Molecular Detection Of Antibiotic Resistance And Virulence Genes In Staphylococcus Species Isolated From Human and poultry

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Abstract

Staphylococcus species are the most precarious micro-organisms in poultry meat, because they cause multiple health problems, risks to consumers and the economy of the country. Since they act as antibiotic resistant bacteria that carry virulence genes and antibiotic resistance genes which can be transmitted from food producing animals as poultry to humans through various modes the major one being food chain and cause health hazards for the consumers. Therefore, the current study was aimed to investigate the incidence rate of Staphylococcus species isolated from broiler chicken and hospitalized patient, detection the antimicrobial susceptibility of these isolates by using Vitek2 system and confirmation the presence of genes encoding for pathogenicity and antimicrobial resistance in detected isolates by PCR. To achieve these 200 samples were collected from poultry farms and hospitalized patient (100 for each) in dissimilar districts in Assiut province, to be subjected to bacteriological examination. The results showed that the incidence of Staphylococcus species was 35% and 45% in poultry and human samples respectively on mannitol agar. Vitek2 system divided poultry isolates to 11 isolates as coagulase-positive Staphylococci (CoPS) and 24 isolates as coagulase-negative staphylococci (CoNS). Whereas 20 human isolates alienated as CoPS and 25 isolates assigned as CoNS. Antibiogram cleared that 45.7% and 53.3% of Staphylococcus isolates from poultry and human were identified as methicillin-resistant Staphylococci respectively, also Staphylococcal spp. cleared a resistance to different types of antimicrobials such as penicillin, tetracycline clindamycin. Vitek2 system showed a significant ability to differentiate among Staphylococcus species and distinguish its antimicrobial susceptibility which was complicated by conventional method. PCR results showed that the Staphylococcal isolates of poultry and human were harboring genes encoding for pathogenicity (coa, hld and pvl) and antimicrobial resistance (mecA, vanA, cfr and BlaZ) while none of the isolates harbored set and seh genes. So the obtained results emphasized the importance of reducing the unwarranted use of antimicrobial agents and application of sanitary hygienic measures in poultry production.

Keywords: Staphylococcus species, vitek2 system, poultry, human.
Introduction

*Staphylococcus* are widely distributed in water, soil and air, in addition to its isolation from various animal species, including poultry and its genus is present in 70 species (Götz et al., 2006). Poultry meat is the most important source of human food poisoning (Kadariya et al., 2014). Staphylococcal food-borne disease induced by poultry meat became an evident problem reflected negatively on the industry of poultry, causing drawbacks on public health and making difficulty for the medical and veterinary organizations (Teramoto et al., 2016).

*Staphylococcus* species, generally dubbed as coagulase-negative-*staphylococci* (CoNS) and acquired its value as they have been responsible for multiple infections in humans and animals (Vuong and Otto, 2002). *Staph.aureus* is the most critical species within this genus, recognized as coagulase-positive *Staphylococcus* as well as, be one of the causes of food intoxication (Cunha, 2009). This microorganism is the etiological factor causing several avian diseases such as arthritis, septicemia, omphalitis...etc (Smyth and McNamee, 2001). These *Staphylococcal* infectious diseases of chickens are an economic threat and they are viewed as worldwide burden (Lowder et al., 2009).

CoNS are causing nosocomial infections in neonatal intensive care units and uncommon food poisoning bacteria (Tong et al., 2015). Various researches on CoNS and more than 15,000 references, reflecting the increasing of medical influence of these bacteria (Becker et al., 2014) due to the possible spreading of antimicrobial resistant bacteria and antimicrobial resistant genes (Chajęcka-Wierczowska et al., 2015), although, CoNS is documented as a very useful bacteria in the technology and hygiene of food production and preservation (Suškovič et al., 2010).

The virulence genes are communally responsible for the pathogenicity of this organism, like *Staphylococcal* protein A (spa), coagulase protein (coa), *Staphylococcal* enterotoxins A to E collagen adhesion gene (cna), toxic shock syndrome toxin 1 (tst), exfoliative toxins (eta, abt), and leucocidins (Pereira et al., 2009).

The appreciation of antimicrobial resistance in food borne pathogens inventing from food producing animals such as chicken that has been recorded in developing countries (Van et al., 2007). The proclivity for *Staphylococci* to develop antimicrobial resistance is a reason for great worry in both human and animals (Vanderhaeghen et al., 2010).

The undesirable antimicrobial resistance has mostly been risked as result of unreasonable use of antibiotics in producing animals (Adesiji et al., 2014). Amongst all types of resistance, methicillin resistant *Staphylococcus aureus* (MRSA) is considered as critical, as it had been confirmed as the source of acquired infections associated with high rate of bacterial mortality worldwide (Tiemersma et al., 2004).

Automated systems VITEK 2 analyses have proven to be an accurate technique to differentiate Staphylococcal species (Sukru et al., 2018). It is used for the identification of isolated colonies to the genus and species levels (Jackson et al., 2013). Rapid and accurate methods for identification of food borne pathogens are important for microbiological safety. In previous recent years, polymerase chain reaction (PCR) was proven as the most suitable method for fast, sensitive and
unrestricted detection of pathogenic bacteria in food (Kim and Kim, 2017).

The aim of the present study was isolation and identification the Staphylococcus species isolated from poultry and human by using VITEK2 system, also detection of genes encoding for pathogenicity (coa, hld, sei, pv1 and seh) and antimicrobial resistance genes (mecA, cfr; vanA and BlaZ) by PCR.

Materials and Methods

Samples collection

A total of 200 samples were collected aseptically from poultry farms and university hospital in Assiut province (100 for each) during period between March to September 2018. Different organs were obtained from healthy and diseased birds such as caecum, liver and tarsal joints according to Monecke et al. (2013). Also patient swabs (Abscess, conjunctivitis, otitis, pneumonia and urinary tract infections) were gathered according Strommenger et al. (2008).

Preparation of samples Poultry samples:

Slices of liver, tarsal joints and tissues were immersed in test tubes containing nutrient broth for overnight (Mkize, 2016) and nearly one gram of gut content was put in a centrifuge tube containing 9 ml of sterile phosphate-buffered saline (PBS) pH 7.4, and mixed by vortex with glass beads (4 mm in diameter) for 3 minutes. Debris was expelled by centrifugation at 700xg for 1 minute (Seidavi et al., 2010). One milliliter of supernatant was inoculated in a tube contained 9 ml Brain Heart Infusion broth (BHI) and incubated at 37°C for 24h.

Patient samples:

The patient swabs were immersed in a tube contained 9 ml Brain Heart Infusion broth (BHI) and incubated at 37°C for 24h.

Isolation and identification of Staphylococcus species

A loopful of BHI broth was streaked on mannitol salt agar at 37°C for 24h (Gharajalar and Shahbazi, 2018). The obtained colonies were plated onto Sheep blood agar, Baird-Parker agar for identification of Staph. aureus aureus from other species (Son et al., 2010) and Oxacillin Resistance Screening Agar Base (ORSAB) agar for identification of methicillin-resistant Staphylococcus isolates of poultry and human (Nahimana et al., 2006).

Morphological examination of the suspected colonies was done by using Gram staining and biochemical identification of isolates on the base of catalase activity, coagulase (rabbit plasma) and oxidase test (Fijalkowski et al., 2016).

Identification of Staphylococcus species and antibiotic susceptibility using VITEK 2 compact®

The identification of Staphylococcus spp. was done by VITEK GP (Gram Positive) on all obtained isolates from mannitol salt agar according to the cards with reference number 21342, also antimicrobials susceptibilities were done conferring to antibiogram cards AST-GP 67 with reference number 22226 (Sukru et al., 2018).

Detection of virulence and antimicrobial resistance genes in Staphylococcal species by PCR

DNA was extracted from Staphylococcal isolates according to Mansour et al., (2017), after refreshment of these isolates in 5 ml of BHI broth at 35°C (+2°C) for 16-24hrs by using QIAamp DNA Mini kit (Qiagen, Germany, GmbH) on bases of manufacturer’s instruction. Monoplex PCR amplification reaction was performed in a final volume of 25µl, composed of 6µl DNA, 12.5 µl Mastermix (Emerald Amp GT PCR), 1µl for each
primer for 16s rRNA gene and 4.5 µl of PCR grade water. The PCR reactions were implemented in thermal cycler (MJ Research, Inc. Watertown, MA) with the following program: initial denaturation at 95°C for 5 min followed by 45 cycles of 95°C for 45sec, 50°C for 45sec and 72°C for 1min with a final extension at 72°C for 10 min. The amplicons size (bp) were detected by electrophoresis on 1.5% agarose gel (BioshopR, Candainc.) stained with ethidium bromide, then visualized in a UV transilluminator. Also, specific primers for detection of virulence (coa, sei, seh, hld and pvl) and antimicrobial resistance (mecA, vanA, cfr and BlaZ) were used (Table 1).

**Table (1):** Nucleotide sequences of primers and amplicons size (bp)

| Gene | Oligonucleotide sequences (5-3) | Amplicon size (bp) | References |
|------|--------------------------------|-------------------|------------|
| 16SrRNA | F: AAC TCT GTT ATT AGG GAA GAA CA R: CCA CCT TCC TCC GGT TTG TCA CC | 250 | (Zhang et al., 2004) |
| coa | F:ACCCACAAGGTACTGAATAACG R:TGCTTTTCAGTTGTTCAGTC | 987 | (Veras et al., 2008) |
| sei | F:CAACTCGAATTTCACCACAGGTACC R:CCGGCAGTCCAATCTCCATCTCTGTG | 466 | (Pereira et al., 2009) |
| seh | R: CAA CTTG ATC TAG TCA CTG F:GTC GAA TGA GTA ATC TCT AGG | 360 | (Monday and Bohach, 1999) |
| hld | F: AAGAATTTTTATTTTATTAAGGAGGAGTG R: TTAGTGAATTGTTCACGTGTGCA | 111 | (Jarraud et al., 2002) |
| pvl | F:ATCATAGGGGAAATTGCTGGACATGATCCA R:GCATCAAGTGTATGATAGGATAGCAAAAGC | 433 | (McClure et al., 2006) |
| mecA | F:GATGAATGACTGAACGTCCGATAA R:CCATCTCACCACATTGATGGTCTGCAAA | 480 | (Spanu et al., 2004) |
| vanA | F: GCCGCGGTCCACTTGATAGATA R: TGAGCAACCCCCAACAAGTA | 314 | (Nam et al., 2013) |
| cfr | F:TGAAGTAAAAGCAGGTTGGGGAGTCA R:ACCATAATGGACCACAAGCAGGC | 746 | (Kehrenberg and Schwarz, 2006) |
| BlaZ | F ACTTCAACACCTGTGTTCR TGACCACCTTTATCAGCAACC | 173 | (Martineau et al., 2000) |
Results

Results of conventional method

The data illustrated in table (2) showed the result of bacterial examination for 200 samples were taken from poultry and human samples (100 for each) on mannitol salt agar, as followed, *Staphylococcus* spp isolated from 35% and 45% of poultry and human samples respectively. On the other hand, tube coagulase test divided the poultry and human isolates into 31.4% and 44.4% coagulase positive and 68.6% and 55.6% were coagulase negative respectively. While ORSAB agar detected methicillin-resistant *Staphylococi* in 37.14% (13/35) and 42.22% (19/45) from staphylococcal isolates respectively.

Table (2): Incidence of *Staphylococcus* spp. isolated from poultry and human

| Source     | Number of examined samples | Suspected isolates on mannitol agar | Coagulase test Positive | Negative | ORSAB agar positive |
|------------|----------------------------|------------------------------------|--------------------------|----------|---------------------|
| Poultry    | 100                        | 35(35%)                            | 11(31.4%)                | 24(68.8%)| 13(37.14%)          |
| Human      | 100                        | 45(45%)                            | 20(44.4%)                | 25(55.6%)| 19(42.22%)          |
| Total      | 200                        | 80(40%)                            | 31(38.75%)               | 49(61.25%)| 32(40%)            |

Result of VITEK 2 Compact

By using VITEK 2 system, 35 poultry isolates could be differentiate into17 *staphylococcal* species, the highly identified species were *Staph.aureus* 14.3% (5/35) followed by *Staph.lentus* 8.5% (3/35), whereas the least species were *Staph.lugdunensis, Staph.simulans* and *Staph.capitis* (2.86% for each). On the other hand from 45 human isolates could differentiate 19 *staphylococcal* species, the main species were *Staph.aureus* 31.1% (14/45) trailed by *Staph.haemolyticus* and *Staph.cohnii* 3/45 isolates for each (6.7%) (Table3).

Antibiogram for VITEK 2 Compact

Compact antibiogram device was used for detection the antimicrobial resistance. The result of antibiogram showed in table (4) reveled that antimicrobial resistance profile of the 35 *Staphylococcus* isolates from poultry samples to different antibiotics was investigated; none of the isolates were completely sensitive to the 13 tested antibiotics. High percentage of resistance was observed in tetracycline 28 (80%), clindamycin 26 (74.3%), penicillin 22 (62.8%) and erythromycin 18 (51.4%). While, low resistance was noticed to gentamicin 6 (17%) and trimethoprim / sulfamethazole 7 (20%) between tested antibiotics.

Further that the antibiogram results showed that the least resistant species was *Staph.simulans* that was resistant to 3 antibiotics (tetracycline, clindamycin and quinupristin/dalfopristin). While, *Staph.aureus* and *Staph.lentus* were the most resistant species to the 13 tested antibiotics. While, methicillin-resistant *staphylococci* were identified in 16 isolates (8 as methicillin resistant coagulase positive *staphylococci* (MRCoPS) and the other 8 were methicillin resistant coagulase negative *staphylococci* (MRCoNS)).
system also cleared that vancomycin resistant *Staphylococcus* species was detected in 14 isolates (Table 4). The result of antibiogram showed in Table (5) revealed that antimicrobial susceptibility profile of the 45 *Staphylococcus* isolates from human samples to 13 types of antibiotics.

### Table (3): Results of *Staphylococcus* identification by using Vitek system

| *Staphylococcus* Species   | Source | Number | Coagulase | Percentage(*) |
|----------------------------|--------|--------|-----------|---------------|
|                            |        |        | Positive  | Negative      |
| *Staph.aureus*             | Human  | 14     | 14        | 0             | 31.11         |
|                            | Poultry| 5      | 5         | 0             | 14.29         |
| *Staph.chromogenes*        | Human  | 0      | 0         | 0             | 0.00          |
|                            | Poultry| 2      | 0         | 2             | 5.71          |
| *Staph.hyicus*             | Human  | 2      | 2         | 0             | 4.44          |
|                            | Poultry| 2      | 0         | 2             | 5.71          |
| *Staph.hominis*            | Human  | 2      | 0         | 2             | 4.44          |
|                            | Poultry| 2      | 0         | 2             | 5.71          |
| *Staph.haemolyticus*       | Human  | 3      | 0         | 3             | 6.67          |
|                            | Poultry| 2      | 0         | 2             | 5.71          |
| *Staph.arlettae*           | Human  | 1      | 0         | 1             | 2.22          |
|                            | Poultry| 2      | 0         | 2             | 5.71          |
| *Staph.warneri*            | Human  | 2      | 0         | 2             | 4.44          |
|                            | Poultry| 2      | 0         | 2             | 5.71          |
| *Staph.pseudointermedius*  | Human  | 2      | 2         | 0             | 4.44          |
|                            | Poultry| 0      | 0         | 0             | 0.00          |
| *Staph.lentus*             | Human  | 2      | 0         | 2             | 4.44          |
|                            | Poultry| 3      | 0         | 3             | 8.57          |
| *Staph.epidermitis*        | Human  | 2      | 0         | 1             | 4.44          |
|                            | Poultry| 0      | 0         | 0             | 0.00          |
| *Staph.capitis*            | Human  | 1      | 0         | 1             | 2.22          |
|                            | Poultry| 1      | 0         | 1             | 2.86          |
| *Staph.vitulinus*          | Human  | 1      | 0         | 1             | 2.22          |
|                            | Poultry| 2      | 0         | 2             | 5.71          |
| *Staph.simulans*           | Human  | 0      | 0         | 0             | 0.00          |
|                            | Poultry| 1      | 0         | 1             | 2.86          |
| *Staph.auricularis*        | Human  | 1      | 0         | 1             | 2.22          |
|                            | Poultry| 0      | 0         | 0             | 0.00          |
| *Staph.cohnii*             | Human  | 3      | 0         | 3             | 6.67          |
|                            | Poultry| 2      | 0         | 2             | 5.71          |
| *Staph.saprophyticus*      | Human  | 2      | 0         | 2             | 4.44          |
|                            | Poultry| 0      | 0         | 0             | 0.00          |
| *Staph.schleiferi*         | Human  | 1      | 1         | 0             | 2.22          |
|                            | Poultry| 2      | 2         | 0             | 5.71          |
| *Staph.sciuri*             | Human  | 2      | 0         | 2             | 4.44          |
|                            | Poultry| 2      | 0         | 2             | 5.71          |
| *Staph.xylosus*            | Human  | 2      | 0         | 2             | 4.44          |
|                            | Poultry| 2      | 0         | 2             | 5.71          |
Quinupristin/dalfopristin
Sulfamethoxazole/Trimethoprim
Erythromycin
Gentamycin
ciprofloxacin
Clindamycin
moxifloxacin
Vancomycin
Tetracycline
rifampicin
Oxacillin
Penicillin
(*) The percentage of each *Staphylococcus* spp. was calculated from the total number of isolates: poultry isolates(35) and human isolates(45)

**Table (4): Distribution of Staphylococcus species isolated from poultry samples according to their species diversity and multidrug resistance pattern**

| *Staphylococcus* sp. (n = isolates) | Antibiotics | Resistance Pattern |
|------------------------------------|-------------|--------------------|
|                                    | Oxacillin   | Penicillin         | Gentamicin | Sulfamethoxazole/Trimethoprim | Clindamycin | Erythromycin | Tetracycline | Vancomycin | Moxifloxacin | Levofloxacin | Rifampicin | Ciprofloxacin |
| Staph. aureus(5)                   | 4           | 4                  | 1           | 2                         | 5           | 4          | 2           | 2           | 5           | 2           | 3           |
| Staph. hyicus(1)                   | 2           | 2                  | 1           | 1                         | 2           | 0          | 1           | 1           | 1           | 0           | 0           | 2           |
| Staph. schleiferi(2)               | 1           | 0                  | 0           | 0                         | 2           | 1          | 1           | 2           | 2           | 1           | 1           | 2           |
| Staph. intermedius (2)             | 1           | 2                  | 1           | 1                         | 1           | 0          | 2           | 1           | 0           | 0           | 0           | 2           |
| Staph. lentus (3)                  | 2           | 3                  | 1           | 1                         | 3           | 2          | 3           | 2           | 1           | 1           | 2           | 2           |
| Staph. hominis(2)                  | 0           | 0                  | 0           | 0                         | 0           | 1          | 0           | 1           | 1           | 0           | 0           | 1           |
| Staph. chromogens (2)              | 0           | 2                  | 0           | 0                         | 0           | 2          | 1           | 0           | 0           | 2           | 0           | 0           |
| Staph. warneri(2)                  | 1           | 1                  | 0           | 0                         | 2           | 0          | 1           | 1           | 2           | 2           | 0           | 2           |
| Staph. haemolyticus (2)            | 0           | 2                  | 0           | 0                         | 2           | 1          | 0           | 0           | 0           | 2           | 1           | 0           |
| Staph. arlettae (2)                | 1           | 0                  | 0           | 0                         | 2           | 0          | 2           | 1           | 0           | 0           | 0           | 0           |
| Staph. sciuri (2)                  | 0           | 2                  | 1           | 1                         | 2           | 2          | 1           | 0           | 0           | 2           | 1           | 0           |
| Staph. xylosus (2)                 | 1           | 0                  | 0           | 0                         | 2           | 0          | 2           | 0           | 0           | 1           | 0           | 0           |
| Staph. vitulinus (2)               | 1           | 2                  | 1           | 1                         | 0           | 2           | 2           | 1           | 1           | 0           | 0           | 1           |
| Staph. clintonii (2)               | 0           | 0                  | 0           | 0                         | 2           | 2          | 1           | 0           | 0           | 2           | 1           | 0           |
| Staph. capitis(1)                  | 1           | 1                  | 0           | 0                         | 1           | 1          | 1           | 1           | 1           | 1           | 0           | 1           |
| Staph. simulans(1)                 | 0           | 0                  | 0           | 0                         | 1           | 0          | 1           | 0           | 0           | 0           | 0           | 0           |
| Staph. lugdunensis (1)             | 1           | 1                  | 0           | 0                         | 1           | 1          | 1           | 1           | 1           | 1           | 1           | 1           |
| Total                              | 35          | 16                 | 22          | 6                         | 7           | 26         | 18          | 28          | 14          | 12          | 13          | 17          | 11          | 13          |

The high ratio of resistance to penicillin (84.4%), clindamycin (73%), tetracycline (66.7%) and rifampicin (64.4%). Also, penicillin and clindamycin recorded the high percentages of resistance (84.4%) and (73%) respectively. Whereas gentamicin and trimethoprim/sulfamethazol showed the low resistance (26.7%) for both from tested antibiotics.
Further that the antibiogram results showed that the least resistant specie was *Staph. auricularis* that was resistant to 6 antibiotics (penicillin, clindamycin, vancomycin, ciprofloxacin, gentamicin and trimethoprim/sulfamethazole). On the other hand, *Staph. aureus* was the most resistant specie to the 13 tested antibiotics. Methicillin-resistant *staphylococci* were detected in 24 isolates, (11 were (MRCOPS) and the other 13 were (MRCONS)). Vitek2 system also detected vancomycin resistant *staphylococcus* species in 12 isolates.

**Table (5):** Distribution of *Staphylococcus species* isolated from human samples according to their species diversity and multidrug resistance pattern

| Staphylococcus sp. (n = isolates) | Antibiotics |  |  |  |  |  |  |  |  |  |  |  |
|----------------------------------|-------------|---|---|---|---|---|---|---|---|---|---|---|
|                                  | Oxacillin   | Penicillin | Gentamicin | Trimethoprim/sulfamethazine | Clindamycin | Erythromycin | Tetacycline | Vancomycin | Moxifloxacin | Levofloxacin | Rifampicin | Ciprofloxacin |
| **Coagulase Positive**           |             |             |             |             |             |             |             |             |             |             |             |             |
| *Staph. aureus* (14)             | 8           | 13          | 2           | 3           | 11          | 11          | 6           | 4           | 5           | 12          | 3           | 5           |
| *Staph. hyicus* (2)              | 2           | 2           | 1           | 1           | 2           | 1           | 2           | 0           | 1           | 1           | 1           | 1           |
| *Staph. pseudointermedius* (2)   | 0           | 2           | 1           | 1           | 2           | 2           | 1           | 0           | 1           | 1           | 2           | 1           |
| *Staph. intermedius* (1)         | 0           | 1           | 1           | 0           | 1           | 0           | 1           | 0           | 1           | 1           | 1           | 1           |
| *Staph. schleiferi* (1)          | 1           | 0           | 0           | 1           | 1           | 1           | 1           | 1           | 0           | 0           | 1           | 1           |
| **Coagulase Negative**           |             |             |             |             |             |             |             |             |             |             |             |             |
| *Staph. haemolyticus* (3)        | 3           | 2           | 1           | 0           | 3           | 2           | 3           | 2           | 0           | 2           | 2           | 2           |
| *Staph. cohnii* (3)              | 1           | 2           | 0           | 0           | 2           | 0           | 2           | 1           | 0           | 1           | 2           | 1           |
| *Staph. hominis* (2)             | 1           | 1           | 0           | 0           | 1           | 1           | 0           | 1           | 1           | 1           | 1           | 0           |
| *Staph. warneri* (2)             | 1           | 1           | 0           | 0           | 2           | 1           | 1           | 1           | 1           | 1           | 1           | 1           |
| *Staph. lentus* (2)              | 1           | 1           | 1           | 1           | 1           | 1           | 1           | 1           | 0           | 1           | 1           | 0           |
| *Staph. saprophyticus* (2)       | 1           | 2           | 0           | 0           | 1           | 2           | 1           | 0           | 1           | 2           | 1           | 1           |
| *Staph. sciuri* (2)              | 1           | 2           | 1           | 1           | 1           | 1           | 1           | 1           | 0           | 1           | 1           | 1           |
| *Staph. xylosus* (2)             | 1           | 2           | 1           | 1           | 1           | 2           | 0           | 1           | 1           | 0           | 1           | 1           |
| *Staph. arlettae* (1)            | 1           | 1           | 1           | 1           | 1           | 1           | 1           | 0           | 1           | 1           | 1           | 1           |
| *Staph. epidermidis* (1)         | 1           | 2           | 0           | 0           | 2           | 0           | 2           | 1           | 1           | 0           | 1           | 1           |
| *Staph. capitis* (1)             | 0           | 1           | 0           | 0           | 1           | 1           | 0           | 1           | 0           | 1           | 0           | 1           |
| *Staph. vitulinus* (1)           | 0           | 1           | 1           | 1           | 1           | 0           | 0           | 1           | 1           | 1           | 1           | 1           |
| *Staph. auricularis* (1)         | 0           | 1           | 1           | 1           | 1           | 0           | 0           | 1           | 0           | 0           | 0           | 0           |
| *Staph. lugdunensis* (1)         | 1           | 1           | 0           | 0           | 1           | 1           | 0           | 0           | 1           | 1           | 1           | 0           |
| **Total**                        | 45          | 24          | 12          | 12          | 33          | 28          | 30          | 12          | 16          | 19          | 29          | 18          | 19          |
Results of PCR

Ten *Staphylococcus* isolates (5 from poultry and 5 from human) were selected randomly for testing by PCR. 16s rRNA gene primers confirmed the presence of staphylococcal DNA in these isolates (Table 6&Fig.1), also different virulence genes were detected in staphylococcal isolates; *coa* gene was detected in four isolates (2 poultry and 2 human), *hld* gene detected in 6 isolates (2 poultry and 4 human) and *pvl* gene detected in two isolates (one for poultry and other in human) (Table 6 and Fig.2,3 and4) respectively. On the other hand, antimicrobial resistance genes were detected in isolates as followed, each of *mecA* gene and *vanA* gene were detected in 6 isolates (3 poultry and 3 human for each gene) while *BlaZ* gene was detected in eight isolates (4 poultry and 4 human), also *cfr* gene detected in 4 isolates (2 poultry and 2 human) (Table 6 and Fig.5,6,7and8) respectively. While *sei* and *seh* genes not detected in the tested samples.

Despite of the harmony between a results of Vitek system and PCR than conventional method as recorded in table (6) but PCR cleared a high accuracy in detection of *mecA* and *vanA* gene in human isolate no.(8), however Vitek system identified this isolate as vancomycin resistant strain only.

**Table (6):** The distribution of virulence and antimicrobial resistance genes in the tested isolates

| Isolates no. | Source | Species            | Resistance genes                  | Vitek 2 | Conventional | PCR |
|-------------|--------|--------------------|-----------------------------------|---------|--------------|-----|
|             |        |                    | ORSAB    | Coagulase | coa | hld | pvl | mecA | BlaZ | vanA | cfr |
| 1           | Poultry| *Staph.lentus*     | vancomycin resistant              | +ve     | -ve          | -   | -   | -   | +    | +    | +   |
| 2           | Poultry| *Staph.aureus*     | vancomycin resistant              | -ve     | +ve          | +   | +   | +   | +    | +    | +   |
| 3           | Poultry| *Staph.aureus*     | vancomycin resistant              | -ve     | +ve          | +   | -   | -   | +    | +    | -   |
| 4           | Poultry| *Staph.lugdunensis*| methicillin resistant             | +ve     | -ve          | -   | -   | -   | +    | +    | -   |
| 5           | poultry| *Staph.warneri*    | methicillin resistant             | -ve     | -ve          | -   | -   | -   | +    | +    | -   |
| 6           | human  | *Staph.cohnii*     | methicillin resistant             | +ve     | -ve          | -   | -   | +   | +    | +    | -   |
| 7           | human  | *Staph.lentus*     | methicillin resistant             | -ve     | -ve          | -   | +   | +   | +    | +    | -   |
| 8           | human  | *Staph.aureus*     | vancomycin resistant              | -ve     | +ve          | +   | +   | +   | +    | +    | +   |
| 9           | human  | *Staph.aureus*     | vancomycin resistant              | +ve     | +ve          | +   | -   | +   | +    | +    | +   |
| 10          | human  | *Staph.haemolyticus*| vancomycin resistant             | -ve     | -ve          | -   | -   | -   | +    | -    | -   |
Fig (1): Agarose gel electrophoresis of 16s rRNA gene amplification at 250 bp, Lane 1: Ladder, (100 bp), Lanes 2 to 6: positive poultry isolates, Lanes 7 to 11: positive human isolates.

Fig (2): Agarose gel electrophoresis of coa gene amplification at 987 bp, Lane 1: Ladder (100 bp), Lanes 2 to 6: poultry isolates, included lane 3 and 4 were positive isolates, Lanes 7 to 11: human isolates, included lanes 9 and 10 were positive isolates.

Fig (3): Agarose gel electrophoresis of hld gene amplification at 111 bp, Lane 1: Ladder (100 bp), Lanes 2 to 6: poultry isolates, included lane 2 and 3 were positive isolates, Lanes 7 to 11: human isolates, included lane 8, 9, 10, 11 were positive isolates.

Fig (4): Agarose gel electrophoresis of Pvl gene amplification at 433 bp, Lane 1: Ladder (100 bp), Lanes 2 to 6: poultry isolates, included lane 3 was positive isolate, Lanes 7 to 11: human isolates, included lane 9 was positive isolate.
Fig (5): Agarose gel electrophoresis of *mecA* gene amplification at 480, Lane 1: Ladder (100bp), Lanes 2 to 6: poultry isolates, included lane 3,5 and 6 were positive isolates, Lanes 7 to 11: human isolates, included lane 7, 8 and 9 were positive isolates.

Fig (6): Agarose Gel electrophoresis of *vanA* gene amplification at 314bp, Lane 1: Ladder (100bp), Lanes 2 to 6: poultry samples, lane 2,3 and 4 positive, Lanes 7 to 11: human samples, lane 9,10 and 11 positive.

Fig (7): Agarose gel electrophoresis of *BlaZ* gene amplification at 173 bp, Lane 1: Ladder (100bp), Lanes 2 to 6: poultry isolates, included lane 2,3,4,6 were positive isolates, Lanes 7 to 11: human isolates, included lane 7,8,9,10 were positive isolates.

Fig (8): Agarose gel electrophoresis of *cfr* gene amplification at 746bp, Lane 1: Ladder (100bp), Lanes 2 to 6: poultry samples, included lane 2 positive, Lanes 7 to 11: human samples, included lane 9 and lane 10 positive.

**Discussion**
Poultry meat and its products are considered as major vehicles for the transmission of food borne pathogens to humans due to cross contamination events at a farm level and also at retail level (Capita et al., 2007). Moreover, a really main aspect representing a serious health risk to consumers is the antibiotic resistance of *Staphylococcus* strains isolated from poultry (Bywater et al., 2004). The infections caused by these micro-organisms cannot be treated with common antibiotics (Phillips et al., 2004). Also, *Staphylococcus* strains isolated from poultry harbor virulence genes inducing life-threatening human infections (Abdalrahman et al., 2015).

There are a different traditional diagnostic methods for identification of *Staphylococcus* spp. such as growing on mannitol salt agar, coagulase and acetoin production (Kateete et al., 2010), in this study *Staphylococcus* species recovered from 35% and 45% in poultry and human samples respectively on mannitol salt agar (Table 2). Higher percentage of *Staphylococcus* spp. in poultry farms (52.5%) were observed by Onaolapo et al. (2017) lower incidence (10.8%) were recorded by Marek et al.(2016). Ghias et al.(2016) isolated *Staphylococcus* with high rate (55%) from pus samples of patients skin.

Coagulase test divided the poultry and human isolates into 31.4% and 44.4% coagulase positive and 68.6% and 55.6% were coagulase negative respectively. Higher results were observed by Islam et al. (2014) and Ghias et al. (2016). Oxacillin Resistance Screening Agar Bas (ORSAB) improved the recovery of methicillin-resistant *Staphylococci* in 37.14% and 42.22% from staphylococcal isolates from poultry and human respectively (Table 2). In comparison to our result, Simor et al.(2001) recorded a higher predictive value for isolation of MRSA from different clinical samples (76%). The elevation in ratio of contamination by pathogenic species of *Staphylococcus* might have resulted to the hatchery, farm surroundings and by tools used in the farms. It has also been recorded that the isolation of *Staphylococci* in poultry and its products are often connected to low hygienic methods during slaughtering, cutting, storage and shopping (Karmi, 2013). The phenotypic differentiation of *Staphylococcus* spp. is considered a complicated case due to the absence of precise biochemical markers. Nevertheless, phenotypic analyses can’t reach to complete identification for *Staphylococcus* species, also, these method are relatively time consuming and most importantly, difficult to analyze results. So, the use of automated devices such as Vitek 2 system has become routine in human and veterinary microbiology fields to overcome the traditional methods problems (Sasaki et al., 2010).

In the current study, by using VITEK 2 system, poultry isolates could be differentiated into 17 staphylococcal species, the highly identified coagulase positive specie was *Staph.aureus* (14.2%) and coagulase negative specie was *Staph.lentus* (8.5%) (Table 3), this result in accordance with Wieliczko et al.(2002) who found that the most frequently coagulase positive specie was *Staph.aureus* and among coagulase-negative species were *Staph.lentus*, *Staph.xylosus* and *Staph.cohnii*. On the other hand, Marek et al.(2016) detected the most isolated species were *Staph.cohnii* (23.50%), *Staph.aureus* (15.89%) and *Staph.lentus* (13.90%).

Human isolates could be differentiated into 19 staphylococcal species, the highly identified species were *Staph.aureus* 14 isolates (31.1%) followed by *Staph.haemolyticus* and *Staph.cohnii*...
isolates for each species (6.7% for each) by VITEK 2 system (Table 3). Higher results were obtained by Abd El-Tawab et al. (2017) who isolated Staph. aureus from human samples with percentage 67.5%. On the other hand, Delmas et al. (2008) detected the most frequently occurring species were Staph. epidermidis (20%) followed by Staph. saprophyticus and Staph. warneri (10% for each). The difference in percentage of Staphylococcus isolation may be due to different reasons consist of (human and animal sources), geographical situation, numbers of samples and a variation routine of isolation (Fagundes and Oliveira, 2004).

The data recorded in table (4 and 5) demonstrated that the isolates of poultry and human showed multidrug resistance (resistant to ≥ 3 class of antibiotics) high percentages of resistance were observed to tetracycline, clindamycin, penicillin and gentamycin. The high most resistant specie was Staph. aureus, it showed a resistance for 13 types of antimicrobials, this result reinforced by Onaolapo et al. (2017) who used different biochemical parameters such as disk diffusion, microgen staph kit and other tests and found their isolates resisted more than 3 family of antibiotics. Nearly related results of antimicrobial susceptibility have been recorded by Leonard and Markey (2008); Otalu et al. (2011); Pesavento et al. (2007) and Waters et al. (2011).

Vitek2 system detected methicillin-resistant Staphylococci in 45.7% of poultry isolates and 53.3% of human isolates (Table 4&5) while ORSAB detected methicillin-resistant Staphylococci in 37.1% of poultry isolates and 42.2% of human isolates (Table 2). This result confirmed that Vitek2 system is more accurate in the detection of resistant strains of Staphylococcus than ORSAB (Sukru et al., 2018), moreover, it was more rapid in getting of the results (12hrs) than ORSAB (24hrs) (Malaviolle et al., 2008). PCR considered a gold stander in identification Staphylococcus spp., and became more essential to overcome the difficulties of conventional methods. In our results 16SrRNA gene confirmed the presence of staphylococcal DNA in our isolates (Table 6 and Fig.1). The role of this gene was reinforced by many authors (Ghebremedhin et al., 2008 and Johnson et al., 2016), also, coagulase gene (coa gene) has a title role in identification of these species (Table 6 and Fig.2), this result supported by Vintov et al. (2003) who found that coa gene can be used for research purposes to explored diversity and polymorphism of Staphylococcus, also, Bharadwaz et al. (2015) decided that coa gene was considered as a marker for identification of Staph. aureus strains and other novel species for instance Staph.intermedius, Staph.delphini, Staph.shleiferi as coagulase positive species.

Different virulence genes were harvested by Staphylococcus spp, one of the most important virulence gene was haemolysin gene (hld) which is exoproteins that are produced by Staphylococci, haemolysin is responsible for the increased dissemination and virulence of these species. In present study, hld gene detected in 2 isolates from poultry and 4 isolates from human (Table 6 and Fig.3). Abdulrahman et al. (2015) found the incidence of hla gene was (75.6%) in the 168 Staph. aureus isolates from poultry, also, Rossato et al. (2018) detected hla gene in 87.6% from 177 nosocomial MRSA strains isolated from patients.

Panton-Valentine leukocidin (pvl) was a cytotoxin gene and has a major role in the pathogenicity of this bacteria, this toxin form pores in the membrane of host defense cells, and be able to cause severe necrotic pneumonia, tissue infections furthermore to its ability to cause life threatening and associated with
community-acquired MRSA infections (Motamedi et al., 2015). Kraushaar and Fetsch (2014) elucidated *pvl* incidence among (MRSA) in retail poultry meat and slaughter employee and emphasized the impact of this animal reservoir on human healthcare. In this study, *pvl* gene was detected in one isolate from each poultry and human isolates (Table 6 and Fig.4), Tawfiq (2018) detected *pvl* gene in three isolates from fresh chicken and Jackson et al. (2013) detected *pvl* gene in one retail beef, in addition to Durand et al. (2006) and Thabit et al. (2017) detected *pvl* gene in community-acquired infection isolates.

In the current study multidrug resistance was perceived, a number of genes have been clarified for detection of antimicrobial resistance in different species of *Staphylococcus* (Table 6). Resistance to methicillin is determined by the presence of the *mecA* gene encoding PBP2a which has a very low affinity to β-lactam antibiotics (Rice, 2012). Wendland et al. (2015) reported the significance of methicillin resistant *Staphylococcus aureus* (MRSA) in poultry, as it was the most consumed protein responsible for wide spreading of MRSA among humans, that could be fatal and associated with multi-drug resistance. Our results cleared that *mecA* gene was detected in 3 out of 5 isolates from each of poultry and human samples by PCR. *Staph. aureus* was the most specie harbored this gene (Table 6and fig.5). This results supported by Ali et al. (2017) and Abdalrahman et al. (2015) who recorded a high percentage of *mecA* gene was detected in *staph aureus* isolates, also, Mulders et al. (2010) detect *mecA* gene in 26 out of 466 (5.6%) *Staph. aureus* isolates of individuals. Coelho et al. (2007) found that 12 out of 80 *Staph. aureus* isolates (15%) of human have *mecA* gene. Molecular detection became a necessary tool because methicillin resistance is often heterogeneously expressed in vitro and provides consistent results because the protocol is basically standardized and has progressed as an proficient tool for epidemiological investigations (Strommenger et al., 2006), these result supported our results which cleared that PCR detected the *mecA* gene in isolate that not detected as methicillin-resistant *Staphylococci* by in the vitek2. Also, these results mean that *macA* gene was present but can’t express about itself (Hoopes, 2008). However, Shan et al. (2016) asserted the role of Vitek in predicting by MRSA even if the accuracy rate is not perfectly reached 100%. Higher mortality, greater morbidity were recorded in patients infected with methicillin-resistant *Staphylococci*, they utilize more healthcare resources compared with those who have infections caused by methicillin-susceptible *Staphylococci* (Itani, 2016). The previous data emphasized the increasing role of *Staphylococci* in poultry infections, which suggested that the safety risks associated with their occurrence in the food consumed by humans, induced hospital infections with a high mortality rate (De Silva et al., 2002; Piette and Verschraegen, 2009).

Vancomycin resistant *Staphylococcus aureus* (VRSA) were a protuberant pathogens that cause a wide range of infections in different hosts (Grundmann et al., 2010). These strains carry the *vanA* gene that responsible for depressing the cell wall affinity for Vancomycin (Sibbald et al., 2006). By using PCR assay, we detected *vanA* gene in 3 out of 5 isolates from each of poultry and human samples (Table 6and fig.6). Martins et al. (2013) detected *vanA* gene in 3 samples out of 15 of chilled industrialized uncooked chicken parts and Okolie et al. (2015) found *vanA* in 22 isolates out of 155 from chicken carcasses. On other hand, Saadat et al. (2014) detected *van A* in 34% of clinical isolates in hospital, while Khudaier(2018) detected *van A* in 4 human isolates out of 163 (2.5%). Taponen
and Pyörälä (2009) reported that the most common mechanism of *Staphylococcus* resistance to antibiotics is the production of β-lactamase due to the presence of BlaZ gene that coded for an alteration of penicillin-binding protein 2a which reduced the affinity for β-lactam antibiotics. The Clinical and Laboratory Standards Institute (CLSI) recommending the detection of BlaZ gene specially in infected cases needing penicillin treatment (Testing and Testing, 2016).

Results obtained in this study indicate the presence of BlaZ gene in different staphylococcal spp. obtained from poultry and human samples (Table 6 and Fig. 7), this results related to Ferreira et al. (2017); Mkize (2016); Pyzik et al. (2019) and Whichard et al. (2007). Other antimicrobial resistant gene was *cfr* gene was detected in different *Staphylococcus* spp. in our isolates (Table 6 and Fig 8). This gene encodes an rRNA methyltransferase which modifies the adenine at position 2503 in 23SrRNA (Kehrenberget al., 2005). It confers a resistance not only to linezolid, but also to phenicols, lincosamides, pleuromutilins and streptogramin A antibiotics (Long et al., 2006).

It is worth to record that surprisingly a high percentage of strains were resisted to several types of antibiotics used in this study. From a clinical view this is a vital observation, as resistant bacteria can transmit genes coding for antibiotic resistance to other bacteria by transduction, conjugation or transformation. This may lead to a spreading antibiotic resistance rapidly in the *Staphylococcus* population. Moreover, the role of poultry as an important source of infection by antibiotic-resistant *Staphylococcus* strains to humans which receiving a high attention (Marek et al., 2016). These information can be used to inform public health official to enforce judicious use of antimicrobial agents in human and veterinary medicine (Cummings et al., 2013).

**Conclusion**

Finally we concluded that the unsanitary measured make poultry meat consider as a good vehicle for different species of *Staphylococcus* specially MRSA and VRSA strains which causes a lot of problems for consumers including life-threatening human infections. As, *Staphylococcus species* isolated from poultry carried virulence genes which played a role in hosts infections, and they also harbored resistance genes toward different antimicrobial agents. Thus, can be transmitted from poultry to humans by various modes the main of which was food chain.

It is for this reasons amongst many other reasons, most researchers have interested studying the antimicrobial resistance. So we recommended decreasing the unwarranted use of antimicrobials in poultry production and educate people about the use of antibiotics, hygiene when preparing food and dangers of eating half cooked meat.

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**Conflict of interest**

The authors declare that they have no conflict of interest.

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