# ZOO ANIMALS

# Meticillin-resistant *Staphylococcus aureus* (MRSA) associated dolphin mortality and the subsequent facility decolonisation protocol

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#### SUMMARY

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This case reports the presence of meticillin-resistant Staphylococcus aureus (MRSA) in a colony of cetaceans maintained under human care. MRSA isolates of the same strain were cultured from multiple organs of two dolphins that died with septicaemia. Following these mortalities and in consideration of the zoonotic potential of this pathogen, a decolonisation protocol was developed and applied to reduce the risk of exposure to humans and animals. After monitoring for MRSA presence in the animals, environment and staff, a strict sanitation protocol was applied for 15 months, with the aim of controlling MRSA. This protocol reduced the incidence of this pathogen and its involvement in acute clinical cases. The transmission between cetaceans and the implication of human reservoirs are discussed as important issues for veterinarians, facility managers and public health officials.

#### BACKGROUND

Staphylococcus aureus is a significant pathogen causing a wide range of lesions and diseases in humans and animals, from mild skin infections to life-threatening bacteraemia.<sup>1</sup> Its pathogenicity is enhanced when resistance develops to antibiotics such as meticillin: currently this resistance is reported worldwide in bacteria from a large diversity of hosts other than humans, including food and companion animals, and in water samples<sup>27, 29</sup>.<sup>2-5</sup> Despite this, there are few reports of meticillin-resistant Staphylococcus aureus (MRSA) isolation in wildlife in Europe (Monecke and others 2014) and it is even more rarely described in the marine environment. MRSA has been detected during health assessments of free-ranging coastal bottlenose dolphins (Tursiops truncatus) from the Southeastern United States,<sup>6-8</sup> as well as from mass stranded shortfinned pilot whales (Globicephala macrorhynchus) in Florida.9 MRSA was also isolated from the blowhole of a captive bottlenose dolphin at necropsy.<sup>10</sup> None of these isolates from cetaceans were associated with disease, yet the potential zoonotic implication of this pathogen is of concern. This concern has been raised previously when MRSA was isolated from a seal in a marine mammal rehabilitation facility in the USA.<sup>11</sup> Concern is due to increasing contact between humans and dolphins, as well as implications for waterborne sources increasing spread of this important zoonotic organism. This report describes the presence of MRSA in a colony

of cetaceans maintained under human care, and the subsequent decolonisation procedure to reduce risk to humans and animals, both important issues for veterinarians, facility managers and public health officials to be aware of  $^{28}$ .

#### CASE PRESENTATION

This case involved a colony of 10 cetaceans that in 2009 were separated into two groups housed in independent facilities in Italy. Group A was composed of four females and one male bottlenose dolphin (T truncatus) and a female Risso's dolphin (Grampus griseus); group B consisted of four male bottlenose dolphins. In both facilities, dolphins were maintained in outdoor concrete pools with filtered water disinfected using sodium hypochlorite. Both facilities also housed other terrestrial mammals and livestock. Animal management procedures were identical at the two facilities complying with the current Italian zoo licensing regulations (D. Lgs 73/2005 and D.M. 469/2001). Regular health evaluations were performed through blood analyses, blow cytology and microbiology, and gastroenteric swabs (first gastric chamber and rectum) on animals under behavioural training. In May 2012, two individuals originating from group A (a nine-day-old bottlenose dolphin calf and the Risso's dolphin) died with bacterial systemic disease (endotoxic shock), and MRSA was isolated from both cases. The Risso's dolphin suffered from chronic debilitating conditions and died in septicaemic shock with severe neutrophilic leucocytosis, moderate hypovolaemia and severe hyposideraemia. The day before death the animal was lethargic with abnormal respiratory rate. Necropsy revealed Gram-positive bacterial embolism in several organs (lung, liver and brain) associated with acute purulent inflammatory changes. The bottlenose dolphin calf died after showing a mild increased respiratory rate for approximately 20 hours. Necropsy showed evidence of acute enteric inflammatory changes and haemorrhagic petechiae on the thoracic and abdominal serosae. Megakaryocytic pulmonary emboli support the diagnosis of an endotoxic shock likely related to MRSA infection.

Genetic typing of MRSA strains by spa-typing pulsed-field gel electrophoresis<sup>12</sup> and multilocus sequence typing<sup>13 14</sup> showed that the strains in both animals were identical.

The purpose of this case study is to document the efficacy of a decolonisation protocol on the

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epidemiology of MRSA in the dolphin colony that was tested between 2007 and 2015, by comparing culture results before and following the decolonisation efforts.

#### INVESTIGATIONS

Distribution of MRSA in dolphins was examined by culturing dolphin blowholes from 2007 to 2015. Distribution in human staff and the environment was examined by culturing water samples and human pharyngeal swabs between 2012 and 2015. Water samples were collected with sterile bottles once a month and tested with the UNI 10678/98 method. Human samples were collected by swabbing the pharynx every six months, and prior to arrival for new staff. Sterile swabs were placed and transported in Amies bacterial transport medium and cultured within 48 hours on sheep blood TSA (trypticase soy agar) agar incubated at  $36^{\circ}C+/-1^{\circ}C$  with 7 percent CO<sub>2</sub> for five days.

Dolphin blowhole exhalates were collected every four to six weeks in sterile plastic specimen cups (Zamboni, Forli, Italy) by drying the external blowhole with clean gauze, waiting for the animal to breathe once, before drying again and then asking the animal to breath out seven to eight times and collecting the exhalates in the cup. Sterile swabs were then collected from the cup and placed in Amies bacterial transport medium with and without charcoal (Deltalab, Rubi, Spain) and were cultured within 48 hours on sheep blood agar incubated at 37°C with 5 per cent CO, for five days (IDEXX Laboratories, Leipzig, Germany). If cultures yielded isolates identified as S aureus through colony morphology, and test of sensitivity to antibiotics showed resistance to amoxicillin-clavulanic acid, a specific meticillin-resistant staphylococcus aureus (MRSA) screening was requested and PBP2a (penicillin-binding protein 2a) agglutination in lattice was performed to identify the S aureus as MRSA. Meticillin resistance of staphylococci is determined by the mecA gene. This gene encodes an additional penicillin-binding protein (PBP2a). The presence of this gene product confirms the presence of a MRSA.

#### TREATMENT

After two dolphins died and MRSA was cultured from these animals, a decolonisation procedure<sup>10</sup> was adopted from 2012 to identify the possible source of infection, and reduce or control its dissemination.

An action plan in response to positive culture was designed as follows:

#### Dolphins

Dolphins were sampled every four to six weeks. If a dolphin was culture positive without symptoms, the sampling frequency was increased to biweekly until it was culture negative again. The staff was separated into groups and was instructed to wear the protective tools provided (gloves Satinex powder free, masks FFP1, glasses EN1661F, protective clothing and waterproof interchangeable shoes; Zamboni) to be used until the animals were culture negative. Positive asymptomatic animals were not treated with antibiotics. All dolphins were regularly supplemented with oral immune stimulants (Broncho-Vaxom, Takeda Italia, Rome, Italy).

#### Environment

If environmental samples were MRSA positive, thorough disinfection was carried out by using sanitising compounds (chlorhexidine and alcohol) applied in an alternating routine to diminish the risk of compound resistance. Use of pressure washer machines and other cleaning tools that vaporise the dust and spread moist around the pools was prohibited, since the spread of steam might facilitate the transmission of pathogens.

#### Water in the tanks

If water samples were MRSA positive, a thorough check of the life support system was performed, the tank water system was isolated and a sodium hypochlorite supersaturation performed in order to disinfect the tank and the related life support system. In this situation, the animals were moved into a different pool. After disinfection, sodium thiosulphate was added to pool water to neutralise the chlorine compounds and the water also partially changed before animals were reintroduced.

#### Staff

All staff were sampled every six months, and any new staff were sampled prior to entry, with only culture negative staff joining the facility. The medical doctor (MD) was informed of cases of positive isolation from the staff and he/she then acted accordingly. All human results were treated confidentially.

#### Transport

All animals were tested prior to departure and strict quarantine protocols were applied during transport, when staff wore gloves, masks FFP1, glasses EN1661F, protective clothing and waterproof shoes. Transport was carried out by truck or plane according to the Italian (DM 469/2001) and IATA (International Air Transport Association) regulations and veterinarians and trainers accompanied the animals for the entire transportation.

#### Upon arrival

The transported animals were considered as sick animals and were isolated from the others. The transport water was disinfected and discharged according to local regulations. The staff used protective clothing for every contact with the animals and were not allowed to enter the water. Animals were kept separate for at least six months before introducing individuals to each other.

#### OUTCOME AND FOLLOW-UP

Dolphin blowhole swabs collected between 2007 and 2009 revealed a transient presence of S aureus with resistance to different antibiotics in 8 out of 10 dolphins. One adult female in group A, in June 2007, was positive for S aureus that was resistant to oxacillin. In 2008, another adult female (positive for MRSA) gave birth to a calf that died within eight days of life, and postmortem blowhole culture from this calf revealed presence of MRSA. This culture was not associated with any pathological findings. In the same year, two further adults were positive for MRSA in blowhole samples, without displaying any clinical signs. Between 2008 and 2009, a multiresistant S aureus was intermittently isolated from the blowhole of three adult male dolphins that were then moved to group B; none of these strains were then investigated for potential meticillin resistance. The Risso's dolphin blowhole samples were positive for S aureus that was resistant to oxacillin and/or amoxicillin and clavulanic acid since 2005 (when the animal was stranded and was rescued). Samples examined in the following years were intermittently positive for MRSA.

After 2012, when the decolonisation protocol was initiated, culture of blow samples confirmed that most of the individuals

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TABLE 1	General overv	iew of MRSA p	presence in the	e cetaceans te	ested betweer	2007 and 20	16			
	Group A						Group B			
	GGF1	TTF1	TTF2	TTF3	TTF4	TTM1	TTM2	TTM3	TTM4	TTM5
2007–2011	MRSA	MRSA	Neg	Neg	MRSA	MRSA	Neg	MRSA	MRSA	MRSA
2012	MRSA	Neg	Neg	Neg	MRSA	MRSA	Neg	MRSA	MRSA	MRSA
2013		Neg	Neg	Neg	Neg	Neg	Neg	MRSA	MRSA	Neg
2014		MRSA	Neg	Neg	MRSA	MRSA	Neg	MRSA	MRSA	MRSA
2015		Neg	Neg	Neg	MRSA	MRSA	Neg	Neg	MRSA	MRSA
2016		Neg	Neg	Neg	Neg	MRSA	Neg	Neg	Neg	Neg

MRSA is reported when an individual repeatedly tested in that same year showed intermittently positive results.

Neg is reported when an individual tested in that year has always shown negative cultures for MRSA.

F, female; GG, Grampus griseus; M, male; MRSA, meticillin-resistant Staphylococcus aureus; Neg, negative; TT, Tursiops truncatus.

sharing the same environment were colonised by MRSA; five of six in group A and three of four in group B were culture positive. In December 2014, the four males composing group B joined group A. MRSA was found in three individuals, while one was constantly negative. Between November 2013 and February 2015, up to 14–16 samples were collected from each animal (total number 127 samples) and all the animals that were initially negative remained negative for the entire period, while the ones that were positive showed changes from positive to negative results in the subsequent months.

During the application of the decolonisation protocol, between June 2012 and December 2013, environmental samples taken from the pools and the closed environment were negative for MRSA despite positive cultures from the animals. Environmental monitoring revealed positive cultures after the introduction of dolphin group B in 2014 when MRSA was cultured from the pools' water at three and five weeks after the introduction of the four dolphins. One month after arrival, the water in one of the quarantine pools where the animals of group B were maintained was positive for oxacillin-resistant S aureus. The animals were then moved to the major pool of the quarantine area and the minor pool was then fully isolated, sealed and thoroughly disinfected. No further isolation of MRSA has occurred since in the pools. The groups (A and B) were kept in two separated filtration systems for a period of 40 days, before being united in the same filtered water system, with 15 months in total before any direct contact between individuals of the two groups occurred. General overview of MRSA presence in the animals for each year between 2007 and 2016 is reported in table 1.

Within the same period, among the 48 tested members of the staff, two people had a MRSA-positive pharyngeal swab. Both staff members were reported to a MD, who prescribed a temporary separation from the animals, treated the people with antibiotics according to human medical protocols and allowed them to return to work with dolphins only after two culture-negative swabs.

The strict quarantine protocol was applied for transport, handling and management. All staff members engaged in the transport had been tested two weeks before the transport occurred and were provided with individual protective clothing, gloves, glasses and masks for the transport and the subsequent period of acclimation time.

#### DISCUSSION

The protocol was applied after two animals died with underdeveloped or compromised immune systems that allowed this pathogen to spread systemically, resulting in septicaemia. The intention was to control the pathogen's spread in the population and environment, thus avoiding clinical cases associated with its presence. This study reports a successful protocol for decolonisation of the environment where dolphins are maintained that had no negative implications for the animals. It took considerable time to obtain persistent negative results from the majority of the animals. During this time, there has been no clinical disease related to this pathogen and the only individual that remained intermittently positive showed no sign of immunocompromise.

The intermittent culture of MRSA from dolphins during the decolonisation protocol, despite their separation, supports the notion that animals may be subclinically persistently infected, and blowhole culture cannot ensure an animal is free from infection, yet consistently negative cultures suggest an animal is less likely to succumb from clinical disease.<sup>15</sup>

The route of colonisation was not determined during the present investigation, but vertical passage from mother to calf was strongly suspected in the case of the nine-day-old calf that died. In humans, perinatal maternal-infant colonisation is documented during delivery<sup>16</sup> or breast feeding<sup>17</sup> contributing to early colonisation in newborns.

Two staff members that were constantly in contact with the dolphins had been culture positive for MRSA and were moved to other activities until they became culture negative. Particular attention should thus be paid to the possible influence of human MRSA on animals under their care. Transmission of MRSA from animals to workers in contact with them and possible public health risks are well documented.<sup>18–20</sup> Other studies showed that people can shed *S aureus* from skin into the water and thus potentially to other individuals.<sup>21–24</sup>

These observations underline the difficulty of a decolonisation protocol. In particular, efficient and adequate disinfection of the areas surrounding the pools and the water in the presence of living animals is difficult, as shown by the ineffective efforts of sterilisation of waters and tanks which did not influence the epidemiology of this bacterium in the dolphin population. Another limitation of the present study that should be addressed in future studies was the organisation of the facility when social animals are considered. In this experience, a sudden separation of MRSA-positive and negative animals without inducing separation stress response in the animals was not possible due to the long-term social groups and numbers of pools available.

Despite these difficulties, the decolonisation protocol suggested by Faires *et al*<sup>10</sup> was applied, allowing management of the environment and full disinfection of quarantine pools. This protocol limits the passage of contaminated water from one pool to another, thus limiting the dissemination of the organism, and

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it is of particular value in those cases when it is necessary to keep an immune-compromised animal in a pathogen-free environment, in order to successfully treat it without the presence of new multiresistant pathogens. In those cases, to avoid the possibility of human infection, a very strict protocol should be mandatory for the animal care staff. This can also be a useful procedure to control possible transmission in case of epidemics, both in staff and animals. It also underlines the need for good staff training on safety measures to reduce the risk of exposure and transmission<sup>31</sup>. Further improvement to the decolonisation protocol could include development of disinfection procedures adopted in human hospitals, such as the use of topical disinfectant for the upper airways <sup>28</sup>.<sup>25</sup>.<sup>26</sup>

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Competing interests None declared.

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