Occurrence of Multidrug Resistant *Staphylococcus aureus* in horses in Malaysia

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Abstract

A total of 22 *Staphylococcus aureus* were isolated from 50 samples from 8 stable horses. They are positive in the catalase and coagulase tests. Upon testing the cultures with SLIDEX test kit all formed agglutination within a few seconds, confirming they are of *S. aureus*. When cultured onto MSA, all isolates formed yellow colonies. However, none of the isolates produced blue colonies on ORSAB indicating that there were no MRSA among the *S. aureus*. There were 13 isolates which were multiresistant. Eleven are resistant to eight out of ten antibiotics tested. All these isolates were found to originate from stable G. One isolate is resistant to 5 antibiotics while another one isolate is resistant to 3 antibiotics. The rest of the isolates are not multiresistant to the antibiotics tested.

Keywords: *Staphylococcus aureus*, Horse, Malaysia, Multidrug resistant, Occurence, antibiotics, MRSA.

Introduction

The widespread use of antibiotics has been responsible for the development of numerous problems including the emergence of multidrug resistant bacteria. *Staphylococcus aureus* is one of the bacteria that have a dramatic increase in resistance to antibiotics in the last decade. *Staphylococcus aureus* is a normal flora both human and animals. It often colonizes the skin and nose in healthy individuals; however, it can also cause severe diseases. In animals, it causes diseases such as mastitis in most species of domestic animals, pyaemia dermatitis in dogs, botryomycosis in horses, septicaemia and arthritis in poultry (Soltys, 1979; Tortora *et al.*, 1989). The most important strain among the *S. aureus* is the methicillin resistant *S. aureus* (MRSA). MRSA are multidrug resistant *S. aureus* and it is the major pathogen causing nosocomial infections. In the medical field, it was first discovered in 1961. Eleven years later, in 1972, it emerged in animals where it was first isolated from cows with mastitis. Since then, MRSA has been found in a variety of animals including dogs and cats (van Duijkeren *et al.*, 2004; Cefai *et al.*, 1994; Rankin *et al.*,2005), chickens (Lee, 2003), pigs (Voss *et al.*, 2005) and also horses (Hartmann *et al.*, 1997). Among the significant clusters of MRSA in horses are reports made from Canada (Weese *et al.*, 2005), USA (Hartmann *et al.*, 1997), Ireland (O'Mahony *et al.*, 2005), Austria (Cuny *et al.*, 2006) and elsewhere. While there are many reports on the occurrence of multidrug resistance bacteria in horses around the world, the information is lacking in Malaysia. Therefore, this study aims to detect the occurrence of multiresistant *S. aureus* in horses in Kuala Lumpur and Selangor area.

Material and Methods

A total of 50 samples were taken from horses from 8 stable horses (stables A to H) around Kuala Lumpur and Selangor. Swabs were made from the nasal cavity of the horses by rubbing against the nasal cavity using a custom-made sterile cotton swabs. The swabs were placed in universal bottles containing sterile normal saline.

Each swab was streaked onto blood agar and incubated overnight at 37°C. Gram-positive cultures were subcultured and purified. The cultures were then subjected to a series of biochemical test such as catalase test, coagulase test. The SLIDEX test (Oxoid) was also carried out according to manufacturer's instruction. Staphylococcus presumptive colonies were also cultured onto Mannitol Salt Agar (MSA) (Oxoid) plate overnight at 37°C. Confirmed *S. aureus* culture was inoculated onto Oxacillin Resistance Screening Agar Base (ORSAB) agar and incubated overnight at 37°C

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Table - 1 Resistance pattern of *Staphylococcus aureus* isolates

Isolates	Resistance Pattern
K35, K36, K37, K38, K39, K40, K41, K42, K43, K44, K45	E, MY, P, DA, VA, OX, CN, MET
K34	E, MY, P, DO, DA
K2M	E, MY, DA

CN (Gentamycin), E (erythromycin), DA (clindamycin), VA(vancomycin), DO (doxycycline), P (penicillin G), OX (oxacillin), MY (lincomycin), MET (methicillin), C (chloramphenicol)

under aerobic condition for identification of MRSA isolates.

Antibiotic susceptibility test on each *S. aureus* isolate was carried out using the standard Kirby-Bauer method. Diluted solution of pure colony culture was prepared by suspending 2 to 3 colonies in 0.1ml sterilized normal saline. Normal saline was then added until the solution had the same turbidity with 0.5 McFarland standard solutions. This indicates that the suspension that was made contain approximately 108 cells/ml. Then a swab was dipped in the standardized solution and spread onto the Mueller Hinton agar. Then antibiotic disc, gentamycin (CN30), erythromycin (E5), climdamycin (DA2), vancomycin (VA30), doxycycline (DO30), penicillin G (P10), oxacillin (OX5), lincomycin (MY10), methicillin (MET10) and chloramphenicol (C30) were placed on the agar with a maximum of five antibiotic discs per agar. The plate was incubated overnight at 37°C. Upon completion, the diameter of the inhibition was measured and compared to the standard antibiotic susceptibility table (NCCLS, 2002). The isolate was assigned as resistance, intermediate and sensitive towards antibiotic tested.

Result and Discussion

A total of 22 *Staphylococcus aureus* were isolated from 50 samples. They are positive in the catalase and coagulase tests. Upon testing the cultures with SLIDEX test kit all formed agglutination within a few seconds, confirming they are of *S. aureus*. When cultured onto MSA, all isolates formed yellow colonies. However, none of the isolates produced blue colonies on ORSAB indicating that there were no MRSA among the *S. aureus*.

Table I shows the resistance pattern of the *S. aureus* isolates. There were 13 isolates which were multiresistant. Eleven are resistant to eight out of ten antibiotics tested. All these isolates were found to originate from stable G. One isolate is resistant to 5 antibiotics while another one isolate is resistant to 3 antibiotics. The rest of the isolates are not multiresistant to the antibiotics tested.

Staphylococcus aureus is a common normal flora of nasal passages, skin and mucous membranes of both human and animals (cats, dogs, rabbit, cattle, horses, and swine). A total of 22 (44%) *S. aureus* isolates were successfully obtained from 50 samples. The nasal carrier rate of *S. aureus* in this study is comparable to an earlier report done by Chingbu and Ezeronye (2003). However, the rate is a bit higher than the normal population which has reported a 30% carriage (CDC, 2004). This may be attributed to the sampling site (stables) where the place is very dusty therefore the horses might have inhaled dust from the surrounding that contains *S. aureus*.

All *S. aureus* isolates were confirmed by the conventional tests like Gram reaction, cell and colony morphology, catalase and coagulase tests. The commercial SLIDEX test kit was used for confirmation of the species. The MSA was used as the differential agar that conveniently differentiates *S. aureus* with other Staphylococcus species.

No MRSA was found among the confirmed *S. aureus* isolates. This is based on the inability of those isolates to form blue colonies on ORSAB agar. ORSAB agar is a special media containing aniline blue. It can detect mannitol fermentation in staphylococci. Its formulation includes a dual antibiotic supplement (polymixin B and oxacillin) and 5.5% concentration of sodium chloride which combine to reduce the growth of non-staphylococcal organisms and select for MRSA. The media and culturing method was performed accurately as the control MRSA isolates did form blue colonies on the particular agar.

In the antibiotic susceptibility tests, 13 isolates were found to be multi resistant consisting of 12 isolates from G and one from F. This finding is rather alarming because of the high resistance level of these isolates. If these strains do cause diseases, treatment with the antibiotics that they are resistant to might not be useful. It is also a concern if those isolates get transmitted to humans and cause disease. The isolates from stable G also show resistance to vancomycin. This is very worrisome, since vancomycin is normally regarded as the antibiotic of the last resort and it is the choice of medical practitioners for treating MRSA infection. There have now been several cases of Vancomycin

resistant *S. aureus (Wikipedia, 2007)*. If *S. aureus* develops high level resistance to Vancomycin, there is a limited treatment option available. Two drugs (SynercidTM and linezolid) have now been approved for treatment of Vancomycin resistant *S. aureus* (Donaldson, 2003). However, that it is unpredictable to guess how long it will take before this organism is resistant to the newest antibiotics.

All 11 isolates from stable G were found to be resistant to methicillin. However, they fail to form blue colonies on ORSAB. This indicates that the MIC of these isolates is below 2 mg/ml; these making them not categorized an MRSA. In order to confirm the identity of these isolates, a MIC test is required.

In this study, it appears that all isolates were susceptible to chloramphenicol and gentamycin. This indicates in treating the bacteria infection, a good choice for treatment against *S. aureus* infection is using these two antibiotics.

There are several factors that can make *S. aureus* to be resistance to antibiotics. Over prescribing of antibiotics by clinician is one way that can lead to resistance of *S. aureus***.** When there is over usage and incomplete course of antibiotics by patients**,** example when appropriate antibiotics are given to the animals, the owner may only give part of the course of antibiotic and not finish the remainder, possibly leaving bacteria partially treated. This will result in an increase resistance towards the antibiotics. The availability of antibiotics which is this is a big concern internationally where many antibiotics are available without prescriptions. Many pharmacists in these countries act as the caregiver and give out antibiotics based on patients' complaints without adequate diagnosis or testing and lead to the resistance of the bacteria towards antibiotic. High cost and lack of adequate medications also can lead to the resistance to antibiotic. In several lesser-developed countries, many antibiotics are very expensive. This may contribute to only partial use of an antibiotic (Horwitch, 2000).

Even though there is no isolation of MRSA in this study, there are some studies reporting MRSA as emerging disease nowadays that affect the equine industry. Weese and co-workers (2005) reported that the prevalence of MRSA colonization in horses was 4% in 2000 and 8% in 2002. This showed that the prevalence getting higher throughout years. Although the prevalence is low, prevention measures must be taken seriously. The possibility of either the multidrug resistant *S. aureus* or the MRSA as the source of zoonotic *Staphylococcal* infections in humans was suggested many years ago (Scott *et al.*, 1988). Surveillance of animals as well as animal handlers for carriage or occurrence of MRSA and other multidrug resistant bacteria is crucial to better understand the current situation of the dissemination of these bacteria so that an effective control measure can be formulated. Apart from that close co-operation between the human medical and veterinarian is important in order to have a holistic control programme for the ever rising antibiotic resistance bacteria.

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References

1. CDC (2004): Drug resistant *Staph.* spreads across US. www.webmd.com (accessed on 8 June 2007).
2. Cefai, C; Ashurst, S and Owens, C. (1994): Lancet. 344:539-540.
3. Chingbu, C.O. and Ezeronye,O.U.(2003): African J of Biotech. 2(10):374-378.
4. Cuny, C, et.al. (2006):Eurosurveillance 11: 44-47.
5. Donaldson,S.L.(2003): www.flemingforum.org.uk(accessed on 8 June 2007).
6. Hartmann,F.A., Trostle,S.S., Klohnen,A.A.O. (1997): J. Am Vet Med Assoc 211:590-592
7. Horwitch(2000):www.depts.washington.edu/eminf/2000/ resistance/resist2. html (accessed on 8 June 2007).
8. Lee, J.H. (2003): Applied and Environmental Microbiol. 69:6489-6494.
9. NCCLS (2002): Performance standards for antimicrobial disk susceptibility test, approved standards 7th edition, vol 20.
10. O'Mahony, R et.al. (2005): Vet. Microbiol. 109:285-296.
11. Rankin, S, et.al.(2005): Vet. Microbiol.
12. Scott, G.M.et.al. (1988): J Clin Microbiol 37:1459-1463.
13. Soltys, M.A. (1979): Introduction to Veterinary Microbiology. P 91-95. Penerbit Univerisiti Putra Malaysia, Serdang, Selangor.
14. Tortora, G.J.,Funke, B.R. and Case, C.L. (1989) Microbiology: An introduction. 3rd ed p 478-479. Benjamin Cummings Publishing Company, Inc California USA
15. Van Duijkeren,E,et.al.(2004): Vet.Microbiol. 103: 91-97.
16. Voss, A, et.al.(2005): Emerging Infectious Diseases 11:1965-1966.
17. Weese, J.S., et.al. (2005): Emerging Infectious Diseases; 11 (3): 430-435.