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Staphylococcus aureus in Poultry: Prevalence and Antibiogram of Methicillin-resistant *Staphylococcus aureus* in Avian Species in the Southern Provinces of Egypt

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INTENSIVE usage of antibiotics in poultry sectors enabled the consequent emergence of antibiotic resistant bacteria (ARB) and the development of their corresponding antibiotic resistance genes in the environment of human and animal food chains. To determine the antibiotic resistance of MRSA in poultry, 405 different samples (205 broiler farms, 124 backyards, 60 hatchers, and 16 slaughterhouses) were collected from southern Egypt. Identification was carried out by the classical culture methods, and the disc diffusion test was used to determine the antibiotic resistance patterns. Almost, 10% (40/405) of isolated *S. aureus* was identified as coagulase-positive. While 23% (94/405) was coagulase-negative *Staphylococci*. As expected, most of *S. aureus* isolates were susceptible for Vancomycin (95%), sulfamethoxazole trimethoprim (80%), and chloramphenicol (75%). Contrariwise, the high resistance was shown to clindamycin (97.5%), erythromycin (95%), tetracycline (90%), and penicillin and oxacillin (82.5%). MRSA strains were identified as 95% (38/40) of all isolated *S. aureus* by using a conventional PCR directed to the mec-A gene. This high proportion of MRSA in poultry has a considerable risk to public health. So that, the results of this study highlight the need for control programs that encompass primary animal production and the food chain to alleviate the contamination of MRSA for the poultry industry of Egypt, and consequently for humans.

Keywords: Antimicrobial resistance, mec-A gene, MRSA, Poultry, *Staph. aureus*.

Introduction

Staphylococcus aureus (*S. aureus*) is one of the most substantial life-threatening pathogens of suppurative infections in both humans and animals (Lowy, 1998). In poultry, *S. aureus* can be considered an opportunistic pathogen, where it normally inhabits the skin, feathers, respiratory, and intestinal tracts. (Silva et al., 2019). *S. aureus* has been frequently isolated from many foodstuffs, such as dairy products and meat (Irlinger, 2008). Hence, it has been listed as the third largest etiology of food related disorders worldwide (Sasidharan et al., 2011; Adeyeye & Adewale, 2013). Furthermore, it is one of the great public concerns since the treatment of infections is more difficult when encountering resistance (Weese, 2010; Thaker et al., 2013), which contributes to the evolution of methicillin-resistant *S. aureus* (MRSA).

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MRSA acquire mobile *mec-A* gene carried on Staphylococcal cassette chromosome mec (SCC mec) (Lowy, 1998). This gene encodes for mediating β-lactams resistance through altering the penicillin-binding protein (PBP2a or PBP20) (Voss et al., 2005).

The first report of animal-associated MRSA was noted at 1970 in Belgium from bovine mastitis (Calnek et al., 1997). Since 2005, MRSA- animal strains, have started to emerge in the human population (Fluit et al., 2001). MRSA was first reported in poultry in South Korea from chickens suffered from infectious arthritis. Isolates were typed by random amplified polymorphic DNA (RAPD) as highly similar to each other, while sharing the common ancestors of those isolated from bovine milk and humans (Pinho et al., 2001).

Preceding findings reported that MRSA can be transmitted only through nosocomial infection (Lee, 2006), but currently, other studies report that MRSA exists in a wide range of the community and has the potential to be acquired through consumption of contaminated animal products worldwide (Jones et al., 2002; ECDC, 2009).

Poor hygienic and sanitary conditions in addition to contaminated butchers and meat handlers during the process of slaughtering and evisceration of the bird are significant sources for *S. aureus* infection in Egypt (Kitai et al., 2005). Subsequently, it is thought that poultry products are potential reservoir for MRSA infection in human.

Recently, MRSA has been increasingly reported as emerging problem in veterinary medicine, particularly in small animals and poultry (Cuny et al., 2000; Kwon et al., 2006). Several reports show the prevalence of MRSA in a variety of poultry farms, slaughter houses, carcases, or food of poultry origin (Nemati et al., 2008; Persoons et al., 2009; Lim et al., 2010). This is leading to an upsurge of reports and interest in MRSA colonization and infection in poultry.

The aim of this study was to evaluate the incidence of *S. aureus* between samples isolated from various avian origin in south Egypt by using cultural and biochemical tests, to determine their diversity between samples and to characterize the isolated strains based on their resistance to antibiotics, and investigate the existence of MRSA strains.

Materials and Methods

Sample collection

A total of 405 specimens were collected from different poultry sectors (205 broiler farms, 124 backyards, 60 hatchers, and 16 slaughterhouses) in the southern part of Egypt from Luxor to Aswan province. Specifically, nasal, tracheal, cloacal, lung, liver, and yolk sac as described in Table1.

Isolation and identification of S. aureus

All samples were transported to the Reference lab for quality control on poultry production (RLQP), animal health institute, Luxor, Egypt. Samples were pre-enriched on buffered peptone water (BPW) overnight as (1:10 dilution) then incubated at 37±1°C for 18± 2h under aerobic conditions (Incucell IP20, Munich, German). After enrichment, the samples were streaked on Bacto-Mannitol Salt Agar (MSA) (Difco, Franklin, USA) and Baird Parker agar (BPA) (Oxoid, Hampshire, England) according to ISO 6888 (Verma et al., 2003) and incubated at 35-37°C for 24h in case of MSA, and for 24-48h in case of BPA.

TABLE 1. Source, type, and number of collected samples

Source	Species	Type of samples	Examined samples
Broiler farms	Chicken	Ts- Cs- Ns- lung- liver	205
Backyards	Chicken- goose- duck- turkey- pigeon	Ts- Cs	124
Hatcheries	Baby chicks	Yolk sac	60
Manual slaughter house	Chicken	Ts- Cs- lung- liver	16

Ts is tracheal swabs, Cs is cloacal swabs, and Ns is nasal swabs.

The colonies were observed for the typical appearance of *Staphylococcus* spp. (Cheesbrough, 2005) and those with typical cultural characteristics were further streaked on blood agar for purification. Smears from the suspected colonies were Gram stained and examined microscopically under the oil immersion lens. Typical or suspected colonies were streaked onto the surface of a semisolid agar medium, followed by plating on TSA medium for further biochemical identification, including slide catalase (Gillespie, 1943), coagulase (Wayne, 2011), oxidase, and colony pigmentation.

Antimicrobial sensitivity test

Antibiogram assays were performed for 16 commonly used antibiotics as listed in Table 2. The susceptibility test was determined by disc diffusion technique according to Thornsberry (1990). Interpretation of the inhibition zones was applied according to McClure et al. (2006).

TABLE 2. Antimicrobial discs adopted

Antimicrobial discs	Code	Disc potency (mg/disc)
Penicillin G	P	10 I.U
Ampicillin	AM	10µg
Amoxycillin	AML	30µg
Oxacillin	OX	1µg
Vancomycin	VA	30µg
Gentamicin	GN	10µg
Amikacin	AK	30µg
Erythromycin	E	15µg
Tetracycline	TE	30µg
Enrofloxacin	EN	5µg
Norfloxacin	NOR	10µg
Clindamycin	Cm	2µg
Ttimethoprim-Sulphamethoxazol	Tr-Sp	25µg
Chloramphenicol	C	30µg
Neomycin	N	30µg
Streptomycin	S	25µg

Detection of mec-A gene by conventional PCR

Genomic DNA was extracted using a QIAmp DNA mini kit (QIAGEN, Hilden, Germany) following the manufacturer's directions and then, polymerase chain reaction (PCR) was conducted to amplify the *mec-A* gene by using Emerald Amp GT PCR master mix (Takara). Oligonucleotide primers targeting *mec-A* gene of MRSA were described as follow: mec-A/F (GTA GAA ATG ACT GAA CGT CCG ATA A) and mec-A/R (CCA ATT CCA CAT TGT TTC GGT CTA A) (Irlinger, 2008).

PCR was completed in a total reaction volume of 25µl, including 12µL of Emerald Amp GT PCR master mix (2x premix, RR310A kit), 1µL each of 10µM forward and reverse primer, 4.5µL double-DW, and 6µL DNA sample. The PCR conditions were conducted as follows: A preliminary denaturation step at 94°C for 5min; followed by 35 cycles of amplification with denaturation at 94°C for 45sec, annealing at 50°C for 45sec and extension at 72°C for 45sec, and a final extension step at 72°C for 10min. ATCC 25923 and ATCC 43300 were employed as quality control for both methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistance *S. aureus* (MRSA), respectively. All reactions were performed in a Veriti™ thermal cycler (Thermo Fisher Scientific, USA). The PCR products were electrophoresed on 2% agarose gels, mixed with ethidium bromide, and viewed under UV light.

Results

Overall prevalence of Staphylococcus aureus

Based on cultural and biochemical characteristics, results revealed that about 10% (40/405) of all poultry samples were identified as coagulase-positive (CoP) *S. aureus*. Most of the samples were isolated from chickens as broiler and baby chicks with proportion 12% (38/315) of the total prevalence followed by geese 5.6% (2/36) and manual slaughter house 12.5% (2/124). Individual prevalence of *S. aureus* among different avian origin was distributed as follow: 4.54% (2/44) from liver, 0.82% (1/122) from tracheal swabs (T.S), 8% (13/162) from cloacal swabs (C.S), 33.3% (1/3) from nasal swabs (N.S), and 38.3% (23/60) from yolk sac as described in Table 3.

Antimicrobial sensitivity assay

Staphylococcus aureus isolates were screened for their antibiotic susceptibility pattern by phenotypic profile on 16 antibiotics as illustrated in Fig. 1. The highest sensitivity was recorded toward vancomycin (95%), followed by sulfamethoxazole trimethoprim (80%) and chloramphenicol (75%). Alternatively, the highest resistance was confirmed against clindamycin (97.5%), followed by erythromycin (95%), tetracycline (90%), penicillin and oxacillin (82.5% each).

TABLE 3. *S. aureus* prevalence among avian species from different tissues

Species	No. samples	*S .aureus*/No	%	Source	No. samples	*S. aureus*/No	%
Chickens	315	38	12	Liver	44	2	4.54
Turkey	6	0	0	lung	14	0	0
Pigeon	10	0	0	T.s	122	1	0.82
Duck	38	0	0	C.s	162	13	8
Goose	36	2	5.6	N.s	3	1	33.3
Total	405	40	9.88	Yolk sac	60	23	38.3
				Total	405	40	9.88

Ts is tracheal swabs, Cs is cloacal swabs, and Ns is nasal swabs.

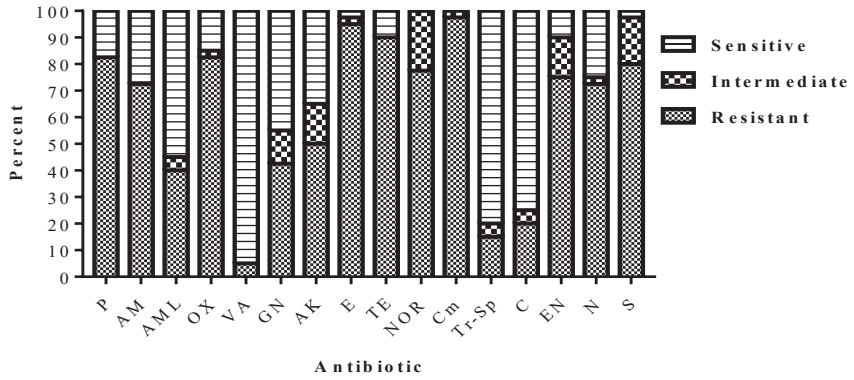

Fig. 1. Phenotypic profile of antimicrobial sensitivity assay

Prevalence of mec-A gene

The overall prevalence of mec-A gene among coagulase-positive *S. aureus* was 95% (38/40). While, individual incidence was 92% (12/13) from broiler farms, 96% (22/23) from baby chicks and 100% (2/2) from backyard and slaughter house as listed in Fig.2.

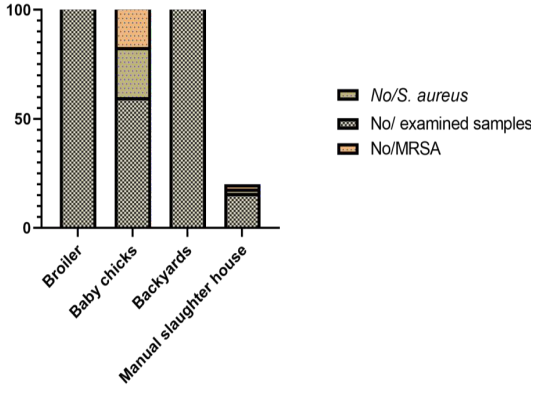

Fig. 2. Prevalence of *S. aureus* and MRSA among avian host

Discussion

The poultry industry in Egypt with a population of 1 billion broilers which appraised to produce 1.5 metric tons (MT) of poultry meat during the period of 2014 (Hassan, 2014). Egypt ranks first in the Middle East with 25 billion Egyptian pounds for poultry investment (Hassan, 2014). However, Poultry are a substantial source of infection for many pathogens including *Staphylococcus*.

Commercial chicken meat has been recognized as one of the most significant vehicles for transmission of food borne pathogens, ARB, and antibiotic resistant genes (Pletinckx et al., 2011). From the 405 samples inspected in this study, 40 isolates were obtained, mainly from chickens (12%), and this was equal with findings obtained by El-Jakee et al. (2008) who isolated *S. aureus* from poultry litter in Behera, Egypt. Other poultry species such as geese showed incidence rates of 6% (2/36), while pigeons, turkeys, and ducks were negative for CoP *S. aureus*. These findings were different than Benrabia et al. (2020) who recorded high prevalence of CoP *S. aureus* from turkey farms (73.6%) in Algeria, followed by breeding hens (52.8%), laying hens (48.8%), and broilers (48.4%).

On the contrary, our screenings were higher than that of Schraft et al. (1992) who reported 8% incidence of *S. aureus* from chickens with musculoskeletal abscess.

The isolation and prevalence data are variable and differ between various countries. Nevertheless, comparisons could still be made, noting that there are limiting factors due to inherent differences in sampling techniques, type of samples, sampling seasons, geographical locations and number of samples which was a significant shortage in our study due to high cost of analysis. As we mentioned above, the poultry industry is a vital source of economy in Egypt, and frequent use of veterinary antibiotics such as penicillin, tetracycline, and erythromycin, for therapeutic and prophylactic purpose, no doubt is a major reason for the existence of antibiotic resistant bacteria (ARB) that can spread through the food chain (Aarestrup et al., 2000; Nemati et al., 2008). Extensive adoption of various antibiotics leads to occurrence of MRSA in local avian, meanwhile it also has global concern. Like other countries, Egypt is also facing emerging dangers posed by *S. aureus* and MRSA and knowledge is quite limited on the prevalence of such pathogen in Egypt compared to the world because of the lack of advanced monitoring system and tools. Hence, this study was carried out to address the prevalence of MRSA among different sources of avian origin in south of Egypt. Moreover, the antibiotics susceptibility pattern of these isolates was studied. Accordingly, the forty *S. aureus* isolates were examined for their susceptibility toward 16 antimicrobial agents. As expected, most of the samples were sensitive to vancomycin (95%) followed by trimethoprim sulphamethoxazole (80%).

Vancomycin is frequently considered the first drug option for infections by resistant bacteria. However, by the 1990s, MRSA showed decreased sensitivity to vancomycin and eventually vancomycin resistant *Staphylococcus aureus* (VRSA) strains were observed (Boneca & Chiosis, 2003; Petrelli et al., 2008). Therefore, continuous monitoring of antibiotic utilization should be conducted.

Persistent resistance of MRSA to β-lactam antibiotics have caused severe problems in human and veterinary medicines. Our findings showed high resistance for β-lactam antibiotics including penicillin and oxacillin (82.5%). As investigated before by Marshall & Levy (2011), there was a significant correlation between oxacillin resistance and other antimicrobials such as erythromycin, clindamycin, and gentamycin. Similarly, our findings showed the same correlation between oxacillin and clindamycin (97.5%), erythromycin (95%), and tetracycline (90%).

As well established that penicillin was the initial antibiotic used for treatment of *S. aureus* infections. However, in the late 1950s, penicillin resistance started to become a concern globally (Gajdács, 2019). Since then, other synthetic β-lactams including methicillin and oxacillin have been used to avoid the pervasive spread of penicillin-resistant *S. aureus*. In addition to being resistant to almost β-lactams, MRSA are often related to other categories of antibiotic resistance, especially aminoglycosides, macrolides, lincosamides and fluoroquinolones, besides, vancomycin and linezolid (Shlaes & Projan, 2009). By comparison to others studies we can infer that our antibiogram findings were quite similar or a little higher or lower than other studies due to frequent change in the susceptibility pattern of the organism with time.

According to (CDC, 2013) a significant correlation has been reported between oxacillin sensitivity and predicting methicillin resistance, as 100% of isolates carrying *mec-A* (the most prevalent gene for MRSA prediction) gene were phenotypically resistant to oxacillin. However, evaluation of our findings showed that 82% of isolates showed resistance to oxacillin, 95% of isolates were *mec-A* gene carriers (Figs. 1, 2). This means that phenotypic profile may not accurately predict the prevalence of MRSA. To avoid misinterpretation, PCR can be used to detect *mec-A* gene as a diagnostic tool with standard criteria for identification of MRSA strains (Batista et al., 2013).

Screening the prevalence of MRSA will be of much utilize in early prevention and control of MRSA colonization and spreading in poultry flocks specially immunocompromised birds and community acquired infections.

In the current investigations, *mec-A* gene was identified in 100% (2/2 each) from manual slaughterhouses and backyards. While ninety-two percent of isolates (12/13) were recorded

from broiler farms. In baby chicks suffering from omphalitis, we found that 96% (22/23) were carriers for *mec-A* gene as illustrated in Fig .1. Our findings were higher than those of Adeyeye & Adewale (2013) who isolated 4/50 (8%) of MRSA strains from broilers. Likewise, this finding was higher than that of Nemati et al. (2008) who found that 2/14 (14 %) of Belgian farms were positive for MRSA.

According to antecedent studies were conducted in European countries, including Denmark, around 35% of slaughter houses were MRSA positive (Perez-Roth et al., 2001) and that was quite similar to our results. On the other hand, our findings were lower than those of Mulders et al. (2010) who recorded of 28% prevalence of MRSA from poultry farms in Malaysia. Furthermore, Falagas et al. (2013) isolated MRSA from turkey flocks in Netherlands at the range of (62-80%). In Germany, transmission of MRSA from livestock to humans arises frequently from occupational animal contact (Köck et al., 2014). Among 466 persons tested for MRSA in Dutch poultry slaughterhouses, 26 individuals were positive, which specifies a higher exposure risk to MRSA compared to the non-occupational Dutch people (Mulders et al., 2010). Additionally, MRSA has been found in a diversity of meats products including turkey, raw chicken, veal, pork, mutton or lamb, beef, and rabbit (Kitai et al., 2005; Kluytmans, 2010; Kwon et al., 2006; Van Den Broek et al., 2009). In general, the prevalence of MRSA was: turkey (35%), chicken (16%), veal (15%), pork (11%), and beef (11%), respectively (De Boer et al., 2009). During a sectional study of MRSA in poultry food and its products, MRSA was identified in 5 isolates from 318 food specimens in Spain (Lozano et al., 2009), also MRSA was found in 2% out of 114 examined samples in the USA (Abdalrahman et al., 2015). While the occurrence of nasal MRSA colonization between swine farmers were different, i.e., 19% in Taiwan, 5.5% in Malaysia, and 15% in China (Graveland et al., 2011).

Prevalence of animal-associated MRSA in the Netherlands was recorded as high as 6% in poultry slaughter house workers, even though high restrictions have been conducted for antibiotic manipulation (Pletinckx et al., 2011). Moreover, MRSA has been reported in Northern Africa, including Tunisia with incidence rates of 16-41% between 2002 and 2007 respectively. In Libya, the infection rate of MRSA was 31% in 2007, while it was 55% in Ethiopia. Between 2003 and 2005, MRSA has been identified as high as 52% from multicenter in Egypt (Falagas et al., 2013). However, such data should be interpreted with attention due to the shortage of specimen analysis, diversity in the study area, and finally, the limited technical resources. The influence of MRSA may also vary significantly depending on the hygienic protocol and monitoring program in the study area. Testing only few specimens from each sector due to limited resources is a significant limitation of the study, and the results may not be representative of the full range of MRSA present on Egyptian poultry farms.

Conclusions

Appraising *S. aureus* prevalence in poultry flock is vital for future risk assessment in poultry production and associated occupational risks. Therefore, understanding the dynamic changes in antibiotic resistance by a sustainable surveillance program of antimicrobial resistance profile of *S. aureus* in Egypt in connection with the medical and veterinary conditions is essential for proper improvement of *S. aureus* control. Regular genotypic analysis of *S. aureus* from human and chicken origin is needed to find the relation in-between, because of the potential for transmission of *S. aureus* multidrug-resistant to humans by the consumption of poultry or any byproduct containing such strains. Future studies are needed to detect the frequency of MRSA in a wider area in Egypt and to include occupational workers to investigate its possible zoonotic transmission that would assist in appraising the human-health risks induced by MRSA.

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Methodology. Mohamed W. Abd Al-Azeem: Validation, Methodology, Supervision. Ashraf El ghoneimy: Supervision, Visualization. Israa M. A. Mohamed: Writing - review & editing.

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الميكروب العنقودى الذهبى فى الدواجن: مدى تواجد وانماط المقاومة للمضادات الحيوية للميكروب العنقودى الذهبي المقاوم للميثيسيلين فى الدواجن فى جنوب مصر

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ادى الاستخدام المكثف للمضادات الحيوية فى قطاع صناعة الدواجن الى ظهور البكتيريا المقاومة للمضادات الحيوية وانتشار جينات المقاومة للمضادات الحيوية فى بيئة السلسلة الغذائية للانسان والحيوان . لتحديد مقاومة المضادات الحيوية لبكتريا المكور العنقودي الذهبى المقاوم للميثيسيلين فى الدواجن تم جمع 405 عينة مختلفة من جنوب مصر(205 عينة من مزارع دجاج التسمين، 124عينة من دواجن تربية منزلية, 60 عينة من مفرخات، 16 من رياشات)

تم الفحص والعزل بالطرق القياسية واستخدام اختبار الحساسية الانتشارية للمضادات الحيوية لتحديد مدى حساسية الميكروب للمضادات الحيوية (لتحديد انماط المقاومة)، 10% من عزلات الميكروب العنقودى الذهبي كانت موجبة لاختبار coagulase بينما 23%من معزولات كانت سالبة لاختبار coagulase، معظم العزلات كانت حساسة للفانكوميسين بنسبة 95%، وسلفاميسكسزول وترايميسوبريم بنسبة 80% وكلورامفينيكول بنسبة 75% بينما اظهرت العزلات مقاومة عالية للكلنداميسين بنسبة 97.5%، الارثروميسين بنسبة 95%، والتتراسيكلين بنسبة 90%، بينسيلين واوكساسيلين82.5%. 95% من عزلات الميكروب العنقودى الذهبى كانت ايجابية لوجود mec-A باختبار البلمرة المتسلسل، وهذه النسبة العالية

من بكتريا MRSA فى الدواجن لها مخاطر كبيرة على الصحة العامة لذلك فان نتائج هذه الدراسة تسلط الضوء على الحاجة إلى برامج المكافحة والتى تشمل الانتاج الحيوانى الاولى وسلسلة الغذاء لتخفيف التلوث ب MRSA لصناعة الدواجن فى مصر وبالتالى للانسان.