**Molecular Characterization of MRSAIsolated from Camels and their Surrounding Environment**

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To investigate the effect of the environment in MRSA transmission to camels, this study was performed in North Coast area and Al America District (Mariot Station), Egypt on camels (*Camelus dromedarius*), comprising 30 females and 10 males aged between one and five years old. A total of 100 various samples including nasal swabs (40), milk (30), soil (15) and water (15) were collected from 40 camels to isolate methicillin-resistant *Staphylococcus aureus* using oxacillin resistance screening agar basal medium (ORSAB) beside determination of antibiogram pattern of the recovered isolates and molecular detection of some genes responsible for antibiotic resistance. It was found that the recovery rate of MRSA was 12.5, 6.67, 13.3 and 6.67% from the examined samples, respectively. Multidrug resistance was displayed by all MRSA isolates with 100% resistance to Cefoxitin and penicillin and with 80% resistance to Gentamycin, while 90% of the isolates were susceptible to Doxycycline, Linezolid and Ofloxacin and 70% to Sulfamethoxazole/Trimethoprim. Finally, MRSA strains were discovered in camels and the surrounding area, suggesting that they may contribute to the spread of the pathogen among animals and people as well as within the community. Disinfecting soil and water using a selective, effective disinfectant may be a solution to this issue.

**Key words:** MRSA, Camels, Isolation, PCR

**1. Introduction**

The world population of one humped camels is around 35 million are only found in nations in Africa and the Middle East, according to the most recent data available in 2020 **(FAOSTAT, 2020).**

It was once believed that most illnesses that usually affect animals did not affect camels. However, recent findings have demonstrated their vulnerability to a wide range of infections. Additionally, increased interaction with and use of camel meat and milk is a significant source of zoonotic disease transmission to humans **(El Harrak et al., 2011).**

The dromedary camel, commonly referred to as "the desert ship," is a versatile animal that was formerly solely used for transportation, as a beast of burden, and as a draught animal for agriculture. But as the population grows, it is transitioning to become a food animal that is utilized for milk, meat, and skins **(Kadim et al., 2008).** Camels may be an important source of MRSA for humans since they are raised in close proximity to people. There hasn't been much research done on camels, and what is known about the part that camels play in the transmission of zoonotic illnesses is scant.

*Staphylococcus* *aureus* are listed among the most important camel zoonotic diseases and infections reported globally **(Jaradat et al., 2013).** *S. aureus* is a ubiquitous bacterium that colonizes the epidermis, nares, and other mucosa of many different animal species. It is often linked to asymptomatic carriage. However, if there is a decline in immune function, it may act as an opportunistic pathogen and cause a wide range of illnesses, from sepsis and toxic shock to skin and soft tissue infections**(Iverson et al., 2015).**

*S. aureus* typically causes mastitis, skin infections (including skin abscesses and necrosis), respiratory tract diseases (pneumonia), and endometritis in ruminants and pseudo-ruminants as camels. As a result, it reduces livestock productivity and causes significant economic losses **(Monecke et al., 2014).**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a key factor in the worldwide rise in nosocomial infections, which are also among the most challenging to cure. Methicillin-resistance in staphylococci constitutes resistance to all of the β-lactam antibiotics and their derivatives **(CLSI, 2009).** The major mechanism is the acquisition of the *mec*Agene that codes for additional penicillin-binding protein 2a (PBP2a) **(Mukarami et al., 1991).**

MRSA may escape through manure and then be transported by the air as well as frequently spreads through polluted surroundings. When farm animal faeces is scattered over the ground, there is a chance that MRSA will grow and harm water supplies, crops, and other agricultural products. Animal colonization over an extended period of time is feasible without the emergence of clinical signs. The likelihood of a subsequent infection rises when *S. aureus* colonizing clones are present. Infection can lead to a wide variety of suppurative infections. The skin disorders and wound infections from which MRSA has been particularly recognized include fistulas, surgical wound infections, severe pyoderma, abscesses, and dermatitis. Also, it has been linked to various conditions such bacterial pneumonia, rhinitis, sinusitis, otitis, bacteremia, septic osteomyelitis, omphalo-phlebitis, metritis, mastitis (including gangrenous mastitis), and urinary tract infections. Additionally, it is possible to consider cattle as a reservoir for a human MRSA strain that is on the rise **(Alp and Damani, 2015).**

Good hygiene is a key general prevention and control measure in homes, hospitals, and animal healthcare facilities since MRSA-contaminated surroundings act as a reservoir for infection. To prevent the transmission of MRSA, people and animals should both adhere to a responsible antibiotic treatment plan. Animals with proven MRSA infections should be cared for separately from other animals.

Therefore, the purpose of this study was to evaluate the presence of Methicillin-Resistant *S. aureus* and its antimicrobial resistance in camels and the surrounding environment in North Coast, Matrouh Province, Egypt using culture-based phenotypic and molecular approaches.

**2. Material and Methods:**

This study was performed in North Coast area and Al America District (Mariot Station), Egypt on camels (*Camelus dromedarius*), comprising 30 females and 10 males aged between one and five years old during 2022.

**2.1. Samples:**

A total of 100 various samples including nasal swabs (40), milk (30), soil (15) and water (15) were collected from 40 camels to isolate methicillin-resistant *Staphylococcus aureus* using oxacillin resistance screening agar basal medium (ORSAB).

Nasal swabs were collected from secured camels where swab was rotated four times in each anterior opening then placed in a sterile test tube with nutrient broth covered with sterile cotton. Before milking, udders were cleansed, dried, and each teat end was scrubbed with a piece of cotton dampened with betadine. Milk samples were then taken. The initial milk streams were thrown away. The samples were brought to the lab after being collected and put in an icebox. 20 ml of each milk sample were centrifuged at 10.000 x g for one minute in a sterile test tube. The sediments were collected, and 5 ml of nutritional broth was then added. Finally, they were simply incubated for 12 hours at 37°C **(Schalm et al., 1971).**

According to **Clegg et al., (1983),** a total of 15 soil samples were taken from yards, specifically from damp areas and organic matter loads at a depth of 5 cm. One gram was weighed, triturated thoroughly with 99 ml of sterile BPW in the laboratory, aseptically filtered through sterile gauze, and the filtrate was cultured for 24 hours at 37 °C for bacteriological analysis.

By using 20 ml sterile plastic syringes, 15 water samples were taken. Each sample was marked with its source, location, kind of watering system, and sampling date **(APHA, 1998).**

**2.2. Isolation and identification of Methicillin Resistant *Staphylococcus aureus* (MRSA):**

Prepared samples were inserted into nutrient broth tubes were incubated for 6 hours then they were inoculated and streaked on Oxacillin Resistance Screening Agar Base **(ORSAB**) (a selective medium for detection of MRSA) (**Oxoid Basingstoke, UK).** The ORSAB plates were incubated at 37 °C for 24-48 hours and then observed for growth of MRSA that appeared as intense blue glistering small round colonies. Isolates were identified as Gram positive non-motile cocci arranged in cluster and they were coagulase and catalase positive.

**2.3. Molecular confirmation of MRSA by PCR assay:**

DNA extraction was performed by boiling method **(Sambrook et al., 1989)** after subculturing the presumptive MRSA isolates on nutrient agar plates. The extracted DNA was amplified using species-specific primer targeting *mec*A and *mec*C genes.

**Table (1): Oligonucleotide primers sequences of MRSA**

|  |  |  |  |
| --- | --- | --- | --- |
| **Target gene** | **Nucleotide sequence (5ꞌto 3ꞌ)** | **Amplicon Size (bp)** | **Reference** |
| ***mec*A** | GTTGTAGTTGTCGGGTTTGGCTTCCACAT ACCATCTTCTTTAAC | 350 | **Wielders et al., (2002)** |
| ***mec*C** | GAAAAAAAGGCTTAGAACGCCTCGAAGATCTTTTCCGTTTTCAGC | 138 | **Stegger et al., (2012)** |

The uniplex PCR reaction mixture, with a total of 25µl, consisted of 12.5 µl of 2X TOP simple TM DyeMIX-nTaq. 1 µl of each primer of 10 µM working concentration (Europhins Scientific laboratories, Japan), 5.5 µl of nuclease-free water and finally 5 µl of DNA template. The reaction mixture was moved to the thermal cycler and cycled once at 94°C for 2 min, followed by 35 cycles at 98 °C for 10 s, 58 °C for 30 s, and 68 °C for 1 min, and then a final extension once at 68 °C for 7 min. PCR products (Five µL) were analyzed by electrophoresis in 1x TBE electrophoresis buffer and on 1.5% (w/v) agarose gel at 100 V for 60 min. A gene ruler 100 bp ladder was utilized to conclude the sizes of DNA products.

**2.4. Phenotypic assessment of antimicrobial resistance pattern:**

The Kirby-Bauer disc diffusion method was used to assess the MRSA isolates' susceptibility to antimicrobials on Muller Hinton agar (Oxoid, Hampshire, UK). Eight antimicrobial agents (Oxoid, Hampshire, UK) were used including Penicillin G (10 U), Cefoxitin (30 µg), Linezolid (30 µg), Ofloxacin (5 µg), Azithromycin (15 µg), Gentamycin (10 µg), Doxycycline (30 µg) and Trimethoprim-Sulfamethoxazole (1.25/23.75 µg). The inhibition zones' diameters around each antibiotic disc tested, sensitivity and resistance categorization were estimated as described in Clinical and Laboratory standard institute **(CLSI, 2018).**

**2.5 Statistical analysis:**

ANOVA statistical analysis was carried out using SAS software **(SAS, 2014).**

**3. Results**

**Table (2):** Recovery rate of MRSA from the investigated samples on ORSAB

|  |  |  |  |
| --- | --- | --- | --- |
| **Samples** | **No. of samples** | **Positive** | |
| **No.** | % |
| **Nasal swabs** | 40 | 5 | 12.5 |
| **Milk** | 30 | 2 | 6.67 |
| **Soil** | 15 | 2 | 13.3 |
| **Water** | 15 | 1 | 6.67 |
| **Total** | **100** | 10 | **10.0** |
| Chi2 = 7.28\*\* \*\* Significant at (P < 0.01)  Positive MRSA samples differ significantly among sources of samples | | | |

**Table (3):** Antimicrobial sensitivity testing of MRSAisolates (n= 10) obtained from the investigated samples

|  |  |  |
| --- | --- | --- |
| **Antimicrobial agents** | **Sensitive isolates** | |
| **No.** | **%** |
| **Azithromycin** | 3 | 30.0 |
| **Cefoxitin** | 0 | 0.00 |
| **Doxycycline** | 9 | 90.0 |
| **Gentamycin** | 2 | 20.0 |
| **Linezolid** | 9 | 90.0 |
| **Ofloxacin** | 9 | 90.0 |
| **Penicillin G** | 0 | 00.0 |
| **Sulfamethoxazole/Trimethoprim** | 7 | 70.0 |
| Chi2 = 20.16\*\* \*\* Significant at (P < 0.01) | | |

**Table (4):** Percentage of some resistance genes in *MRSA* isolates

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Samples** | **No. of tested MRSA isolates** | ***mec*A** | | ***mec*C** | |
| **No.** | **%** | **No.** | **%** |
| **Nasal swabs** | 5 | 5 | 50.0 | 4 | 40.0 |
| **Milk** | 2 | 2 | 20.0 | 1 | 10.0 |
| **Soil** | 2 | 2 | 20.0 | 1 | 10.0 |
| **Water** | 1 | 1 | 10.0 | 0 | 00.0 |
| **Total** | 10 | 10 | 100.0 | 6 | 60.0 |

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| **Fig. (1):** Agarose gel electrophoresis for PCR product of *mec*Aand *mec*C genes for characterization of MRSA strainsfrom the examined isolates. The amplified amplicon size was of 350 and 138 bp and for *mec*Aand *mec*C genes*,* respectively.  **Lane M:** 100 bp DNA markers.  **Lane C+:** Control positive.  **Lane C-:** Control negative.  **Lanes 1 to 10:** MRSA isolates. |

**4. Discussion:**

The pathogenicity of *Staphylococcus aureus*, which causes a wide range of illnesses in both people and animals, is mostly attributed to a confluence of genetic traits that mediate virulence, invasiveness, immune evasion, and drug resistance**(Chua et al., 2014).**

The rise of methicillin-resistant *Staphylococcus aureus* linked with livestock has been documented more often in recent years, raising concerns about the hazards of zoonotic transmission, particularly for those who work around animals **(Guardabassi et al., 2013),** but also because of the potential for these strains to enter the public through the food supply **(Kluytmans, 2010).**

As presented in **Table (2)**, the overall prevalence of MRSAin the current work was 10% where the highest recovery rate was recorded in soil samples (13.3%, 2 isolates) followed by nasal swabs (12.5%, 5 isolates) then milk and water samples (6.67% for each). **Alzohairy (2011)** claimed a greater frequency of camels in Saudi Arabia of 35.5%. This study's MRSA recovery rate from camel nasal swabs was also lower than that of **Al-Doughaym et al. (1999),** who reported a 34.1% MRSA recovery rate from nasal swabs from pneumonic camel lungs. However, it was higher than reported by **Yusuf et al. (2017),** who found that the prevalence of MRSA IN pneumonic camels in Nigeria was only 5%.

On the other hand, Staphylococcus spp. were discovered in 152 of the 159 camels analysed (95.6%), and a total of 258 swabs (81%) were positive, according to Ben Chehida et al.'s (2021) analysis of 318 nose and rectal swabs taken from 159 seemed healthy camels from Tunisia. 16 of these isolates (6.2%) were coagulase-positive Staphylococcus (CoPS) and were identified as S. aureus by biochemical and molecular testing. These were isolated from 14 camels (8.8%) and co-transmitted by two camels in the nasal and rectal mucosa. Spa typing and PFGE were used to characterize all S. aureus isolates that were found to be methicillin-susceptible Staphylococcus aureus (MSSA).

Multidrug resistance was displayed in **Table (3)**. It was observed that all MRSA isolates with 100% resistance to Cefoxitin and penicillin and with 80% resistance to Gentamycin, while 90% of the isolates were susceptible to Doxycycline, Linezolid and Ofloxacin and 70% to Sulfamethoxazole/Trimethoprim. This result was consistent with the findings of **Tahnkiwale et al. (2002),** who noted a significant incidence of penicillin and oxacillin resistance by MRSA bacteria obtained from cattle, followed by erythromycin, Co-trimoxazole, gentamicin, and cephalothin resistance. Amikacin, gentamicin, and ciprofloxacin were all extremely effective against the majority of the isolates. MRSA isolates also exhibited high resistance to the antibiotics vancomycin (73.3%) and oxacillin (80%), which is consistent with a report by **Al-Doughaym et al. (1999)** that found isolates to be 100% resistant to penicillin, 93.33% resistant to ampicillin, 53.34% resistant to vancomycin, and 40% resistant to oxacillin. Similar conclusions were reached by **Kataria (2008),** who discovered that every isolate of clinical cow mastitis was penicillin-resistant.

In the study by **Onanuga et al. (2006)** 70% of the MRSA isolates were susceptible to ofloxacin, ciprofloxacin, sparfloxacin and gentamicin and resistant to ampicillin, cephalexin and clindamycin. **Alzohairy (2011)** has reported the highest rate of multidrug resistant MRSA from camels (41.1%) than other animals.

All of the isolates' penicillin resistance is consistent with Staphylococcus's well-documented natural resistance to -lactam antibiotics. Because penicillin resistance may be plasmid-borne in some cases and spread swiftly to many additional strains, by the 1980s over 90% of *S. aureus* had developed penicillin resistance.

The fact that MRSA showed such a high level of antibiotic resistance patterns in this study is really worrying. Despite the fact that it has frequently only been isolated from humans, MRSA has recently been discovered in a variety of different animal species, suggesting that animals may act as important reservoirs for the infection **(Van Duijkeren et al., 2004).**

The majority of isolates' in vitro sensitivity to Doxycycline, Linezolid, and Ofloxacin (90%) is suggestive of the medications' potential effectiveness in treating MRSA infections, and it may also be an indication that these antibiotics were not overused or misused in the research setting.

Methicillin-resistant *S. aureus* (MRSA) includes *S. aureus* that has acquired a gene, called *mec*A, giving them resistance to methicillin and essentially to all other β-lactam antibiotics. Molecular MRSA identification was accomplished by PCR detection of *mec*A gene.

PCR has shown to be a potent research tool, and clinical microbiology laboratories are increasingly using it for the sensitive and precise identification of bacteria and antibiotic resistance genes.

The *mec*Agene is carried within a mobile genetic element called the *staphylococcal* cassette chromosome *mec* (SCCmec). SCCmec elements are highly diverse in their structural organization and genetic content, and have been classified into types and subtypes from type I to type XI **(García et al., 2013).**

It was found that all recovered MRSA isolates throughout the current work were positive for *mec*Adetection by PCR while only 6 isolates was found to carry *mec*Cgene **(Fig., 1).**

There are no accessible statistics on the clinical significance of *mec*C positive MRSA in Egypt. Due to the lack of *mec*A in resistant isolates, screening for *mec*C should be part of monitoring for MRSA identification. Additionally, a number of studies have shown that *mec*C-positive MRSA is highly common in animals, indicating that they may serve as an infection reservoir and that farmers who come into contact with animals may be at risk of acquiring these isolates (**Abdulgader et al., 2015).**

The discovery of MRSA in camels by this study raises public health concerns since camels have become a frequent source of food in various regions of the country due to the widespread use of camel milk and meat. Equally significant is the possibility that the spread of MRSA in low-income areas will have a significant impact on the price of antibiotic therapy and the unsuccessful management of severe *S. aureus* illnesses.

**5. Conclusion:**

It is probable that the MRSA recovered from camels in this study came from people who are frequently in touch with them while grazing, feeding, and other activities given that human S. aureus isolates have been discovered to have a high percentage of methicillin resistance. Due to the staphylococci ability to change their pattern of resistance over time, MRSA may continue to be a problem in the future.

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