**Molecular Detection of Integron in *Staphylococcus aureus* Isolated From Ruminants.**

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**ABSTRACT**

*Staphylococcus aureu*s is one of the most important pathogens of humans and animals, asymptomatically colonizes the anterior nares of humans and animals and it is considered one of the most significant etiological agents of intramammary infection in dairy ruminants, causing both clinical and subclinical mastitis, causing wound infection and dermatitis.

A total number of 164 samples (mastitic milk, nasal swabs, wound swabs, abscesses′ contents) were collected from ruminant animals (cattle, sheep and goat) either individually or from farms in Alexandria governorate and subjected to bacteriological examination.

Seventeen *Staphylococcus aureus* isolates were identified morphologically, biochemically. Antibiogram profile of the isolates were carried out against 11 antimicrobials. 16/17 (94%) of isolates showed resistance to at least one antimicrobial, 11/17(64.7) of the isolates showed resistance to three or more of antimicrobials (multi-drug resistance), the highest sensitivity was observed for vancomycin and amoxicillin/clavulanic acid 17/17 (100%) while the highest resistance was observed for penicillin 14/17 (82.4%), and gentamicin 9/17(53%). Eight out of seventeen isolates (47%) showed resistance to oxacillin/cefoxitin (methicillin resistant *S.* *aureus*) all of them were multi drug resistant (MDR).

Ten of MDR *S.* *aureus* isolates were molecularly screened for integron. Class 1 integron cassette were detected in 1/10 of tested isolates which exposed to further sequence analysis revealing *dfr*A15 gene cassette which encodes trimethoprim resistance.

**Conclusion:** this study report, for the first time, the detection and identification of class 1 integron containing *dfr*A15 gene in *S. aureus* isolates which were deposited in the GenBank as *Staphylococcus aureus* strain AR2020 dihydrofolate reductase 15 (*dfrA*15) gene with accession number MW036489.

**Key words:** *Staphylococcus aureus*, Ruminants, Multi drug resistance, Integron, gene cassette.

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**1. INTRODUCTION**

*Staphylococcus aureus* is involved in a wide variety of diseases in humans and animals, and it is considered one of the most significant etiological agents of intra-mammary infection in dairy ruminants, causing both clinical and subclinical infections (**Carfora et al.2016**). *S. aureus* infection in cattle causing (mastitis, udder impetigo), in sheep causing (mastitis, tick pyaemia in lambs, benign folliculitis in lambs and dermatitis), in goats causing (mastitis, dermatitis), in poultry causing (arthritis and septicemia in turkeys, bumble foot, Omphalitis in chicks) (**Quinn et al 2011**). *S.* aureus can be found in healthy cows (carriers) on the teat skin, nasal cavity, and rectum **(Roberson et al.1994).** The emergence of antimicrobial resistance among *S. aureus* has been suggested to cause delay in antibiotic treatment of bovine mastitis. This resistance against wide range of antimicrobial classes may be attributed to the indiscriminate use of these agents in the treatment of bovine mastitis (**Oliver and Murinda 2012**). Misuse of antibiotics in treating bacterial infections has led to the selection of resistant strains, and unfortunately, the risk of transfer of the resistance gene to sensitive bacteria is growing **(Xu et al.,2011).** Integrons are genetic sets capable of integrating mobile genetic elements called gene cassettes and displacing them. Since integrons contain a promoter, they can express genes existing in a gene cassette. Therefore, integrons act both as gene expression vectors and as a natural cloning system **(Mazel, 2006)**. Although the role of class 1 integrons in the spread of antibiotic resistance genes among Gram-negative bacteria is clear, little was known about the prevalence of class 1 integrons in Gram positive bacteria, especially in *S. aureus* (**Clark et al.,** **1999**). Class 1 integron/cassettes may contribute significantly to the horizontal transfer of antimicrobial resistance genes among different bacterial species from different sources or geographical locations (**Partridge et al., 2009**).

The present study aimed to carry out molecular detection of integron in *S. aureus* isolated from ruminants and to achieve this follow the following:

* Investigate *S. aureus* from different lesions of ruminants (cattle, sheep and goat).
* Screening of the *S. aureus* isolates from different sources for antibiotic resistance and Molecular detection of integron (gene cassette) from the MDR *S. aureus* isolates.

**2. MATERIAL AND METHODS**

**2.1. Collection of samples:**

A total of 164 ruminant animals (59cattle, 83 sheep and 22 goats) were examined. The samples represented as milk samples from cows, ewes and goats suffering from clinical mastitis, nasal swabs from apparently healthy cattle, sheep and goats, wound swabs and pus samples were collected under aseptic conditions and transported to Animal Health Research Institute, Alex provincial lab in an ice box then immediately processed for *S. aureus* isolation.(table, 1).

**Table (1):**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample | Cattle | Sheep | Goat | Total |
| Mastitic milk | 31 | 17 | 5 | 53 |
| Nasal swabs | 15 | 35 | 8 | 58 |
| Abscess content(pus) | 1 | 11 | 5 | 17 |
| Wound swabs | 12 | 20 | 4 | 36 |
| Total | 59 | 83 | 22 | 164 |

**2.2. Isolation of *S. aureus***

The nasal swabs, wounds swabs and pus samples were inoculated in to 5ml of Trypticase soya broth (TSB) and incubated at 370 C for 24hrs for enrichment. Enriched samples were streaked on Mannitol salt agar (Hi-Media, Mumbai) plates and incubated at 370C for 24hrs. Plates with yellow color colonies were selected and identified **(Kateete et al., 2011)**

A loopful of milk sample was streaked on Baird - Parker agar (Hi-Media, Mumbai), incubated at 370C for 24 - 48 hrs. (**Quinn et al., 1994)**.

**2.3. Identification of *S. aureus* isolates*:***

Suspected colonies were subcultured on 5% sheep blood agar and incubated at 37ᵒc for 24-48 hrs., then examined for gross colony morphology, hemolytic activity, microscopical identification of Gram stained film and biochemical characterization using catalase, coagulase test (slide, tube) according to **Quinn et al. (2002)**

**2.4. Antibiogram profile of *S. aureus:***

Antimicrobial susceptibility test was performed according to Kirby-Bauer disc diffusion method (**Bauer et al., 1966**). Using Mueller-Hinton agar (HiMedia Laboratories,Mumbai, India) against 11 different antimicrobial disks (HiMedia Laboratories, Mumbai, India): Oxacillin (OX) 5µg, Cefoxitin (CX) 30 µg, Penicillin-G (P) 10 µg ,Amoxicillin/clavulanic acid(AMC) 30µg ,Tetracyclin(TE) 30µg, Cefotaxime (CTX) 30 µg, Ciprofloxacin (CIP) 5 µg, Amikacin(AK) 30µg, Erythromycin (E) 15µg ,Vancomycin (VA) 5 µg and Gentamicin (CN) 10µg. The inhibitory zone diameters were measured and interpreted according to Clinical and Laboratory Standards Institute (**CLSI, 2007**).

**2.5. PCR and sequencing of integron in *S. aureus* isolates:**

A total number of 10 morphologically and biochemically identified *S. aureus* isolates were screened by PCR for the presence of integron using *hep*35&*hep*36 primers coding for integrase gene and the gene cassettes within class 1 integrons were ampliﬁed using 5'-CS and 3'-CS primer pairs. Chromosomal DNA was extracted using QIAamp DNA Mini Kit Catalogue no.51304. Oligonucleotide primers procured from Metabion (Germany), their sequences, amplicon sizes and cycling conditions for each target gene were listed in Table (2). PCR assay was carried out and amplified PCR products were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) according to **Sambrook et al. (1989)** and **WHO (2000)**. The PCR products were then sequenced and identiﬁed using the basic local alignment search tool (BLAST) program for gene cassette screening strategy.

**Table (2): Oligonucleotide primers sequences (Metabion,Germany)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Reference** | **Length of aplified product** | **Primer sequence**  **(5'-3')** | **Target gene** |
| **White *et al*. 2000** | 491 bp | TGCGGGTYAARGATBTKGATTT | ***Integron***  ***(hep 35 and hep 36 primers)*** |
| CARCACATGCGTRTARAT |
| **Sow *et al*., 2007** | Variable | GGC ATC CAA GCA GCA AG | **class 1 integron cassettes(5'*-CS* and3'-*CS)*** |
| AAG CAG ACT TGA CCT GA |

**2.6. Computer analysis of the sequence data**

The data of DNA sequenced data of amplified gene was identified by the database of Genbank using the BLAST program available at the NCBI BLAST homepage (Http:// ww.ncbi.nlm.nih.gov/BLAST/).

**3. RESULTS**

**Table (3): *S. aureus* isolated from examined samples**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Animal** | **Cattle** | | **Sheep** | | **Goat** | | **Total** | |
| Type of sample | No. of samples | +ve | No. of samples | +ve | No. of samples | +ve | No. of samples | +ve |
| Mastitic milk | 31 | 4 | 17 | 1 | 5 | 0 | 53 | 5 |
| Nasal swabs | 15 | 2 | 35 | 3 | 8 | 1 | 58 | 6 |
| Abscess content(pus) | 1 | 0 | 11 | 1 | 5 | 0 | 17 | 1 |
| Wound swabs | 12 | 1 | 20 | 3 | 4 | 1 | 36 | 5 |
| Total | 59 | 7 | 83 | 8 | 22 | 2 | 164 | 17 |

**Table (4): Antibiogram profile of *S*. *aurues* isolates.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Antimicrobial agent | Disc potency | Susceptible(**S**) | | Intermediate(**I**) | | Resistant(**R**) | |
| Penicillin-G (**P**) | 10 µg | 3 | 17.6% | 0 | 0 % | 14 | 82.4 % |
| Oxacillin (**OX**) | 5µg | 9 | 53% | 0 | 0 % | 8 | 47 % |
| Amoxicillin/clavulanic acid (**AMC**) | 30µg | 17 | 100% | 0 | 0 % | 0 | 0% |
| Vancomycin (**VA**) | 5 µg | 17 | 100% | 0 | 0 % | 0 | 0% |
| Ciprofloxacin (**CIP**) | 5 µg | 15 | 88.2% | 2 | 11.8% | 0 | 0 % |
| Cefoxitin (**CX**) | 30 µg | 9 | 53% | 0 | 0 % | 8 | 47 % |
| Cefotaxime (**CTX**) | 30 µg | 11 | 64.7% | 6 | 35.3 % | 0 | 0 % |
| Tetracyclin (**TE**) | 30µg | 9 | 53% | 0 | 0% | 8 | 47 % |
| Erythromycin (**E**) | 15µg | 9 | 53% | 2 | 12 % | 6 | 35 % |
| Amikacin (**AK**) | 30µg | 14 | 82.4% | 2 | 11.8 % | 1 | 5.9 % |
| Gentamicin (**CN**) | 10µg | 8 | 47% | 0 | 0 % | 9 | 53% |

**Table (5): Detailed antimicrobial sensitivity of different *S. aureus* isolates isolated from Ruminants**

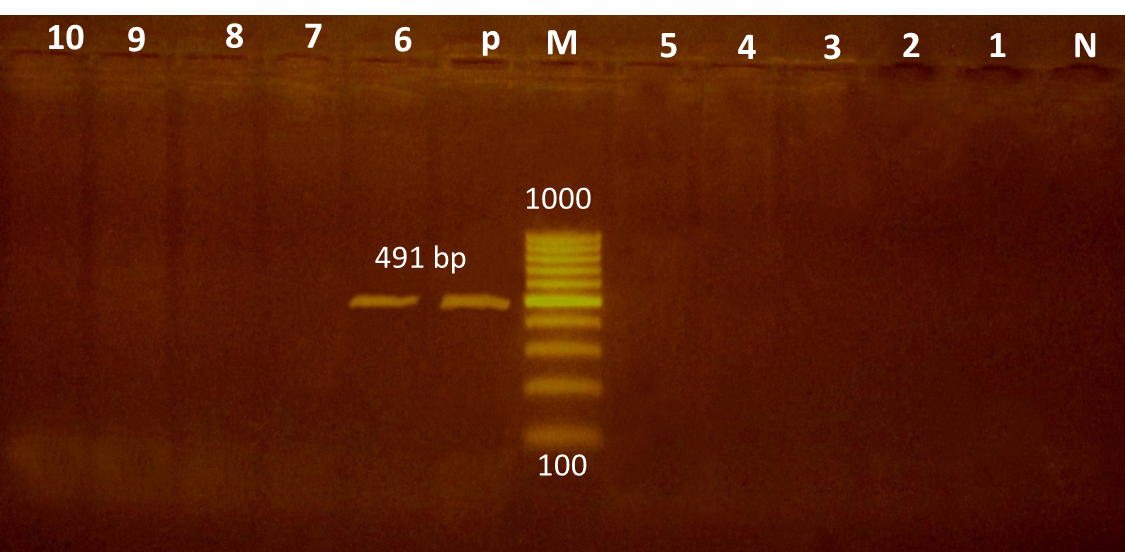
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Disc**  **Isolate** | | **P** | **OX** | **AMC** | **VA** | **CIP** | **CX** | **CTX** | **TE** | **E** | **AK** | **CN** |
| 1 | SP | R | R | S | S | S | R | I | R | R | S | R |
| 2 | GW | R | S | S | S | S | S | S | S | S | S | S |
| 3 | SW | R | R | S | S | S | R | I | R | R | S | R |
| 4 | SW | R | R | S | S | S | R | S | S | S | R | R |
| 5 | SW | R | R | S | S | S | R | I | S | R | S | R |
| 6 | CW | R | R | S | S | S | R | I | R | R | S | S |
| 7 | SN | S | S | S | S | S | S | S | S | S | I | R |
| 8 | GN | R | S | S | S | S | S | S | S | S | S | S |
| 9 | CN | R | S | S | S | S | S | I | R | S | S | R |
| 10 | SN | S | S | S | S | S | S | S | S | S | S | S |
| 11 | SN | R | R | S | S | I | R | S | R | I | I | R |
| 12 | CN | R | S | S | S | S | S | S | S | I | S | S |
| 13 | SM | R | R | S | S | I | R | S | S | S | S | R |
| 14 | CM | R | S | S | S | S | S | S | R | R | S | S |
| 15 | CM | R | S | S | S | S | S | S | R | R | S | S |
| 16 | CM | R | R | S | S | S | R | I | R | S | S | S |
| 17 | CM | S | S | S | S | S | S | S | S | S | S | R |

**Isolate ID**: **G** (Goat); **S** (Sheep); **C** (Cattle); **P** (Pus); **W** (Wound)**;** **N** (Nasal); **M** (Milk).

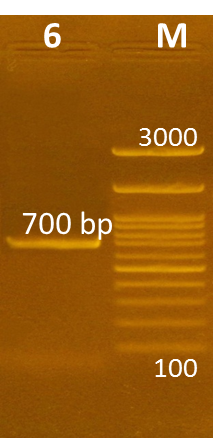
Total examined *S. aureus = 17*

**Detection of integron:**

Ten isolates were selected for detection of integron, all of the selected isolates were MDR isolates, 1/10 (10%) show presence for integron molecularly with degenerate primers designed to hybridize to conserved regions of integron encoded integrase genes intI1, intI2 and intI3 using *hep* 35 and *hep*36 gene, the isolate number (6) which was isolated from cattle wound swab from a dairy cattle farm in the region of Borg El-Arab, Alexandria governorate was (+ve), then this isolate was examined for detection of integron 1 gene cassette and contains a single gene cassette of a size About 700 bp , and by analysis of the gene cassette sequence reveals dihydrofolate reductase (*dfr*A15) which responsible for trimethoprim resistance. The GenBank accession number of the *Staphylococcus aureus* strain (AR2020) dihydrofolate reductase15 (*dfr*A15) gene sequences determined in this study is MW036489.



**Fig. (1)** Ethidium bromide stained agarose gel electrophoresis containing the PCR products along with 100bp DNA ladder (lane M). P: positive control; N: negative control which is (Nuclease free water).*Staph aureus* isolates (lanes 1:10), isolate 6 show positive amplicon at size of 491 bp.



**Fig. (2)** Amplification products of class 1 integron cassette with 100bp DNA ladder (lane M). positive integron *Staph aureus* isolate (lane 6) show positive amplicon at size of 700 bp.

**4. DISCUSSION**

*S. aureus* is a common nasal and skin colonizer of healthy humans and of different animal species. However, it is also one of the most important opportunistic pathogens responsible for a variety of infections in both humans and animals. It is frequently associated with mastitis in cattle, sheep, and goats, causing chronic infections in cows and clinical and subclinical infections in goats and sheep **(Peton et al. 2013)**. Staphylococcal organisms have a remarkable ability to become resistant to antimicrobials, as evidenced by the acquisition of drug resistance genes soon after the organisms were exposed to new antimicrobials **(Enright 2003)**. As shown in table (5) 16/17 of the isolates (94 %) show resistance to at least one anti-microbial, 11/17 of the isolates(64.7%) show multi-drug resistance. (5/6, 83.3%) of the isolates of wound swabs and pus were MRSA/MDR, the highest resistance were observed for penicillin (6/6, 100%), followed by oxacillin/cefoxitin (5/6, 83.3%), the highest susceptibility observed for amoxicillin-clavulanic acid, vancomycin and ciprofloxacin (100%) The anti-microbial resistance profile of these isolates indicated that methicillin-resistant *Staphylococcus aureus* (MRSA) is frequently involved in animal wound infections and such bacteria are resistant to more than one class of antimicrobials and this causing difficulty in treatment of infections due to the multi-drug resistance. These results are in conformity with the reports of **Okeke et al.2005** and **Kaur et al.2015.** The results showed increase of resistance in *S. aureus* isolates from wound and pus samples against penicillin(6/6, 100%), oxacillin/cefoxitin MRSA(5/6, 83.3%), erythromycin(4/6, 66.7%) and tetracycline(3/6, 50%) and this in agreement to the results obtained by **Tiwari et al. 2016** where isolated *S. aureus* showed 100% resistant to penicillin, 95.53% to methicillin and the MRSA strains showed multidrug resistance pattern.

(2/6, 33.3%) of the *S. aureus* isolates which were isolated from nasal swabs were MDR and (1/6, 16.7%) was MRSA. The highest resistance were observed for penicillin (4/6, 66.7%), followed by gentamicin (3/6, 50%), the highest susceptibility observed for amoxicillin-clavulanic acid, vancomycin (100%), followed by ciprofloxacin, cefotaxime, cefoxitin, oxacillin(5/6, 83.3%) and tetracycline, erythromycin and amikacin(4/6, 66.7%).

(1/6, 16.7%) of the *S. aureus* isolates from nasal samples was MRSA less than the results obtained by **Alzohairy 2011** who reported that 57% of *S. aureus* isolated from nasal swabsof farm animals were MRSA and more that the results obtained by **Gharsa et al. 2012** and **Mourabit et al.2020** who revealed that 7% and 0% of *S. aureus* isolates from nasal swabs of ruminants were MRSA. The antimicrobial susceptibility of *S. aureus* isolates isolated from nasal swabs of apparently healthy animals showed low resistance levels for antimicrobials and this agreed with studies of **Zhou et al. 2017** and **Mourabit et al.2020**.

Of the 5 *S. aureus* isolates isolated from mastitis milk (4/5, 80%) were MDR and (2/5, 40%) were MRSA. The highest resistance were observed for penicillin (4/5, 80%), followed by tetracycline (3/5, 60%), the highest susceptibility observed for amoxicillin-clavulanic acid, vancomycin and amikacin (5/5, 100%), followed by ciprofloxacin, cefotaxime, (4/5, 80%) and gentamicin(3/5, 60%). And this agreed with **Amal Awad et al. 2017** who reported that the examined *S. aureus* isolates which were isolated from bovine mastitis milk samples showed high resistance against penicillin (83.3%) and a lower resistance was observed against gentamicin (23.8%), amikacin (16.7%) and ciprofloxacin (14.3%) and multidrug resistances were detected in 83.3% of the isolated *S. aureus*.

Difference in the percentage of resistance could be due to geographical variation and frequency of drug usage.

The high level of MDR stains in the wounds, pus and mastitic milk samples may be attributed to the over-prescription and misuse of antimicrobials used for the treatment of bacterial infections animals in Egypt have been associated with the exacerbation of antimicrobial resistance among these pathogenic bacteria as mentioned by **Dahshan et al. 2015**.Whilethe resistance for *S. aureus* isolates from nasal sawbs was lower than the level of resistance in the *S. aureus* isolates from wounds, pus and mastitis milk samples this may be attributed to that these nasal isolates were isolated from apparently healthy animals thus not exposed to extensive treatments with antimicrobials.

As shown in table(4) the highest resistance in the 17 *S. aureus* isolates was observed for penicillin 14/17 (82%) was nearly similar to reports of **Amal Awad et al. (2017),** **Soares et al. (2017)** and **Mohammed et al. (2018)** who revealed that the resistance of *S. aureus*  isolated from bovine mastitis milk samples against penicillin was (83.3%, 83.6% and71.7%) respectively, and less than those obtained by **Thaker et al. (2013)** and **Ismail (2017)** who revealed that the resistance for penicillin among *S. aureus* isolates from milk samples was 100%, this result is not surprising because the wide prescribtion of penicillin in the veterinary field. Also the resistance to penicillin is related to the production of plasmid-encoded beta-lactamase enzyme (penicillinase) as mentioned by **Lowy (2003)** that more than 90% of staphylococcal isolates now produce penicillinase. The gene for β-lactamase is part of a transposable element located on a large plasmid, often with additional antimicrobial resistance genes (e.g., gentamicin and erythromycin).

Acquisition and spread of antibiotic resistance among staphylococcal strains is a major concern in treatment of staphylococcal infections in humans and animals. The evolution of multidrug resistance is relatively fast due to horizontal or lateral gene transfer, which is influenced by a wide range of mobile genetic elements. In particular, integrons comprise a substantial proportion of these elements and are often found in plasmids and/or transposons that enhance spread of resistance genes (**Malachowa and DeLeo, 2010).**

Only one *S. aureus* isolate from 10 examined isolates proved to be positive for integron which isolated from cattle wound swab. The obtained result was lower than that reported by **Hosseini et al. (2020)**, **Goudarzi et al. (2019)**, **Li. and Zhao (2018)** who revealed the presence of integron 1, integron 2 in Staphylococcus isolates with percentages of (39.6%,3.7%; 34.1%,14.3% and 85.1%, 0%) respectively. On the other hand, **Ammar et al. (2016)** and **Al-Ashmony et al. (2016)** revealed that none of *S. aureus* investigated in their studies harbored class 1 integrons.

The differences in the prevalence of integron genes can be due to the differing geographic regions, the bacteria strains, or the indiscriminate use of antibiotics.

The low prevalence of integrons in our study may be that it carried on other genetic elements as plasmids while we screened the integron in the genomic DNA and this agreed with **Xu et al. (2011)**

The integron positive isolate were further examined for the detection of integron 1 gene cassette and it was containing a single gene cassette of a size About 700 bp , and by analysis of the gene cassette sequence revealed dihydrofolate reductase (*dfr*A15) which responsible for trimethoprim resistance.

The GenBank accession number of the *dfr*A15gene sequences determined in this study is MW036489. dihydrofolate reductase (*dfr*A15) was not detected before in the previous studies on the gene cassettes of the integron bearing *S.* *aureus* isolates that agree with **Li. and Zhao2018** who reported presence of genes coding for resistance to trimethoprim (*dhfr*V, *dfr*A1 and *dfr*A12), aminoglycosides) *aad*A1, *aad*A5, *aad*A4, *aad*A24, *aac*A4, *aad*A2 and *aad*B), chloramphenicol (*cml*A6) and quaternary ammonium compound (*qac*H), gene and he mentioned that those gene cassette arrays have not been reported in bovine milk-associated *S. aureus* isolates previously, although all of these gene cassette arrays exist in other types of bacteria and **Xu et al.2007** who detected *aad*A2 gene and *dfr*A12-*orf*F-*aad*A2 by **Ahmed et al.2005**.

The sequence of the *dfr*A15gene detected in *S.* *aureus* isolate of the current study was compared to those deposited in GenBank, homology of nucleotides ranged up to 100%, to those detected in Gram negative bacteria as *Vibrio cholera*, *Enterobacter hormaechei*, *Vibrio alginolyticus*, *Proteus mirabilis*, *Acinetobacter* *baumannii*, *Salmonella* *enterica*, *Morganella* *morganii*, *Pseudomonas* *aeruginosa*, *klebsiella* *pneumonia*, *shigella* *dysenteriae*, *Citrobacter* *freundii*, *Aeromonas* *caviae* and *E. coli* that agreed with **Clark et al. 1999** and **Ahmed et al.2005** who reported that arrays of gene cassettes detected in staphylococci, had been previously reported in clinical isolates of various negative bacteria, with identical sequences and **Chen et al. (2011)** who reported that the transfer of resistance genes that may occur between gram-positive and gram-negative organisms could lead to the construction of diverse resistance to the usual antibiotics.

**CONCLUSION**

-The present study highlights the prevalence of MDR and Methicillin Resistant *Staphylococcus aureus* strains in ruminants in Alexandria governorate.

-To our knowledge, this is the ﬁrst study to report the occurrence of *S. aureus* isolate that carry a class 1 integron containing the *dfr*A15gene cassette.

-In the present study, class 1 integron was identified with dihydrofolate reductase15 gene cassette (*dfr*A15) which confers resistance to trimethoprim in one isolate of *S. aureus* obtained from a cattle wound swab using PCR and DNA sequencing.

**5. REFERENCES**

Abo-Shama, U. H. 2014. Prevalence and antimicrobial Susceptibility Of *Staphylococcus aureus* isolated from Cattle, Buffalo, Sheep and Goat`s raws milk in Sohag Governorate, Egypt. Assiut Vet. Med. J. 60(141)63-72.

Ahmed, A. M., Nakano, H., Shimamoto, T. 2005. Molecular characterization of integrons in non-typhoid Salmonella serovars isolated in Japan: description of an unusual class 2 integron. J. Antimicrob. Chemother. 55:371–374.

Al-Ashmony, A. L., Al-Sawy, A.F., Torky, H. A. 2016. Genotypic Molecular Detection of Certain Genes Encoding Virulence Determinates and Antibiotic Resistance in *Staphylococcus* aureus Isolates from Mastitis Cows. Alex. J. Vet .Sci. 49(2): 90-98.

Alzohairy, M. A. 2011. Colonization and antibiotic susceptibility pattern of methicillin resistance Staphylococcus aureus (MRSA) among farm animals in Saudi Arabia. J. Bacteriol. Res. 3(4) 63-68.

Amal Awad, Ramadan, H., Nasr, S. , Ateya, A., Samar Atwa, 2017. Genetic characterization, antimicrobial resistance patterns and virulence determinants of *Staphylococcus aureus* isolated form bovine mastitis. Pak. J. Biol. Sci. 20: 298-305.

Ammar, A. M., Attia, A. M., Abd El-Aziz, N. K., Abd El Hamid, M. I., El-Demerdash, A. S. 2016. Class 1 integron and associated gene cassettes mediating multiple-drug resistance in some food borne pathogens. Inter. Food Res. J. 23(1): 332-339.

Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45(4):493-496.

Carfora, V. ;Giacinti, G. ;Sagrafoli, D. ;Marri, N. ;Giangolini, G. ;Alba, P. ;Feltrin, F. ;Sorbara, L. ;Amoruso, R. ;Caprioli, A. ;Amatiste, S., Battisti, A.(2016): Methicillin-resistant and methicillin-susceptible Staphylococcus aureus in dairy sheep and in-contact humans: An intra-farm study. J. Dairy Sci. 99:4251–4258.

Chen, B., Zheng, W., Yu, Y. et al. 2011. Class 1 integrons, selected virulence genes, and antibiotic resistance in *Escherichia coli* isolates from the Minjiang River, Fujian Province, China. Appl. Environ. Microbiol. 77:148-55.

Clark, N.C., Olsvik, Ø., Swenson, J.M., Spiegel, C.A., Tenover, F.C. 1999. Detection of a streptomycin/spectinomycin adenylyltransferase gene (*aad*A) in *Enterococcus faecalis*. Antimicrob. Agents. Chemother. 43: 157-160.

CLSI. 2007. Performance standards for antimicrobial susceptibility testing; 17th informational supplement.CLSI M100-S17. CLSI, Wayne, PA.

Dahshan, H., Abd-Elall, A.M.M., Megahed, A.M., Abd-El- Kader, M.A. and Nabawy, E.E. 2015. Veterinary antibiotic resistance, residues and ecological risks in environmental samples obtained from poultry farms, Egypt. Environ. Monit. Assess. 187 (2):4218.

Enright, M.C. 2003.The evolution of a resistant pathogen: The case of MRSA. Curr. Opin. Pharmacol. 3(5):474–479.

Gharsa, H., Ben Slama, K., Lozano, C., Gomez-Sanz, E., Klibi. N., Ben Sallem, R., Go´mez, P., Zarazaga, M., Boudabous, A., Torres, C. 2012. Prevalence, antibiotic resistance, virulence traits and genetic lineages of Staphylococcus aureus in healthy sheep in Tunisia. Vet. Microbiol. 156 (2012) 367–373

Goudarzi, M. , Mohammadi, A. , Goudarzi, H., Fazeli, M., Sabzehali, F. 2019. Genetic Variability and Integron Occurrence in Methicillin Resistant *Staphylococcus aureus* Strains Recovered from Patients with Urinary Tract Infection. Arch. Pediatr. Infect. Dis. In Press(In Press):e86189.

Hosseini, S. M., Hadi, N., Bazargani, A., Emami, A., Pirbonyeh, N. 2020. The First Report of Prevalence of Class 1-3 Integrons in Clinical Isolates of *Staphylococcus aureus* in Southwestern Iran: A Multicenter Study. Jundishapur J. Microbiol. 12(11):e90902.

Ismail, Z.B. 2017. Molecular characteristics, antibiogram and prevalence of multi-drug resistant *Staphylococcus aureus* (MDRSA) isolated from milk obtained from culled dairy cows and from cows with acute clinical mastitis, Asian Pac. J. Trop. Biomed. 7 (8): 694- 697.

Kateete, D. P., Namazzi, S., Okee, M., Okeng, A., Baluku, H., Musisi, N. L., Katabazi, F. A., Joloba, M. L., Ssentongo, R., Najjuka, F. C. 2011. High prevalence of methicillin resistant *Staphylococcus aureus* in the surgical units of Mulago hospital in Kampala, Uganda. BMC. Res. Notes. 4, 326.

Li, L., Zhao, X. 2018. Characterization of the resistance class 1 integrons in *Staphylococcus aureus* isolates from milk of lactating dairy cattle in Northwestern China.BMC*.* Vet. Res. (2018) 14:59

Lowy, F. D. 2003. Antimicrobial resistance: the example of *Staphylococcus aureus*. J Clin. Invest. 111: 1265–1273.

Mazel, D. 2006. Integrons: agents of bacterial evolution. Nat. Rev. Microbiol. 4(8):608–620.

Mohammed, J., Ziwa, M.H., Hounmanou , Y.M.G., Kisanga, A., Tuntufy, H.N. 2018. Molecular Typing and Antimicrobial Susceptibility of Methicillin-Resistant *Staphylococcus aureus* Isolated from Bovine Milk in Tanzania. Inter. J. Microbiol. 2018

Mourabit, N., Arakrak , A., Bakkali , M., Zian , Z., Bakkach, J., Laglaoui, A. 2020. Nasal carriage of *Staphylococcus aureus* in farm animals and breeders in north of Morocco. BMC Infect. Dis.20,602.

Okeke, I.N., Klugman, K.P., Bhutta, Z.A., Duse, A.G., Jenkinsetal, P.2005. Antimicrobial resistance in developing countries. Part II: Strategies for containment. Lancet Infect. Dis. 5: 568-580.

Oliver, S.P., Murinda, S.E. 2012. Antimicrobial resistance of mastitis pathogens. Vet. Clin. North Am.: Food Anim. Pract. 28: 165-185.

Partridge, S.R., Tsafnat, G., Coiera, E., Iredell, J.R. 2009. Gene cassettes and cassette arrays in mobile resistance integrons. FEMS. Microbiol. Rev.33(4):757–784.

Peton, V. and Le Loir, Y. 2013. *Staphylococcus aureus* in veterinary medicine. Infect. Genet. Evol. 21:1567–1581.

Quinn, P.J., Carter, M.E., Markey, B.K., Cartey, G.E. 1994. Clinical Veterinary Microbiology. Section 2. Bacteriology, 8. Staphylococcus species. Mosby-Year Book Europe Limited, Lynton House, London, England. 118-126

Quinn, P.J., Markey, B.K., Carter. M.E., Donnelly, W.J., Leonard, F.C., Maguire, D. 2002. Veterinary Microbiology and Microbial Disease.1st Published, Oxford: Blackwell Science Ltd.

Quinn, P.J., Markey, B.K., Leonard F.C., FitzPatrick, E.S., Fanning, S., Hartigan, P.J. 2011. Veterinary Microbiology and Microbial Disease. Second edition.

Roberson, J.R., Fox, L.K., Hancock, D.D., Gay, J.M., Besser, T. E. 1994. Ecology of *Staphylococcus aureus* isolated from various sites on dairy farms. J. Dairy Sci. 77:3354–3364

Sambrook, J., Fritscgh, E.F., Mentiates 1989. Molecular coloning. A laboratory manual. Vol !., Cold spring Harbor Laboratotry press, New York.

Soares, B.S., Melo, D.A., Motta, C. C., Marques, V.F., Barreto, N.B., Coelho, S.M.O., Coelho, I.S., Souza, M.M.S. 2017. Characterization of virulence and antibiotic profile and agr typing of *Staphylococcus aureus* from milk of subclinical mastitis bovine in State of Rio de Janeiro. Arq. Bras. Med. Vet. Zootec.69(4)843-850.

Sow, A.G., Wane, A., Diallo, M.H., Boye, C.S., Aïdara-Kane, A. 2007. Genotypic characterization of antibiotic-resistant *Salmonella Enteritidis* isolates in Dakar, Senegal. J.I.DC. 1(3): 284-288.

Thaker, H. C., Brahmbhatt, M. N., Nayak, J. B. 2013. Isolation and identification of *Staphylococcus aureus* from milk and milk products and their drug resistance patterns in Anand, Gujarat. Vet. world. 2013.10-13

Tiwari, R., Yadav, S. K., Singh, S. 2016. Methicillin resistant Staphylococcus aureus isolated from wounds of livestock and companion animals of Uttar Pradesh India: A preliminary study. Int. J. Pharmacol. 12: 821-829.

White, P.A., McIver, C.J., Deng, Y., Rawlinson, W.D. 2000. Characterisation of two new gene cassettes, aadA5 and dfrA17. [FEMS. Microbiol. Lett.](https://www.ncbi.nlm.nih.gov/pubmed/10620677) 182(2):265-269.

WHO (2002): World Health organization. Department of communicable diseases surveillance and response.

Xu, Z., Li, L., Shi, L., Shirtliff, M.E. 2011. Class 1 integron in staphylococci. Mol. Biol. Rep. 38(8):5261–5279.

Xu, Z., Shi, L., Zhang, C., Zhang, L., Li, X., Cao, Y., Li, L., Yamasaki, S. 2007. Nosocomial infection caused by class 1 integron carrying *Staphylococcus* *aureus* in a hospital in South China. Clin. Microbiol. Infect. 13: 980-984.