

Molecular fingerprinting of methicillin resistant *Staphylococcus aureus* strains isolated from human and poultry in Duhok, Iraq

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been recently identified in poultry and farm workers. The aim of this work was to investigate the epidemiological relatedness of MRSA among chickens and farmworker. MRSA isolates (n=50) from human (n=14) and from chickens (n=36) were tested for molecular epidemiological relatedness between human and poultry. RAPD-PCR was carried out for fingerprinting of MRSA isolates genome. Seven genotypes group (A-G) have been identified. All human MRSA were belonging to genotype A. Whereas, chickens MRSA isolates was belonging to different genotype patterns groups (A-G). To conclude, human MRSA was belonging to one genotype pattern but the chickens MRSA strains were belonging to seven genotypes. The genotype pattern A was the most dominant among all MRSA isolates. It is possible that the chickens play an important role for the human exposure to MRSA by direct contact. Further studies are required to address the relatedness between human and chicken MRSA.

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Introduction

Staphylococcus aureus (*S. aureus*) is an opportunistic pathogen capable of causing different infections in humans and animals (1). In 1961, there were reports on *S. aureus* isolates that had acquired resistance to methicillin and this was so-called methicillin-resistant *S. aureus* (MRSA) (2). MRSA which is a specific strain of the *S. aureus* that has acquired and developed resistance to all beta-lactamase antibiotics including, methicillin, penicillin, and other narrow-spectrum antibiotics (3). Depending on the clinical isolates, up to 50% of *S. aureus* infections are caused by MRSA. Nowadays, MRSA is subdivided into three main categories, hospital-associated MRSA (HA-MRSA), livestock-associated MRSA (LA-MRSA) and Community-associated MRSA (CA-MRSA) (4,5). Many studies have documented that different MRSA nasal carriage rates have been observed in the Iraqi people (6-8). Chicken infectious diseases are a main economic burden on the poultry industry, *S. aureus* infection in commercial

broiler chickens can cause septic arthritis, septicemia, subdermal abscesses (bumble-foot), omphalitis and gangrenous dermatitis (9,10). Animals can become reservoirs for MRSA and spread it to other animals and even human (11). Human in close contact with animals are at high risk for MRSA colonization (12,13). The poultry meat has been implicated as a main source of MRSA in humans (14,15). In a study, a lineage of MRSA in retail chicken meat was observed and suggested to be of human origin (16). Recently, a high prevalence MRSA isolates were observed in both farm workers and chickens in Duhok, Iraq (17). The present study aimed to investigate the epidemiological relatedness of MRSA strains among chickens and farmworker in Duhok, Iraq.

Material and methods

Methicillin resistant *S. aureus* isolates

Staphylococcus aureus and MRSA isolates from adult farm workers (Workers with history of hospitalization,

surgery, dialysis or residence in a long-term care facility, and history of previous isolation of MRSA were excluded) and chickens (Commercial broiler collected from different farms in all districts in Duhok city) were previously isolated and identified using conventional bacteriological, biochemical and molecular methods (17). 50 MRSA isolates (14 from Human and 36 from chickens) were selected and screened for the molecular typing.

RAPD-PCR typing of methicillin resistant *S. aureus*

Randomly amplified polymorphic DNA (RAPD)-PCR technique was applied for the identifying genomic variation and establishing strain-specific fingerprints of the isolated MRSA strains. Short synthetic oligonucleotide primer (Eric2: 5'AAGTAAGTGACTGGGGTGAGCG3') with a random sequence about 22 bases in length was used for this purpose (18,19).

Genomic DNA extraction

DNA was extracted from *S. aureus* isolates using DNA isolation kit according to the company's guidelines (AccuVi Bio, UAE). In brief, 1 ml of an overnight broth culture was centrifuged at 14,000 rpm for 30s and 250 µl of suspension solution was added to the cell pellet. Next, 250 µl of lysis solution and 12 µl of proteinase k were added and incubated at 55°C for 30 min. 500 µl of binding solution was added and centrifuged at 8,000rpm for 1 min. The flow through was discarded and the mixture was washed twice by 500 µl of wash solution. Then, the DNA was eluted in 200 µl elution buffer at 6,000 rpm for 1 min. The purity and concentration of DNA of each isolate was measured by using Nanodrop device (Thermo scientific, USA).

Polymerase chain reaction (PCR)

PCR reactions were carried out in a C1000 thermal cycler (Bio-Rad, USA). PCR amplification reactions were achieved in a final volume of 20 µl. The components, volumes, and concentrations used were: 10 µl of deoxyribonucleotide master mix (Promega, USA); 2 µl *S. aureus* genomic DNA (final concentration 50-100 ng/ml), 1 µl for primer (Eric2) in a final concentration 10 pmol/µl, and nuclease-free water to a final volume of 20 µl. The PCR conditions were: 1 min for initial template denaturation step at 95 °C, followed by 30 cycles of denaturation at 94°C for 45 s; annealing for 27°C for 1 min, and extension at 72°C for 2 min. These cycles were followed by a final extension step 72°C for 5 min followed by hold step at 4°C.

Gel electrophoresis

DNA bands of the amplified PCR products were separated on agarose gel according to their sizes. The electrical power was turn on 65 V. for 50 min 1x TBE buffer. Ethidium bromide at a final concentration of 5 µg

ml⁻¹ was used to stain the agarose gels. The separated fragments were visualized using a U.V. illumination at 366 nm wavelengths (HVD life science, Austria).

Results

At the present study, the genomic variation among human MRSA and chicken MRSA was evaluated utilizing Eric2 primer in randomly amplified polymorphic DNA (RAPD)-PCR technique. Among the 50 MRSA tested, seven different random amplification polymorphic DNA patterns (genotypes) have been identified and termed A, B, C, D, E, F, and G (Fig 1). Random amplified polymorphic DNA (RAPD) method was generated for MRSA strains to detect genetic relationship in an epidemiological sense. It implicates the amplification of arbitrarily selected chromosomal DNA sequences with a short primer with a random sequence not useful to a specific region of the DNA target, but capable of hybridization at random chromosomal sites, the amount and locations of these arbitrary sites will differ among various strains, producing a different RAPD-PCR profile based on the number and sizes of the fragments detected by electrophoresis.

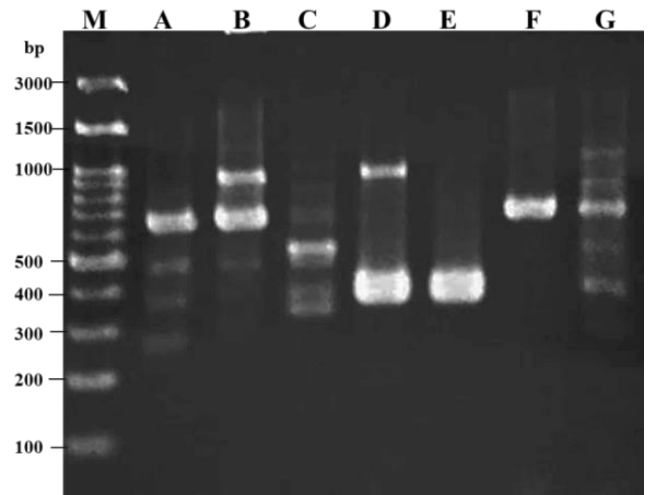


Figure 1: RAPD-PCR profiles and the banding pattern of MRSA selected from two sources and generated with primer Eric2 with a different molecular weight about (200-1000bp). Lane M is DNA ladder, lane A represent MRSA isolates from workers and chickens samples, lanes B-G MRSA isolates represent chickens samples.

All tested human MRSA (n=14) were belonging to genotype group A, whereas 10 out 36 chicken MRSA were belonging to group A. While the remaining 26 chicken MRSA typing isolates were from B, C, E, F and, G groups while all human isolates detected negative for these groups (Table 1).

Table 1: Genotype patterns generated by RAPD PCR for MRSA isolates

Genotype	MRSA		No. of bands
	Chicken	Human	
A	10	14	4
B	5	0	3
C	6	0	5
D	4	0	2
E	5	0	1
F	4	0	2
G	2	0	5

Discussion

Staphylococci can cause different patterns of infections in human such as, bone infection, heart infection and serious skin infections (20,21). *S. aureus* enterotoxins can cause food poisoning, toxic shock syndrome and urinary tract infections (22,23). MRSA is a common bacterial pathogen responsible for a variety of infections and widely distributed among societies (24,25). Poultry *S. aureus* can become a zoonotic risk factors and cause food poisoning in human especially in case of insufficient cooking of chickens portions (26). Recently MRSA have been found in farms animals particularly in chickens, the incidence of MRSA in Duhok, Iraq was 27.3% (17). In Jordan, MRSA was detected in 4% in poultry (27). While in UK broiler farms was about (74%), in Germany about 21%, and high levels of poultry MRSA have also been found in Denmark, Austria and Norway and (28).

At the present study, seven different RAPD patterns (genotypes) were identified. All human MRSA were belonging to one genotype (group A) and 27/8% (10/36) of chicken MRSA was belonging to this group. While the remaining chicken MRSA were identified as B, C, E, F and, G groups. This may show the close relationship between isolates, and may have deviated from same precursor or comes from human and vice versa through handling of contaminated chickens with bacterium or other transmission facility at different stages of farming. This could indicate that there was no transmission of MRSA among human and chicken and may be due to hygiene precautions of workers (15).

MRSA have been found in farms animals particularly in chickens (27). Animals can become reservoirs for MRSA and spread it to other animals and even human (29). MRSA infection and colonization has been noticed in human after a time of directly and close contact with infected animals (30). A number of LA-MRSA strains such as CC398, are shown to be transmitted between species, particularly after inhalation of contaminated dust, which could contain a large numbers of bacteria and these suggested to be a major way of the dissemination of these organisms in close hall system (31).

People commonly become colonized with LA-MRSA strains, after physical contact with live birds and farms environments or consumption of improper cocked meats (32,33), and also can be transmitted between people and family members, particularly who live together within household (34). Animals usually acquire the organisms from the mothers and can also horizontally acquire these organisms during growing life from other animals or from the environment. However evidence from parent MRSA colonization shows a little influence on their offspring after a time, because MRSA can change their rates more than once from birth to full growth, this, has proved by prevalence of *S. aureus* between different sampling points (35).

The transmission of MRSA between chickens in farms are most commonly occur due to contamination of farms inhabitation and the organisms may shed into the feeds and water vessel from the nostrils, or through their fecal materials, the organisms shed on the surface ground of household, as well as through vaccinations and treatment of chickens with contaminated injections (35).

Colonization is one of the major risk factors for *S. aureus* infections. In humans *S. aureus* have widely colonized the anterior nares but has also been isolated from healthy skin, axilla, perineum throat, trachea of ventilated patients, cervical swabs and from the urethra of previously catheterized patients (36-38).

Many researchers suggested that MRSA isolates are able to move between humans and animals, however the successful transfer of these bacteria between humans and poultry is not very clear, but the data from a study showed that infection of humans by transmission through direct contact and food products polluted with poultry MRSA is very possible. Lee (15) revealed that six animals isolates were identical to the patterns of certain humans isolates, also, it was reported that a cat's isolate was indistinguishable from the Pulsed-field gel electrophoresis (PFGE) pattern of the human epidemic strain (39). It is possible that the MRSA isolates could transmit from human to chicken and vice versa. Therefore, these isolates can become widespread in the community atmosphere of Duhok province, Iraq, where antibiotics such as vancomycin, and amikacin are not used for animal treatment, these can raise the occurrence of MRSA strains among humans.

Conclusions

All farm workers MRSA isolates showed identical patterns of RAPD-PCR with chicken MRSA isolates. However, not all chicken MRSA isolates were identical to human MRSA. It is possible that the MRSA isolates could transmit from human to chicken and vice versa. Further investigations are required to study the relatedness between human and chicken MRSA using different typing approaches.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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التنميط الوراثي الجزئي للمكورات العنقودية الذهبية المقاومة للمثيسيلين المعزولة من الدواجن وعمال حقول الدواجن في مدينة دهوك، العراق

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الخلاصة

جراثيم المكورات العنقودية المقاومة للمثيسيلين شخّصت حديثاً في الدواجن وعمال حقول الدواجن. كان الهدف من هذه الدراسة هو بحث العلاقة الوبائية بين عزلات المكورات العنقودية المقاومة للمثيسيلين في الدواجن وعمال حقول الدواجن. تم اختبار العلاقة الوبائية الجزئية ل عزلات جراثيم المكورات العنقودية المقاومة للمثيسيلين (٥٠) المعزولة من الإنسان (١٤) والدواجن (٣٦). استخدمت تقنية تفاعل السلسلة المتبلورة للتنميط الوراثي الجزئي للعزلات. تم تحديد سبعة مجاميع من الأنماط الوراثية أ-خ. جميع عزلات المكورات العنقودية المقاومة للمثيسيلين من الإنسان كانت تنتمي إلى مجموعة أ. في حين كانت عزلات جراثيم المكورات العنقودية المقاومة للمثيسيلين المعزولة من الدواجن تنتمي إلى مجاميع أنماط وراثية مختلفة أ-خ. يستنتج إلى أن جميع عزلات جراثيم المكورات العنقودية المقاومة للمثيسيلين للإنسان تنتمي إلى نمط وراثي واحد ولكن عزلات الدواجن تنتمي إلى سبعة أنماط وراثية. النمط الوراثي A كان النمط السائد بين جميع العزلات. من الممكن أن تلعب الدواجن دوراً مهماً لتعرض الإنسان للجراثيم المكورات العنقودية المقاومة للمثيسيلين عن طريق الاتصال المباشر. تتطلب دراسات إضافية لتحديد العلاقة بين عزلات جراثيم المكورات العنقودية المقاومة للمثيسيلين في الإنسان والدواجن.