemia, wound infection and omphalitis . [11].

This result was Slightly higher than that obtained by [12] *Staphylococcus aureus* in food is a consequence of inadequate hygienic

handling and processing, posing a potential risk to public health. The current study aimed to identify and characterize some virulence genes, as well as detection of antimicrobial resistance of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) isolated from retail chicken products and hand swabs from vendors in Egypt. In addition, genetic relatedness of the isolates from chicken and humans was evaluated by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) using

protein A as a target. A total of 110 samples were collected from chicken products ($n=80$) and vendors ($n=30$). Overall, 30 (37.5%) chicken products samples were positive for *S. aureus*, whereas hand swabs from meat handlers revealed that 18 (60%) were positive. Ten *MRSA* strains were characterized by the presence of the *mecA* gene, comprising seven isolates from chicken and three from humans. Virulence-associated factors were evaluated by PCR, revealing that 31.3% of *S. aureus* isolates harbored the Pantone–Valentine leukocidin (PVL) gene.

These results disagreed with [13] who proved that 90 (18.4%) out of 489 (18.4%) of the students were found to be colonized by *S. aureus*. Only 10 (2.04%) of the students were found to be *MRSA* carrier. All *MRSA* isolates were sensitive to Vancomycin. PLV gene was detected in one *MRSA* strain. These results agreed with [14] A total of 409 samples were investigated bacteriologically to detect the occurrence of staphylococci among the diseased animals and human, the highest isolation rate was observed in human samples (36%) followed by chicken (12%) samples. A total of 78 *S. aureus* isolates secured from different animals and human origins were characterized and identified using the most important conventional biochemical tests as anaerobic glucose fermentation, catalase, coagulase, acetone production, novobiocin sensitivity and mannitol fermentation. SpA was extracted from 17 *S. aureus* isolates (6 human and 2 chicken isolates). Concerning the human samples included in this study, 78 sample isolates Staphylococcus isolates out of 160 Total sample isolate, while only 11 (36.6%) isolates were *MRSA*. whether *MRSA* is present in poultry from (2) sampled isolated out of (3) Staphylococcus isolates were *S. aureus* while only 2 (66.6%) isolates were *MRSA*.

Staph. aureus isolated from human samples were higher than in poultry samples. That's to say, 66% of Poultry Skin swab, 10% of Poultry bumble foot and 67.5% of the human nasal swabs, 37.5% of the human Diabetic foot swab were positive for *S. aureus*.

Our result of *MRSA* from total isolates of poultry samples than in human samples. 2 samples (66.6%) of total 3 *S. aureus* from Poultry Bumble foot and 9 (33.3%) of total 27 *S. aureus* from human nasal swabs. staphylococcus aureus out (11) *MRSA* (36.6%) this result can be detected by antibiotic sensitivity Vitek 2 [15].

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Status of the *MRSA* isolates and hence may have an impact on therapeutic approaches conducted to control infections due to such isolates. It is also possible that such additional genetic material increases the virulence of *MRSA* isolates [16].

Added nosocomial pneumonia as an additional type of infection. Nosocomial infections with methicillin resistant Staphylococcus aureus (*MRSA*) became an infection control problem worldwide during the past 20 years. They are mainly associated with hospital associated, clonal lineages (HA-*MRSA*) which have a pronounced capacity for spread in and among hospitals [17].

Conclusion

Data presented in this study showed a broad distribution of identical related *S. aureus* clones are responsible for the resistance of antimicrobial situations in Egypt with a highly prevalence rate of methicillin resistance among the obtained isolates which represent an alarm for a great hazard to public health.

MRSA isolates detected in poultry staphylococci have most been assumed to originate from human sources. In nature, genes on plasmids often encode proteins that protect the bacterium from one or more antibiotics. As bright as the future looks for new diagnostic tools, prospects concerning new developments of anti-staphylococcal drugs for use in poultry & human seem less encouraging.

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Conflict of Interest

There is no one conflict of interest.

Funding Statement

Personal fund.

Ethical Consideration

We apply all ethical guidelines for use of animals in research according to international committee for research ethics.

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التوصيف الجيني لبكتريا الميكروب العنقودي الذهبي المعزول من الدجاج والأنسان المقاومة للميثيلين

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تم إجراء هذه الدراسة على عدد ١٦٠ عينة من دجاج ومرضى بالمستشفيات في محافظتي الجيزة والقاهرة لعزل وتصنيف بكتريا الأستاف أورييس المقاومة للميثيلين .

وكانت نسبة المعزولات من عينات مصدرها إصابة جلد الإنسان وأرجل الطيور ومن مسحات أنفية للمرضى ومن مسحات من أرجل مرضى داء السكري هي ٦٦٪ ، ١٠٪ ، ٦٧,٥٪ ، ٣٧,٥٪ على الترتيب .

وكانت نسبة وجود بكتريا ستاف أورييس المقاومة للميثيلين ٨٣,٣٪ و ٥٠٪ في كل عينات مصدرها الطيور والأنسان على الترتيب .

ووجد أن الجين *mec A* كان موجود بنسبة ١٠٠٪ في عينات المأخوذة من الطيور والإنسان بينما الجين *blaZ* كان موجود بنسبة ٨٣,٣٪ ، ٥٠٪ على الترتيب وكان الجين *spa* موجود في معظم معزولات ميكروب بكتريا ستاف أورييس المقاومة للميثيلين المعزولة من الطيور والإنسان .

ووجد أن نسبة معزولات ميكروب بكتريا ستاف أورييس المقاومة للميثيلين المعزولة (تجويف الأنف) وصلت إلى ٨٠٪ من إجمالي العينات المجمع من الأنف في حال الإصابات البشرية .

ويعمل إختبار تفاعل البلمرة المتسلسل المتعدد (PCR) ومقارنته بإختبار إنتشار المضادات الحيوية (disc diffusion method) على عينات مصدرها الطيور والإنسان وجد أن 11 عينة إيجابية بتفاعل تفاعل البلمرة المتسلسل المتعدد من 15 عينة إيجابية بإختبار إنتشار المضادات الحيوية (73% = 11/15) .

من هذه الدراسة نستنتج أن هناك إنتشار واسع لبكتريا ستاف أورييس المقاومة للميثيلين وهذا يمثل إنذار لمراقبة ولوضع ضوابط وإشترطات لإستخدام المضادات الحيوية للحفاظ على الصحة العامة .

وهذا يدل أن بكتريا ستاف أورييس المقاومة للميثيلين المعزولة من الدواجن تكون سبب من الأسباب الرئيسية لإصابة الإنسان ولذلك يجب تحديد اليات محددة لتشخيص إصابات الطيور بهذه البكتريا لمنع إنتقاله وإصابة الإنسان بها .