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REGULAR ARTICLES

Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in broilers and workers at 'pluck shops' in Trinidad

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Abstract

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is a cause of zoonotic infections in many countries. People with occupational contact with food animal production are at risk of colonization. The aim of this study was to determine the prevalence of MRSA and their frequency of resistance to other antimicrobial agents from broilers and workers at the 'pluck shops' in Trinidad. For isolation of MRSA, choanal, cloacal and pharyngeal swabs taken from broilers and nasal swabs from humans were enriched then plated on CHROMagar MRSA and Brilliance MRSA. MRSA was confirmed using the PBP2a test kit, resistance to oxacillin and cefoxitin and polymerase chain reaction (PCR) for the *mecA* gene. Antimicrobial resistance of the MRSA isolates to 16 antimicrobial agents was determined using the disc diffusion method. Of the 287 broilers and 47 humans sampled, MRSA was isolated at a frequency of 2 (0.7%) and 0 (0.0%) respectively. All the MRSA isolates exhibited resistance to one or more of the 16 antimicrobial agents. The study demonstrated that broilers at 'pluck shops' in Trinidad harbor MRSA. This is the first isolation of MRSA from poultry in Trinidad, West Indies, and this finding is of public health significance since occupational exposure of humans can lead to increased risk of acquiring MRSA infections.

Keywords MRSA · Antimicrobial resistance · Broilers · Workers · Trinidad

Introduction

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is a zoonotic pathogen which has been reported in various animals, including pigs, cattle, poultry and sheep (Verkade and Kluytmans 2014). People working with livestock are at high risk of infections caused by MRSA (Cuny et al. 2015). LA-MRSA primarily belongs to multilocus sequence type 398 but ST9 and ST5 have also been reported (Kraushaar et al. 2017).

In Trinidad and Tobago, the southernmost island in the Caribbean, broilers account for 88% of the meat consumed (Baboolal et al. 2012). Cottage or small-scale poultry processors called 'pluck shops' are widely patronized by consumers for dressed broilers. MRSA has been documented in broilers and the workers in contact with them elsewhere (Mulders et al. 2010).

To date, no published data exist on the occurrence of MRSA in poultry and workers who are in contact with broilers at 'pluck shops' in Trinidad. The current study was therefore conducted to determine the prevalence of MRSA in broilers and workers at 'pluck shops' in Trinidad and their resistance to other antimicrobial agents.

Methodology

This cross-sectional study was conducted between September 2015 and November 2015 in Trinidad. An estimated sample size of 275 and 81 for broilers and workers respectively was calculated using the following formula and reported prevalence rates (Mulders et al. 2010): $n = [t^2 \times p\ (1-p)]/m^2$ (Charan and Biswas 2013) where n = required sample size, t = confidence level at 95% (standard value of 1.96), p = estimated prevalence of MRSA and m = margin of error.

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A convenience sampling approach was used to select the 'pluck shops'. The study was designed to sample nine broilers from each 'pluck shop'; however, in counties with a few numbers of 'pluck shops', six broilers were sampled.

On arrival at each 'pluck shop', a questionnaire was administered to the manager and to each consenting worker. Three swab samples from the cloaca, choana and pharynx were collected from each broiler and bilateral nasal swabs from workers. All participants signed a written consent form. Each sample was then placed in a tube containing 5 ml of Amies transport (Oxoid Ltd., Basingstoke, Hampshire, England), placed on ice and transported to the laboratory within 4 h of sample collection.

MRSA was isolated by incubation in Mueller Hinton broth (Oxoid Ltd., Basingstoke, Hampshire, England) with 6.5% sodium chloride, followed by selective enrichment in phenol red mannitol broth (Becton Dickinson, Le Pont deClaix, France) with 75 mg/L aztreonam (Alfa Aaesar, Ward Hill, Massachusetts, USA) and 5 mg/L ceftizoxime (Tokyo Chemical Industry Company Limited, Kita Ku, Tokyo, Japan). The selective enrichment broth (10 μl) was then inoculated onto CHROMagar MRSA (CHROMagar Limited. Paris, France) and Brilliance MRSA agar (Oxoid Ltd., Basingstoke, Hampshire, England). Characteristic colonies from CHROMagar MRSA and Brilliance MRSA were then plated on 5% blood agar plates (Oxoid Ltd., Basingstoke, Hampshire, England) (Graveland et al. 2009). All incubation was at 37 °C for 18–24 h.

All suspected colonies were identified as *S. aureus* using standard techniques which included tests for catalase, coagulase and Gram staining. Pure cultures of the isolates identified as *S. aureus* were stored at – 70 °C in Brain Heart Infusion broth (Oxoid Ltd., Basingstoke, Hampshire, England) with equal volume of 50% glycerol.

MRSA was determined by conventional PCR to detect the *mecA* gene (Cho et al. 2007), the PBP2a Latex Agglutination test (Oxoid Ltd., Basingstoke, Hampshire, England) and antimicrobial resistance to oxacillin and cefoxitin. ST398 was determined using PCR for the C01 gene (van Wamel et al. 2010).

The susceptibility of the MRSA isolates to the various antimicrobial agents: streptomycin (S, 10 μg), sulphamethoxazole/trimethoprim (SXT, 1.75 μg/23.25 μg), tetracycline (TE, 30 μg), penicillin G (P, 10 IU), rifampicin (RD, 5 μg), clindamycin (DA, 2 μg), gentamicin (CN, 10 μg), erythromycin (E, 15 μg), norfloxacin (NOR, 10 μg), oxacillin (Ox, 1 μg), vancomycin (VA, 30 μg), ciprofloxacin (CIP, 5 μg), cefoxitin (FOX, 30 μg), ampicillin (AMP, 10 μg), chloramphenicol (C, 30 μg) and enrofloxacin (ENR, 10 μg), was read and compared to the chart for zones of resistance (CLSI 2012).

Data for the prevalence study were analyzed using Statistical Package for Social Sciences (SPSS) version 21. Chi-square (χ^2) test was used to determine whether statistically significant associations existed between prevalence and risk factors. All significant differences were determined at alpha (α) = 0.05.

Results

A total of 287 broilers were sampled from 33 'pluck shops' throughout Trinidad. The frequency of detection of MRSA in broilers was 0.7% (2/287) by PCR for the *mecA* gene, PBP2a test and resistance to the antimicrobial agents, oxacillin and cefoxitin. The two MRSA-positive samples were isolated from CHROMagar MRSA, one was from the cloaca and the other choana.

Both MRSA isolates were resistant to cefoxitin, oxacillin, ampicillin and penicillin. One of the two isolates was resistant to clindamycin. The two MRSA isolates were susceptible to rifampicin, erythromycin, tetracycline, gentamycin, streptomycin, norfloxacin, vancomycin, chloramphenicol, sulphamethoxazole/trimethoprim, ciprofloxacin and enrofloxacin. ST398 was not detected in any of the MRSA isolates analyzed.

The demographic data for the 'pluck shops' are shown in Table 1. All the risk factors observed had no effect on the presence of MRSA at the 'pluck shop'.

A total of 47 human samples were collected from 'pluck shops' throughout Trinidad. The frequency of detection of MRSA by PCR, resistance to the antimicrobial agent cefoxitin and the PBP2a test was 0.0% (0/47). However, a frequency of detection for MRSA of 2.1% (1/47) was observed with oxacillin.

Discussion

Although a very low prevalence (0.7%) of MRSA was detected in broilers, this is considered the first documentation of the existence of MRSA in broilers in the country. This is considerably lower than the prevalence of 4.8% for MRSA (Nemeghaire et al. 2013) for broilers in Belgium, 6.9% in the Netherlands (Mulders et al. 2010) and 45.5% in Southeast Nigeria (Ugwu et al. 2015). The rather low prevalence of MRSA detected in broilers in our study is an indication that MRSA strains may not be fully established on broiler farms in the country. The presence of MRSA, albeit low, is a cause for concern since it can be transmitted to humans by direct or indirect contact and also has the potential to cause infections in both broilers and humans, which are difficult to treat.

In our study, the results of other tests used to detect MRSA (PBP2a test, resistance to oxacillin and cefoxitin) were comparable to PCR for the *mecA* gene, which is the gold standard (Pournajaf et al. 2014). This diagnostic strategy will be invaluable, accurate and cost-effective to laboratories in developing countries where the use of PCR may not be possible because

Table 1 Demographic data for 'pluck shops'

Parameters	Frequency no. (%)
Maximum daily throughput	
< 100	5 (15.2)
100–500	23 (69.7)
501–1000	4 (12.1)
> 1000	1 (3.0)
No. of employees	
1–5	26 (78.8)
6–10	5 (15.2)
10–20	2 (6.1)
Time spent in holding bay before slaughter	
< 6 h	5 (15.2)
Overnight	7 (21.2)
> 24 h	12 (36.4)
Other	9 (27.3)
Adequate water supply	
Yes	26 (78.8)
No	7 (21.2)
Frequency of cleaning	
Once a day	4 (12.1)
After each batch or use	1 (3.0)
Other	28 (84.8)
Foot bath present	
Yes	2 (6.1)
No	31 (93.9)
Hand washing facility	
Yes	33 (100.0)
Hand Sanitizer	
Yes	14 (42.4)
No	19 (57.6)

of the cost of the machines, consumables and lack of technical skills to perform the tests.

Contrary to other reports (Geenen et al. 2013; Wendlandt et al. 2013), the dominant livestock-associated strain ST398 was not detected in the two MRSA isolates analyzed. However, ST9 and ST5 strains, not assayed for in the current study, have also been reported in other countries (Kraushaar et al. 2017).

Both MRSA isolates were resistant to cefoxitin, oxacillin, ampicillin and penicillin, all beta-lactams. This was expected since MRSA is resistant to beta-lactams. One of the two isolates was also resistant to clindamycin. Resistance of MRSA from poultry to clindamycin has also been reported in the Netherlands (Mulders et al. 2010) and in Nigeria (Ugwu et al. 2015).

It was a surprise that both MRSA isolates were susceptible to tetracycline contrary to the high frequency of resistance of MRSA strains to tetracycline reported by others (Nemeghaire et al. 2013; Ugwu et al. 2015) and the fact that tetracycline is one of the commonly used antibiotics in Trinidad and resistance is usually associated with frequent use (Schmithausen et al. 2015). However, susceptibility of MRSA to tetracycline in the current report compares favorably with the report in the USA (Abdalrahman et al. 2015) where MRSA isolates were all sensitive to tetracycline. The susceptibility of MRSA strains to the other antimicrobial agents may be due to a lower frequency of use on the farms due to cost and unavailability.

The prevalence of MRSA (0.0%) in 'pluck shop' workers in Trinidad and Tobago was comparable to the reported frequency of 0.0% in Switzerland (Huber et al. 2010) but lower than the 5.6% and 6.9% in the Netherlands (Mulders et al. 2010) and Korea (Moon et al. 2015) respectively.

Resistance to oxacillin and cefoxitin is used in many laboratories to report MRSA in Trinidad. In the present study, one of the human *S. aureus* isolates was resistant to oxacillin; however, it was negative by both PCR for the *mecA* gene and the PBP2a test. This emphasizes the need to subject MRSA strains detected by the disc diffusion method to other more specific tests such as PBP2a tests, prior to instituting antibiotic therapy in humans.

This study has demonstrated the occurrence of MRSA in broilers in Trinidad and poses public health threat to humans because workers in contact with broilers are at increased risk of acquiring MRSA infections.

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Compliance with ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institution, The University of the West Indies.

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from each individual participant included in the study.

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