Characterization of Methicillin Resistance *Staphylococcus aureus* isolated from chicken and human

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A B S T R A C T

Staphylococcus aureus causes a wide range of diseases in human beings, from minor skin infections to severe illnesses such as septicemia, toxic shock, endocarditis, and pneumonia. *Staphylococcus aureus* is a major pathogen of increasing importance due to the rise in antibiotic resistance. S. aureus was an important cause of disease in poultry it could be involved in a wide range of clinical conditions such as septicemia, bone and Joint infections, abscesses and dermatitis.In this study, 160 samples were collected , out of which 80 samples were from poultry and human patients (80 for each). The incidence of infection with *S.aureus* in Bumble foot, Skin swabs in chicken and Diabetic foot swab , Skin sawb and Nasal swab in human were, 10%, 66%, 30%, 40%, 67.5% respectively. Methicillin resistant *S. aureus* (MRSA) were incidence in chicken and human 66.6% and 33.3% respectively. Subjected to PCR for detection of some antimicrobial resistance genes using intrinsic methicillin resistant gene *(mec* A), the IgG binding region of protein A*(spa)* gene were 83.3% and 50 % respectively also beta-lactamase *(blaZ)* gene 100% and 100% respectively were detected in most *MRSA* isolates either from human or chicken samples.

Keywords: Methicillin resistant – *S.aureus* – human – chicken – *mec* A gene – *blaZ* gene –*spa* gene

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1. INTRODUCTION

Methicillin resistant Staphylococcus aureus MRSA has been found to colonize livestock including pigs, cattle and poultry. Since many Of the *MRSA* clone allineages identified in livestock were un-Common 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*). (Köck et al., 2013). 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" (Ashraf A Abd El Tawab1, 2015; Suleiman et al., 2013). Susceptibility testing results for all 275 S. aureus isolates were consistent with cefoxitin screening and mecA gene PCR. (Shan et al., 2016). Further *S. aureus* is Gram positive producing smooth, circular colonies, convex and clustrous; size of the colony may be 0.5-1.5 µm in diameter. Under microscope, it appears like irregular three dimensional brunches 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 (Jahan et al., 2014). Abroad distribution of identical related *S. aureus* clones are responsible for the mastitis situations in Egypt with highly prevalence rate of methicillin resistance among the obtained isolates which represent an alarm for a great hazard to public health. (Ashraf et al., 2016; Ashraf, 2016). The use of antibiotics in farm management (growing crops and raising animals) has become a major area of concern. Its implications is the consequent emergence of antibiotic resistant bacteria (ARB) and accordingly their access into the human food chain with passage of antibiotic resistance genes (ARG) to the normal human intestinal microbiota and hence to other pathogenic bacteria causative human disease. Therefore, we pursued in this study to resistance determining region, *mec*A of , methicillin-resistant *S.aureus* (MRSA) (Osman et al., 2016). All S.

aureus isolates were screened by PCR for *mec A* . *S. aureus* becomes methicillin resistant by the acquisition of the *mec A* gene which encodes a penicillin binding protein (PBP2a) with a low affinity for β-lactamase. The strains producing PBP2a are resistant to all β-lactams (García-Álvarez et al., 2011). The *Spa* types were assigned via the *Spa* typing plugin. The staphylococcal cassette chromosome *mec* (SCC*mec*) types were determined by multiplex PCR as previously described . Representative isolates belonging to different *Spa* types were further analyzed by the Multi Locus Sequence Typing facility. (Harris et al., 2013, Ge et al., 2017).

The aim of this work directed to isolate, identify and detected the genes that causative agent from chicken farms were causing Bumble foot and from human samples were causing nosocomial infections at Giza and Cairo Governorate by using PCR Master Mix beside detection *MRSA* antimicrobial susceptibility pattern by using Vietk 2 system for these isolates.

2. MATERIAL AND METHODS

2.1 Samples collection:

A total of 160 samples from chicken and human were examined in Cairo and Giza Governorate for bacteriological examination. Samples pus from Bumble foot, Skin swabs in chicken and Diabetic foot swab, Skin sawb and Nasal swab in human were collected on 5 ml nutrient broth in screw capped tubes in an ice box and transferred to laboratory for bacteriological examination .

2.2 Bacteriological examination (Oxoid):

Pre-enriched non selective medium (buffered peptone water) was inoculated with the collected samples at ambient temperature and then incubated at 37˚C for 24 hrs 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 37ºC. The developed colonies were picked up and subcultured for purification. The purified colonies were morphologically identified by Gram stain and biochemical tests (Swayne, 1998).

2.3 In-Vitro anti-microbial sensitivity test:

The isolated *MRSA* strains were subjected to the sensitivity test against different antibiotics, using the Vitek 2 system (Chatzigeorgiou et al., 2011).

2.4 Detection of resistance genes of isolated S. aureus:

By using QIAamp® DNA Mini Kitinstructions (Catalogue no. M501DP100) (Sambrook and Russell David, 1989). It was applied on 8 random isolated *MRSA(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 lactmase (*blaZ*), mecithicillin (*mecA*).

3. RESULT

3.1 *Total incidence of S.aureus from chicken and human:*

Staphylococci aureus isolated from human samples are higher than in chicken samples . That's to say of 66% chicken Skin swab, 10% of chicken bumble foot and 67.5% of the human nasal swab, 30% of the human Diabetic foot swab and 40% of the human skin swab were positive for *S.aureus*. Shown in Table (1)

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

MRSA isolates were higher in chicken samples than in human samples . 2 samples (66.6%) of total 3 S.aureus from chicken Bumble foot and 9 (33.3%) of total 27 S.aureus from human nasal swabs. In Table (2) stated that oxacillin, vancomycin, tetracycline, clindamycin, Doxycycline, Rifampicin and Erythromycin, were the most resistance antibiotics against the isolated staphylococcus aureus from chicken sample , on the other hand, Trimethoprim / Sulfamethoxazole, Moxifloxacin, Levofloxacin, Ciprofloxacin, Gentamicin, Nitrofurantoin, Tigecycline and Linezolid were the most sensitive (Table 3).

3.3 *Incidence of mecA ,blaZ and spa gene from MRSA isolates of chicken and human samples by PCR.*

That *mec* A gene and *Spa* gene of *MRSA* isolates either from human sample were 5 positive, 1 negative and chicken sample were 1 positive, 1 negative by PCR and The percent of *(mecA, Spa)* gene PCR positive results were represented in *MRSA* isolates of chicken origin 83.3%. and *MRSA* isolates of human origin positive (*mecA)* gene were 83.3% while, the percent of *MRSA* isolates of chicken was 50%. and shows that *blaZ* gene of *MRSA* isolates either from all human sample were positive and all chicken sample were positive by PCR. and *MRSA* isolates of human origin positivess (*blaZ)* gene were 100% while, the

Table (1): Incidence of *S. aureus* from chicken and human sample

Origin	Type of sample	Total no.of samples	Suspected *S. aureus*	
			NO.	%
Chicken	Pumple foot	30	3	10
	Skin swab	50	33	66
	Nasal swab	40	27	67.5
Human	Diabetic foot swab	10	3	30
	skin swab	30	12	40
	TOTAL	160	78	48.75

Table 1: Incidence of MRSA *S .aureus* isolated from chicken and human samples.

Type of Sample	Total number of *S. aureus* isolates	MRSA	
		NO.	%
chicken Bumble foot	3	2	66.6
Human nasal swabs	27	9	33.3
Total	30	11	36.6

Table 2: The result obtained by using VITEK2 system(ultradiagnositic, bioMerieux complies with ISO13485 and FDA Quality System Regulation (QSR)) for detection antibiotic sensitivity of *S.aureus*.

Antimicrobial	Chicken		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 3: Incidence of positive gene from MRSA isolates of chicken and human isolates by PCR.

Type of isolate	Total No.of examined isolates.	*mec*A		blaZ		Spa	
		No.	%	No.	%	No.	%
Human nasal swabs	6	5	83.3	6	100	5	83.3
Chicken Bumble foot	2	1	50	2	100	1	50

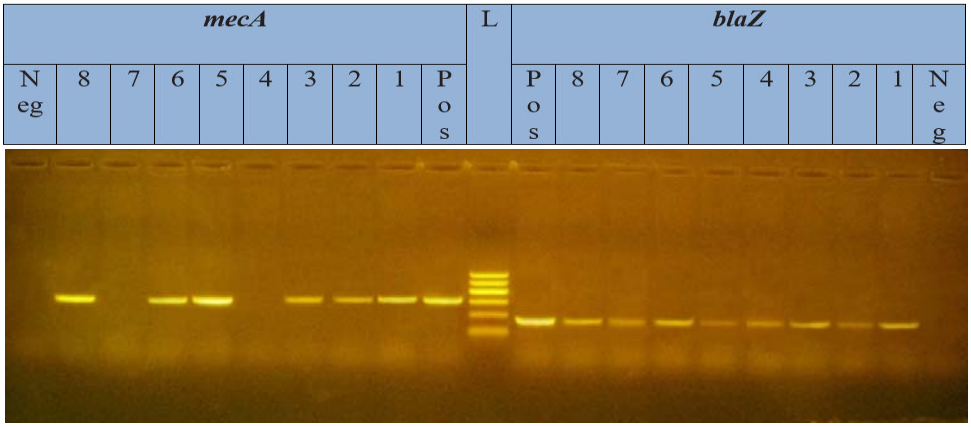

Figure (1): Agarose gel electrophoresis of PCR products after amplification of *mec*A gene at 310bp amplified product. Lane (L): 100-600bp DNA Ladder "Marker" (100 Pharmacia). lanes (1:3 , 5:6 , 8) positive isolates at 310 bp. and lanes (4 , 7): negative isolates at 310 bp. Lane Pos: Positive control (reference strain deposited to gene bank with *MRSA* ATCC 43300 methicillin-susceptible S.aureus ATCC 25923) . 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.

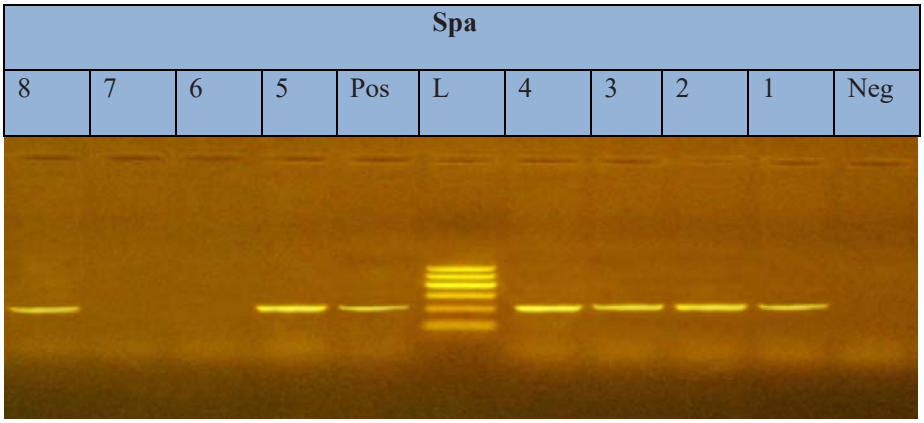

Figure (2): Agarose gel electrophoresis of PCR products after amplification of *spa* (IgG-binding protein) gene at 226 bp amplified product. Lane (L): 100-600 bp DNA Ladder "Marker" (100 Pharmacia). All lanes (1-5): positive isolates at 226 bp. Lane Pos: Positive control (reference strain deposited to gene bank with *MRSA* ATCC 43300 methicillin-susceptible *S.aureus* ATCC 25923). Lane Neg : Negative control and Lane 6-7: Negative isolate.

percent of MRSA isolates of poultry was 100% (Table 4)

3.4. Detection of mecA and blaZ gene in MRSA isolates from chicken and human S.aureus :

Detected of mecA gene in a 310 bp, detected of blaZ gene in 173bp and detected of spa gene 226 bp in MRSA strains from poultry and human in positive samples (Figure 1, Figure 2).

4. DISCUSSION

S. aureus infection has become an increasingly grave problem in industrialized poultry farming. *Staphylococcal* infections including, synovitis with arthritis,osteomyelitis, dermatitis, endocarditis, septicemia, wound infection and omphalitis (Lowder et al., 2009). *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 characterize virulence factors, as well as 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 *mec*A 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 *Panton–Valentine leukocidin (PVL)* gene. This result was some what higher than that obtained by (El Bayomi et al., 2016).

These results disagreed with (Habeeb et al., 2014), A total of 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 (El-Jakee et al., 2008) 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 chicken from (2) sampled isolated out of (3) *Staphylococcus* isolates were *S. aureus* while only 2 (66.6%) isolates were *MRSA*.

Staphylococci aureus isolated from human samples are higher than in chicken samples . That's to say, 66% of chicken Skin swab, 10% of chicken bumble foot and 67.5% of the human nasal swabs, 30% of the human Diabetic foot swab and 40%of the human skin swabs were positive for *S.aureus*.

In the current result of *MRSA* from total isolates of chicken samples than in human samples. (2) samples (66.6%) of total (3) *S.aureus* from chicken Bumble foot and (9) (33.3%) of total (27) *S.aureus* from human nasal swabs. *staphylococcus aures* out (11) *MRSA* (36.6 %) this result can detected by antibiotic sesnsitivity Vitek 2.

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. (Rushdy et al., 2007).

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 . (Cuny et al., 2011)

The sensitivity tests for the isolated *S. aureus* showed strains indicated high resistance to methicillin followed by oxacillin; Nalidixic acid; Ampicillin; Amoxicillin; Cefotaxime and Tobramycin, respectively. Meanwhile, they were highly sensitive to Gentamycin; Enrofloxacin; Norfloxacin; Lomefloxacin and Ciprofloxacin. PCR results cleared that*, femA; mecA* and *hlb* virulence genes were detected in all 5 *S. aureus* studied strains (Ashraf, 2016).

5. CONCLUSION

Data presented in this study showed abroad distribution of identical related *S. aureus* clones are responsible for the resistance of antimicrobial situations in Egypt with highly prevalence rate of methicillin resistance among the obtained isolates which represent an alarm for a great hazard to public health.As bright as the future looks for new diagnostic tools, prospects concerning new developments of antistaphylococcal drugs for use in poultry & human seem less encouraging.

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