Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

SHORT COMMUNICATION

Occurrence and Antibiotic Susceptibility of Methicillin-Resistant *Staphylococcus aureus* Recovered from Oropharynx of Live Cockerels

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ARTICLE HISTORY (16-240)

Received: September 22, 2016
Revised: November 01, 2016
Accepted: December 14, 2016
Published online: December 29, 2016

Key words:
Antibiotic susceptibility profile
Latex agglutination test
Live cockerels
Methicillin-resistant *Staphylococcus aureus*
MRSA

ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) was recovered many times from raw poultry meat or carcasses; however, these were predominantly human-associated strains. Hence, possible human involvement in contamination of carcasses by slaughterhouse workers and other human handlers may not be overlooked in such cases. During this study, efforts were focused on isolation of MRSA from oropharynx of live poultry. Samples were collected from oropharynx of 50 live cockerels. A total of 25 *Staphylococcus aureus* isolates were identified. Only four isolates produced glistening, convex and mucoid colonies of MRSA on selective media. These isolates were further confirmed for methicillin resistance through latex agglutination test and produced catalase and coagulase, β-hemolysis on blood agar. Out of 25 *Staphylococcus aureus* isolates, 16% isolates were identified as MRSA. Antibiotic susceptibility profile of MRSA isolates indicated 100% sensitivity against vancomycin and linezolid whereas 100% resistance was recorded against oxacillin, cefoxitin and penicillin. However, 50% of the MRSA isolates were sensitive to levofloxacin and trimethoprim/ sulphamethoxazole and 25% isolates displayed resistance to tetracycline.

To Cite This Article: Zaheer Z, Hussain I, Rahman SU, Younas T, Zaheer I, Abbas G and Nasir M, 2017. Occurrence and antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* recovered from oropharynx of live cockerels. Pak Vet J, 37(1): 108-110.

INTRODUCTION

Staphylococcus aureus is a well-recognized opportunistic epithelial colonizer of both man and animals. Irrational administration of antimicrobials in veterinary and human medicine has made *S. aureus* resistant to routinely prescribed antimicrobials. One of such resistant strains of *S. aureus* is methicillin resistant *Staphylococcus aureus* (MRSA). MRSA is resistant to antimicrobials due to the presence of *mecA* gene which encodes penicillin binding protein 2a i.e. PBP2a. Resistance to methicillin has made this microbe a significant threat for human and animal health (Grema *et al*., 2015).

Initially MRSA was considered as a nosocomial microbe because in most of the hospitals there was frequent use of antibiotics without even knowing the true status of antibiotic sensitivity for a particular case, leading to the emergence of resistance in microbes inflicting infections. However, in the 1990s it was found to spread beyond human health care centers. Later on, globally MRSA was reported in communities and was also found to possess a potential for zoonosis (David and Daum, 2010).

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been isolated from human beings, many species of animals and their products. Nevertheless, in case of poultry MRSA has been isolated from raw meat and meat products (Karmi, 2013). The sites which are studied for MRSA colonization in live birds were nasal cavity and cloaca (Persoons *et al*., 2009). Since *S. aureus* is reported to frequently colonize the mucosal surfaces therefore, another important site of MRSA colonization in birds may be oropharynx and has not been investigated for the presence of MRSA, as far as could be ascertained. Hence, this study resolves the status of MRSA colonization in oropharynx of live birds.

MATERIALS AND METHODS

During the study, oropharynx samples were collected from 50 healthy Hyline layer breed's cockerels (housed at

a single controlled shed) at the age of 3 weeks with the help of sterile swabs. The swabs were moistened with sterilized saline before collection of samples. Each swab was inserted into the oropharynx of birds and was rotated for 30 seconds and transported to the laboratory in Stuart's medium. The sample swabs were enriched in the mannitol-salt broth at 37°C for 24 hours. The enriched swabs were subsequently cultured on the Staph-110 medium (allows the growth of all types of *Staphylococci*). The colonies of *S. aureus* (obtained from Staph-110) were cultured on the chromagar (CHROMagar, France). Chromagar unlike Staph-110 is specifically manufactured to allow the growth of MRSA in the form of red to mauve colored, pin point, mucoid and convex colonies whereas other microbes on chromagar produce colorless or blue colored growth. Presumptively positive colonies of MRSA produced red to mauve colonies on the chromagar and were subcultured on 5% sheep blood agar at 37˚C for 24 hrs. to observe the hemolysis pattern.

After biochemical testing, confirmation of MRSA isolates was carried out by latex agglutination test. The *mecA* encoded PBP2a was detected using the MRSA latex agglutination test (Oxoid Ltd., UK). The test was performed according to the manufacturer's instructions. A standard *mecA*-negative *S. aureus* strain (ATCC 25923), as well as a standard *mecA*-positive strain (ATCC 43300) were used for quality control. To perform the test presumptively positive MRSA culture from the blood agar were used to detect the presence of PBP2a protein in order to confirm the presence of MRSA.

Antibiogram of the MRSA isolates were determined by Kirby Bauer disc diffusion method. A suspension of each confirmed methicillin resistant *Staphylococcus aureus* isolates was prepared in peptone water to match 0.5 Mcfarland turbidity standards. All confirmed MRSA isolates were subjected to antibiotic susceptibility testing screened by inoculation on Mueller Hinton agar supplemented with 4% NaCl. After inoculation of Muller Hinton agar with MRSA isolates, oxacillin (1 µg), vancomycin (30 µg), linezolid (30 µg), trimethoprim/ sulphamethoxazole (25 µg), tetracycline (30 µg), penicillin (10 µg), levofloxacin (5µg), cefoxitin (30 µg) (Oxoid Ltd., UK) were aseptically placed on the surface of the inoculated plates and incubated aerobically at 37ºC for 18-24hrs. Oxacillin disc in this test was used as representative of methicillin, due to non-availability of the methicillin discs commercially. The zones of inhibition were measured and compared with Clinical and Laboratory Standards Institute (CLSI) guidelines. The study was conducted in compliance with rules and regulations designed by Institutional Bioethics Committee (IBC), University of Agriculture, Faisalabad, Pakistan.

RESULTS AND DISCUSSION

A total of 25 *Staphylococcus aureus* isolates were obtained after performing standard microbiological procedures. The *Staphylococcus aureus* isolates were then plated on the chromogenic culture media (CHROMagar, France). Four out of these *S. aureus* isolates yielded mucoid, glistening, round colonies producing red to mauve pigmentation on the chromagar and were considered as presumptively positive MRSA colonies. These red to mauve colonies were streaked onto the 5 % sheep blood agar (Oxoid Ltd., UK) to observe the hemolysis pattern.

The colonies of presumptively identified MRSA isolates produced beta-hemolysis on the blood agar and were microscopically exhibiting clusters of Gram positive cocci. During biochemical profiling catalase as well as coagulase production and mannitol fermentation was observed.

A total of four samples (from oropharynx) produced agglutination upon exposure to the latex reagent within 3-5 minutes. All of the MRSA isolates were sensitive to vancomycin and linezolid and showed resistance to oxacillin, cefoxitin and penicillin. Two out of four isolates were sensitive to levofloxacin and trimethoprim/ sulphamethoxazole whereas only one isolate displayed resistance to tetracycline, as shown in Table. Results of antimicrobial susceptibility test, confirming multidrug resistance of MRSA isolatesare quite similar to those reported by Kitai *et al*. (2005) and Fessler *et al*. (2011). Furthermore, percentage sensitivity and percentage resistance of the MRSA isolates were calculated, as shown in Figure.

In the present study the MRSA isolates were recovered directly from the birds while the human origin of these isolates was not ascertained. Whereas, in Korea (Lee, 2003), Japan (Kitai *et al*., 2005), Egypt (Karmi, 2013), US (Hanson *et al*., 2011), Germany (Fessler *et al*., 2011) MRSA has been isolated from raw chicken meat or carcasses, which were mainly human-associated strains hence the possibility of human involvement in contamination of carcasses by the slaughterhouse workers may not be ruled out (Grema *et al*., 2015). Moreover, animals may also become a source of MRSA colonization and MRSA associated ailments for human beings, inflicting resistant infections to affected individuals (Verkade and Kluytman, 2014).

Table 1: CLSI standards and antibiotic zone of inhibitions displayed by MRSA isolates

Antibiotic	oxacillin (1 µg)	tetracycline (30 µg)	cefoxitin (30µg)	vancomycin (30 µg)	linezolid (30µg)	levofloxacin (5 µg)	trimethoprim/ sulphamethoxazole (25 µg)	penicillin (10µg)
CLSI standards (mm)	S= ≥ 13 I= 11-12 R= ≤ 10	S= ≥ 19 I= 15-18 R= ≤ 14	S= ≥ 22 I= - R= ≤ 21	S= ≥ 15 I= - R= -	S= ≥ 21 I= - R= ≤ 20	S= ≥ 19 I= 16-18 R= ≤ 15	S= ≥ 16 I=11-15 R= ≤ 10	S= ≥ 29 I= - R= ≤ 28
Zone of inhibition (mm) Isolate 1	0	20	19	18	22	14	18	26
Zone of inhibition (mm) Isolate 2	0	22	17	20	25	23	20	21
Zone of inhibition (mm) Isolate 3	0	12	15	16	22	11	6	22
Zone of inhibition (mm) Isolate 4	0	20	17	18	24	21	9	24

S= Sensitivity, I= Intermediate and R=Resistance.

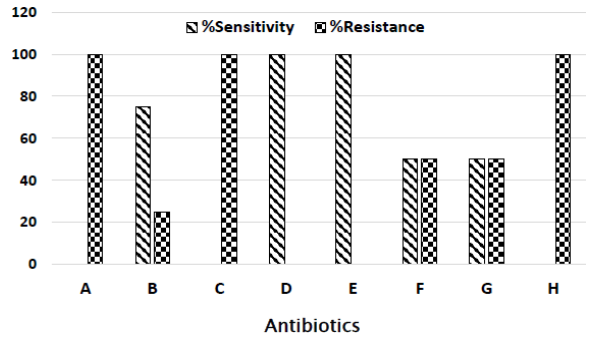

Fig. 1: Sensitivity and resistance percentage of MRSA isolates against various antibiotics (A=Oxacillin; B=Tetracycline; C=Cefoxitin; D=Vancomycin; E=Linezolid; F=Levofloxacin; G=Trimethoprim/ sulphamethoxaole and H=Penicillin).

The occurrence of MRSA in the cloacal area of live birds have been reported from a farm in the South West Nigeria. It was concluded that MRSA from poultry can be transferred to man not only by digestion of contaminated meat but also through contact with live birds and their fecal material (Oke and Oke, 2013). Similarly, MRSA colonization by the cloacal region and nasal cavity of live birds has also been confirmed from a study conducted in Belgium (Persoons *et al*, 2009). However, the current study identifies the occurrence of MRSA inside oropharynx of live poultry.

This study indicated that MRSA can also be harbored inside the oropharynx of live bird itself. A total of 4 MRSA isolates were obtained from 50 samples, indicating the presence of MRSA in oropharynx of live poultry birds, studied for the first time. Latex agglutination test confirmed the presence of PBP2a in all (4) isolates of MRSA. Therefore, this research was designed to confirm the occurrence of MRSA in oropharynx of birds and provides basis for the future investigations regarding molecular characterization.

Author's contribution: IH and SUR supervised the research. Research work, data analysis and write up was conducted by ZZ, TY, IZ, GA and MN. All authors approved the final version of the manuscript.

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