Original Article

Prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in pigs and workers at abattoirs in Trinidad and Tobago

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Abstract

Introduction: Methicillin resistant *Staphylococcus aureus* (MRSA), a major cause of zoonotic infections, has emerged globally in livestock, particularly pigs. People with occupational contact with food producing animals are at high risk of colonization. The aim of this study was to determine the prevalence of MRSA in pigs and abattoir workers throughout Trinidad and Tobago as well as their resistance to other antimicrobial agents.

Methodology: Nasal and skin behind the ear swabs from pigs and nasal swabs from humans were enriched in Mueller Hinton broth with 6.5% sodium chloride, followed by phenol red mannitol broth with 75 mg/L aztreonam and 5 mg/L ceftizoxime. The enriched sample was then plated on both CHROMagar MRSA and Brilliance MRSA. All incubation was at 37ºC for approximately 24 h. Suspect MRSA isolates were confirmed as MRSA using the Penicillin-Binding Protein (PBP2a) test kit and polymerase chain reaction (PCR) to detect the *mecA* gene. Resistance of the *S. aureus* and MRSA isolates to 16 antimicrobial agents was determined using the disc diffusion method.

Results: Of the 929 pigs and 44 humans sampled, MRSA strains were isolated at a frequency of 0.9% (8/929) and 2.3% (1/44) respectively. All isolates exhibited resistance to one or more of the 16 antimicrobial agents.

Conclusions: The study demonstrated that pigs and workers at slaughter houses in Trinidad and Tobago harbour multidrug resistance *S. aureus* and MRSA. This is of public health significance as occupational exposure of humans can lead to an increased risk of infection and therapeutic failure.

Key words: MRSA; pigs; resistance; Trinidad and Tobago; workers.

Introduction

Staphylococcus aureus is a remarkable versatile bacterium behaving both as a harmless commensal and an important pathogen causing serious and life-threatening infections in humans and animals. In animals and humans, antimicrobial agents are used to prevent, treat and control diseases. However, in animals they are also used for sub-therapeutic purposes when administered at low dosages over a long duration to promote growth [1].

The uncontrolled use or abuse of antimicrobial agents in human and veterinary medicine has created a selective pressure for the emergence and dissemination of antimicrobial-resistant bacteria particularly in developing countries [2]. This has resulted in failed treatments and increased morbidity and mortality. These resistant bacteria may be transferred to humans either through the food supply chain or by direct contact with animals and MRSA is one such bacteria [3,4].

MRSA is a strain of *S. aureus* which has acquired the *mecA* gene that encodes for an altered penicillin binding protein (PBP2a) with a low affinity for β-lactam antibiotics [5]. This confers resistance to methicillin and the other β-lactams of which many members are still widely used in both human and veterinary medicine [5,6].

Initially, in the 1960s, MRSA was a nosocomial pathogen. However, in 2005, the term Livestock acquired MRSA (LA-MRSA) evolved when the presence of MRSA in pigs and the transfer to humans were reported for the first time [7]. Since then, LA-MRSA has been documented worldwide and in various animals however, it is particularly associated with pigs, veal calves and poultry [8,9]. In Western Europe, the United States and Canada, LA-MRSA strains primarily

belong to the Multi Locus Sequence Type (MLST) 398 [10] but in Asia they belong mainly to Multi Locus Sequence Type (MLST) 9 [11].

People at risk for LA-MRSA include persons with occupational exposure to livestock such as farmers, abattoir workers, transporters of animals, veterinarians and their families [12]. Risk factors for human colonization by LA-MRSA include duration of exposure of animals with MRSA, contact with live animals, antibiotic use at the farm, livestock density and handling contaminated meat products [12,13]. Studies on pigs have shown that isolates obtained from pigs and their human caretakers are frequently indistinguishable, suggesting transmission between the two [14,15].

In Trinidad and Tobago, MRSA strains have been reported in pigs on farms [16] and humans in the hospitals and community [17,18]. In the study conducted on pigs on farm in Trinidad [16], a prevalence of 2.1% was reported for MRSA in pigs, however MRSA was not detected in their human handlers.

To date, there is a dearth of information on the occurrence of MRSA in pigs and workers at the abattoirs in the country. The current study was therefore conducted to determine the prevalence of MRSA and resistance to other antimicrobial agents amongst *S. aureus* strains recovered from pigs and workers at the abattoirs throughout Trinidad and Tobago.

Methodology

Abattoirs sampled in the study

This cross-sectional study was conducted between August 2014 and December 2015. A total of 44 persons and 929 pigs from 10 different abattoirs throughout Trinidad and Tobago were sampled. Of the 10 abattoirs, five were government-operated public abattoirs, four were commercial private abattoirs and one was government-owned but with private operations. The five public functional abattoirs slaughtered pigs from a wide range of farms in the area. However, the private abattoirs only slaughtered pigs raised on their farms which were located on the same compound as the abattoir. The abattoirs were grouped into two categories based on their maximum daily throughput. Abattoirs with maximum daily throughput of less than 40 pigs were classified as low throughput (LT) while those with maximum daily throughput of more than 40 were considered high throughput (HT).

Sample size determination and sample collection

To determine the sample size for the study, the following formula was used for the calculation:

$$n = [t^2 \times p \, (1\text{-}p)]/m^2 \qquad [19]$$

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.

Using the formula with reported prevalence rates for MRSA in pigs and humans [20,21], the estimated sample size determined for pigs and humans was 1016 and 81 respectively.

A total of 159 pigs were sampled from each of the three HT abattoirs and 80 pigs from the seven LT abattoirs. With the use of sterile swabs (Medline Industries Inc., Mundelein, Illinois, USA), two samples, nasal swab and swab of the skin behind the ear [22], were collected from each pig after stunning or after the jugular vein was cut in abattoirs where stunning was not performed. A questionnaire was administered to the manager of each abattoir to obtain demographic data on the slaughter capacity, species of animals slaughtered, hygiene practices and details on the slaughtering process. At each abattoir, bi-lateral nasal swabs were taken from consenting workers. A questionnaire was also administered to obtain bio-data of the workers, the number of years working with animals and antibiotic usage. Each sample was then placed in a tube containing 5 mL of Amies transport medium (Oxoid Ltd., Basingstoke, Hampshire, England) and transported to the laboratory within 4 h of sample collection.

Isolation of MRSA strains

In the laboratory, each swab was inserted into a test tube containing Mueller Hinton broth (Oxoid Ltd., Basingstoke, Hampshire, England) with 6.5% sodium chloride. After overnight incubation at 37°C, one millilitre of the pre-enriched growth was added to nine millilitres of selective enrichment comprising 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 inoculated selective broth was incubated at 37°C for 18-24 h after which 10 µl was inoculated onto CHROMagar MRSA (CHROMagar Limited, Paris, France) and Brilliance MRSA agar (Oxoid Ltd., Basingstoke, Hampshire, England), then incubated at 37°C for 18- 24 h. The CHROMagar plates were incubated for an additional 24 h if characteristic colonies were not exhibited after the first 24 h incubation. Characteristic colonies on CHROMagar MRSA and Brilliance MRSA were then plated on 5% Blood agar plates (Oxoid Ltd., Basingstoke, Hampshire, England) [23].

All suspected colonies were identified as *S. aureus* using standard techniques, including Gram staining and performing both the tube coagulase and catalase tests. Pure *S. aureus* cultures were stored in Brain Heart Infusion broth (Oxoid Ltd., Basingstoke, Hampshire, England) with 50% glycerol at -80°C.

Detection of MRSA by PCR

Conventional PCR was performed to detect MRSA. The *mecA* gene was amplified using the primer pair RSM2647 – AAA ATC GAT GGT AAA GGT TGG and RSM2648 – AGT TCT GCA GTA CCG GAT TTG C (Integrated DNA Technologies, Inc. Iowa USA). The Amplicon size for the *mecA* gene was 533bp [24].

Conventional PCR was also performed to detect the presence of the ST398-specific C01 gene. The target gene (*C01*) was amplified using the primer pair C01F– CATTCATCACACGTATATTC and C01R - GGTGTTATTCATGGTTAAG (Integrated DNA Technologies, Inc. Iowa USA). The Amplicon size for the *C01 gene was* 140bp [25].

The amplified DNA products were separated by electrophoresis (BioRad Laboratories Inc., Hercules, California, USA) in a 1.5% agarose gel (Phenix Research Products, Chandler, North Carolina, USA) and were visualized under an ultraviolet transilluminator using the AlphaImager HP, Imaging System (Alpha Innotech Corp, San Leandro, California, USA).

Detection of MRSA by PBP2a test kit

The PBP2a agglutination test kit (Oxoid Ltd., Basingstoke, Hampshire, England) was used to identify MRSA strains following the manufacturer's instructions.

Determination of resistance to antimicrobial agents by disc diffusion method

The Clinical and Laboratory Standards Institute (CLSI) method [26] was used to determine antimicrobial resistance of all confirmed *Staphylococcus aureus* isolates (including the MRSA) by the disc diffusion method. The antimicrobial agents were selected based on their use in veterinary and human medicine in Trinidad and on reports in the literature. The antimicrobial agents on discs (Oxoid Ltd., Basingstoke, Hampshire, England) and concentrations used were Streptomycin (S, 10 µg), Sulphamethoxazole/Trimethoprim (SXT, 1.75 µg/23.25 µg), Tetracycline (TE, 30 µg), Penicillin G (P, 10 IU), Rifampicin (RD, 5µg), 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). The susceptibility of the *S. aureus* isolates to the 16 antimicrobial agents was read and compared to the CLSI [26] chart for zones of resistance and susceptibility to antimicrobial agents.

Ethics approval

The approval of the University of the West Indies, St. Augustine Campus Ethics Committee was received before the commencement of the study. All abattoir workers who participated in the study completed the consent forms prior to being sampled.

Statistical analyses

Data for the prevalence study were analysed 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

Detection of MRSA is slaughter pigs

The prevalence of MRSA in slaughter pigs was 1.3% (12/929) by resistance to antimicrobial agents

Table 1. Demographic data on the abattoirs sampled.

Parameter	No. (%)
Type of abattoir	
Government Public	5 (50.0)
Private	5 (50.0)
Maximum daily throughput	
< 10	1 (10.0)
11-30	3 (30.0)
31-50	0 (0.0)
51-100	3 (30.0)
> 100	3 (30.0)
Pigs slaughtered weekly	
< 10	3 (30.0)
11-30	2 (20.0)
31-50	2 (20.0)
51-100	1 (10.0)
> 100	2 (20.0)
Frequency of cleaning	
Once per day	6 (60.0)
Twice per day	3 (30.0)
Other	1 (10.0)
Stunning methods	
Electric stunner	6 (60.0)
Lash on head	2 (20.0)
No stunning	2 (20.0)

(oxacillin and cefoxitin) and 0.9% (8/929) by the phenotypic test (PBP2a) and PCR for the *mecA* gene. Of the 929 pigs sampled, 503 (54.1%) were from the private abattoirs and 426 (45.9%) originated from the government-owned public abattoirs. All the private abattoirs and government-owned abattoirs with private operations had pig farms on the property. The demographic data on the abattoirs sampled is presented in Table 1. All eight MRSA-positive pigs originated from two private abattoirs.

Of the 929 pig samples, 11.4% (106/929) displayed the characteristic mauve colour on CHROMagar MRSA, 0.5% (5/929) were MRSA and 0.1% (1/929) was non-MRSA *S. aureus* (Table 2). Of the 106 samples that exhibited the characteristic mauve appearance on CHROMagar MRSA, only 5 (4.7%) were MRSA and 1 (0.9%) was confirmed as non-MRSA *S. aureus*. A majority, 76.4% (81/106) of the samples were non-*S. aureus* staphylococci and 95.3% (101/106) were non-MRSA isolates.

The anterior nares were the anatomic site from where the five MRSA-positive samples were detected on CHROMagar MRSA. The only MRSA-positive sample isolated from the skin behind the ear was also isolated from the anterior nares. A higher frequency of non-MRSA isolates (97.8%) was recovered from the skin behind the ear than the anterior nares (93.2%) but the difference was not statistically significant (P>0.05).

Seventy-seven (8.3%) of the 929 pig samples exhibited the characteristic blue colour on Brilliance MRSA, of which 3 (0.3%) were MRSA and 12 (1.3%) were non-MRSA *S. aureus*. Of the 77 samples which showed the characteristic colour, 3 (3.9%) were MRSA and 12 (15.6%) were confirmed as non-MRSA *S. aureus*. A majority, 76.6% (59/77) of the samples that showed the characteristic colour on Brilliance MRSA were non-*S. aureus* staphylococci and 96.1% (74/77) were non-MRSA isolates. Of the 3 MRSA-positive

isolates, two originated from the anterior nares and one was from the skin behind the ear. All the non-MRSA *S. aureus* were isolated from the anterior nares. A higher frequency of the non-MRSA isolates was recovered from the skin behind the ear (97.1%) than the anterior nares (96.2%).

There was no statistical significance difference detected for isolation of MRSA between the two-selective media (p = 0.750).

All MRSA isolates from pigs were resistant to cefoxitin, penicillin and ampicillin and a high frequency of resistance (88.9%) was observed to oxacillin and tetracycline. A low frequency of resistance (11.1%) was observed to streptomycin. However, all MRSA isolates were susceptible to clindamycin, rifampicin, erythromycin, norfloxacin, vancomycin, chloramphenicol, sulfamethoxazole/ trimethoprim, ciprofloxacin and enrofloxacin. The modal frequency pattern observed was P-AMP-FOX-OX-TE (77.8%) and the other patterns included P-FOX-AMP and OX-P-TE-FOX-AMP-S. One (11.1%) MRSA isolate exhibited multidrug resistance. ST398 strain was not detected in any of the MRSA isolates analysed.

Detection of MRSA in abattoir workers

The frequency of detection of MRSA by PCR, PBP2a and resistance to antimicrobial agents cefoxitin and oxacillin was 2.3% (1/44).

The demographic data of abattoir workers are represented in Table 3. The only risk factor that had a statistically significantly (p = 0.023) effect on the isolation of MRSA was the recent use of antibiotics.

The MRSA isolate exhibited resistance to oxacillin, cefoxitin, penicillin, ampicillin and vancomycin but was susceptible to rifampicin, chloramphenicol, sulfamethoxazole/trimethoprim, streptomycin, gentamycin, clindamycin, ciprofloxacin, norfloxacin, enrofloxacin and erythromycin.

Table 2. Selectivity of CHROMagar and Brilliance MRSA for isolation of MRSA from pig samples.

Selective agar	Anatomic site	No. of samples tested	Samples that yielded characteristic * appearance. No. (%)	Samples that yielded characteristic appearance			
				Confirmed as MRSA**. No. (%)	Confirmed as non-MRSA *S. aureus*. No. (%)	Positive non-*S. aureus* staphylococci. No. (%)	Total Non-MRSA isolates. No. (%)
CHROMagar	All	929***	106 (11.4)	5 (4.7)	1 (0.9)	81 (76.4)	101 (95.3)
MRSA	Anterior nares		73 (7.9)	5 (6.8)	1 (1.4)	55 (75.3)	68 (93.2)
	Skin behind the ear		46 (5.0)	1 (2.2)	0 (0.0)	41 (89.1)	45 (97.8)
Brilliance	All	929	77 (8.3)	3 (3.9)	12 (15.6)	59 (76.6)	74 (96.1)
MRSA	Anterior nares		53 (5.7)	2 (3.8)	12 (22.6)	38 (71.7)	51 (96.2)
	Skin behind the ear		34 (3.7)	1 (2.9)	0 (0.0)	33 (97.1)	33 (97.1)

*Characteristic appearance: CHROMagar MRSA-mauve, Brilliance MRSA- Blue; **By use of PCR for mecA gene and PBP2a test; ***For each anatomic site, 929 samples were collected.

Frequency of isolation of S. aureus from pigs and resistance of isolates to antimicrobial agents

Overall, *S. aureus* was isolated from 52 (5.6%) of the 929 pigs sampled and the microorganism was recovered from 37 (4.0%) and 27 (2.9%) of the nose and the skin behind the ear samples respectively. The difference was not significantly significant (p = 0.110). The frequency of isolation of *S. aureus* was 0.0% (0/426) and 10.3% (52/503) for pigs slaughtered in government and private abattoirs respectively. The difference was statistically significant (p = 0.002).

The frequency of resistance of *S. aureus* isolated from pigs to the selected antimicrobial agents is shown in Figure 1. Of the 77 isolates tested, all were resistant to one or more of the 16 antimicrobial agents. The highest frequency of resistance was exhibited to penicillin and ampicillin (P and AMP, 100%), followed by streptomycin, enrofloxacin and tetracycline (S, ENR and TE, 84.4%). The lowest frequency of resistance was detected to sulfamethoxazole/trimethoprim (SXT, 5.2%). All isolates were sensitive to vancomycin. The differences in frequency of resistance to the antimicrobial agents were statistically significant (p = 0.000). Multidrug resistant *S. aureus* (resistant to three or more antimicrobial classes) was exhibited by 88.3% (68/77) of the isolates. The highest frequency of resistance was exhibited to β-lactams (100.0%), followed by aminoglycosides (87.0%), fluoroquinolones (84.4%) and tetracyclines (84.4%). A moderate frequency of resistance was observed to macrolides (75.3%) and lincosamides (75.3%). The lowest frequency of resistance was observed to sulphonamides (5.2%). The differences in frequency of resistance to the antimicrobial classes were however not statistically significant (p = 0.088). All isolates were sensitive to glycopeptide.

Table 3. Demographic data of human samples and risk factors for isolation of *S. aureus* and MRSA.

Parameter	No. of humans sampled	No. (%) positive for MRSA	p-value	No. (%) positive for *S. aureus*	p-value
Age					
< 21	1	0 (0.0)	0.157	0 (0.0)	0.489
21-30	12	0 (0.0)		1 (8.3)	
31-40	16	0 (0.0)		0 (0.0)	
41-50	5	1 (20.0)		1 (20.0)	
51-60	8	0 (0.0)		0 (0.0)	
> 60	2	0 (0.0)		0 (0.0)	
Total	44	1 (2.3)		2 (4.5)	
Gender					
Male	40	0 (0.0)	0.090	1 (2.5)	0.721
Female	4	1 (25.0)		1 (25.0)	
Total	44	1 (2.3)		2 (4.5)	
Length of time Working (years)					
< 1	7	0 (0.0)	0.546	0 (0.0)	0.55
1-2	1	0 (0.0)		0 (0.0)	
3-5	11	1 (9.1)		1 (9.1)	
6-10	4	0 (0.0)		1 (25.0)	
> 10	21	0 (0.0)		0 (0.0)	
Total	44	1 (2.3)		2 (4.5)	
Contact with animal					
Live	2	0 (0.0)	0.886	0 (0.0)	0.912
Dead	12	0 (0.0)		1 (8.3)	
Both	27	1 (3.7)		1 (3.7)	
None	3	0 (0.0)		0 (0.0)	
Total	44	1 (2.3)		2 (4.5)	
Illness					
Dermatitis	1	0 (0.0)	0.977	0 (0.0)	0.955
Respiratory problem	2	0 (0.0)	0.955	1 (50.0)	0.090
Any other illness	4	0 (0.0)	0.909	1 (25.0)	0.175
Recent surgery	3	1 (33.3)	0.068	1 (33.3)	0.133
Recent hospitalization	4	1 (25.0)	0.091	1 (25.0)	0.175
Use of antibiotics	1	1 (100.0)	0.023	1 (100.0)	0.045

Frequency of isolation of S. aureus from abattoir workers and resistance of isolates to antimicrobial agents

S. aureus was isolated from 2 (4.5%) of the 44 abattoir workers. The only risk factor that was statistically significantly (p = 0.045) associated with the presence of *S. aureus* was the recent use of antibiotics.

The frequency of resistance to the selected antimicrobial agents by *S. aureus* isolated from humans is shown in Figure 2. The three isolates of *S. aureus* were resistant to one or more of the 16 antimicrobial agents with the highest frequency of resistance exhibited to penicillin and ampicillin (P and AMP, 100%), followed by streptomycin, gentamicin, clindamycin ciprofloxacin, norfloxacin and erythromycin (66.7%). The lowest frequency of resistance was observed to oxacillin, vancomycin and cefoxitin (OX, VA and FOX, 33.3%). The differences in frequency of resistance to the antimicrobial agents were statistically significant (p = 0.000). All isolates were sensitive to tetracycline, rifampicin, chloramphenicol, enrofloxacin and sulfamethoxazole/trimethoprim.

For the 16 antimicrobial agents tested grouped by class, the highest frequency of resistance was exhibited to β-lactams (100.0%), followed by aminoglycosides, fluoroquinolones, macrolides and lincosamides (66.7%). A moderate frequency of resistance was observed to glycopeptides (33.3%). The differences in the frequency of resistance to the antimicrobial classes were not statistically significant (p = 0.280). All isolates were sensitive to tetracyclines, sulphonamides, ansamycins and phenicols.

Discussion

The prevalence of MRSA in pigs at abattoirs throughout Trinidad and Tobago was 1.3% when as determined by resistance to antimicrobial agents (oxacillin and cefoxitin) using the disc diffusion method but 0.9% by use of phenotypic test (PBP2a) and PCR for the *mec*A gene. The slightly higher frequency of detection of MRSA strains by the disc diffusion method which is the method of choice in many laboratories to detect MRSA because it is easy to perform and very cost effective [27] may have implications. The higher frequency of detection of MRSA by the disc diffusion method may lead to an over-reporting of MRSA with resultant unnecessary therapeutic intervention. For that reason, PCR which is the gold standard for detecting MRSA [28] is used, whenever practicable. Based on our findings, both the PBP2a test and PCR detected the same MRSA strains, i.e. 100% agreement of the results, a finding in agreement with the findings of Sakoulas *et al*. [29] who concluded that the accuracy of the MRSA-Screen latex agglutination method for detection of PBP2a approaches the accuracy of PCR and is more accurate than any susceptibility testing method used alone for the detection of MRSA. A diagnostic strategy which uses the disc diffusion method followed by confirmation of MRSA-positive strains with the PBP2a test will be an invaluable, accurate, cost-effective and affordable option. The prevalence of MRSA (0.9%) detected by both the PBP2a and PCR assays in this study is considerably lower than the 83% to 99% in The Netherlands [30] and 70.8% in Germany [31] but higher than the 0.0% reported in Switzerland [32] and comparable to the 0.9% reported in Japan [33]. The low

Figure 1. Frequency of resistance of *S. aureus* isolates from pigs to antimicrobial agents.
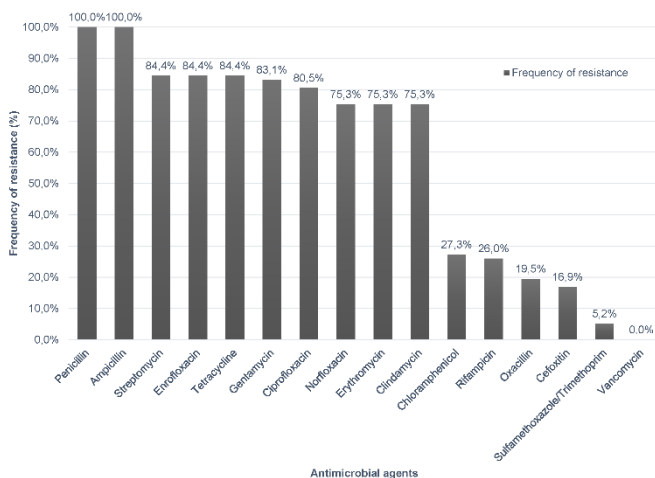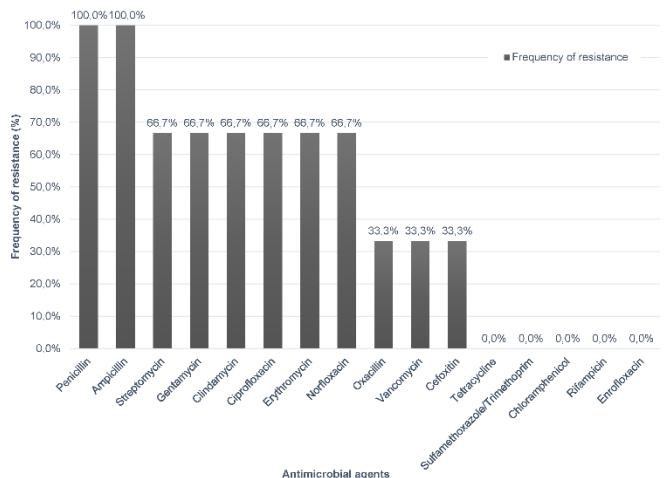

Figure 2. Frequency of resistance of *S. aureus* isolates from humans to antimicrobial agents.

prevalence of MRSA in pigs at the abattoir could reflect the low prevalence (2.1%) earlier detected at the pig farm level in Trinidad and Tobago [16].

In our study, the CHROMagar MRSA and Brilliance MRSA media used to screen for MRSA in pigs have been reported to have high sensitivity and specificity to detect MRSA [34,35]. It is noteworthy that although 106 (11.4%) of the 929 samples tested exhibited the characteristic mauve appearance on CHROMagar as described by the kit manufacturer, only 4.7% of the mauve-appearing colonies were confirmed to be MRSA by PCR. The rather low selectivity of CHROMagar for MRSA was similarly observed by Gordon *et al.* [16] who reported that only 2.7% of the isolated *S. aureus* from pigs sampled on farms that displayed characteristic appearance colour on CHROMagar MRSA, were confirmed to be MRSA strains. Similarly, on Brilliance MRSA agar, of the 77 (8.3%) of 929 samples from pigs that showed characteristic blue appearance on the agar, only 3.9% of the isolates were confirmed positive by PCR, even though Brilliance MRSA has been used to detect MRSA in pigs [22]. It is of concern that both CHROMagar MRSA and Brilliance MRSA had a low selectivity for isolating MRSA. There is therefore a need to further confirm the colonies exhibiting typical appearances on both media by other methods such as PBP2a or PCR, to avoid false-positive results, over diagnosis and over treatment for MRSA.

In the current study, the use of PCR did not detect ST398 strains in the eight isolates of MRSA, a finding at variance with published reports which documented ST398 strains are predominant in LA-MRSA in other countries [10,36]. There is therefore the possibility that strains, other the ST398, not assayed for in the current study, may be present in Trinidad and Tobago.

All MRSA strains isolated were resistant to cefoxitin, penicillin and ampicillin, a finding in agreement with the report that MRSA isolates are usually resistant to βlactams [4,37]. Similarly, a high frequency of resistance (88.9%) was detected to tetracycline amongst MRSA strains in our study, a finding in agreement with published reports [37,38]. The high frequency of resistance exhibited to by MRSA strains to penicillin, ampicillin, streptomycin and tetracycline in our study may be attributed to their frequent use in veterinary practice in the country [16]. More importantly, there is no regulation of the use and availability of antimicrobial agents to livestock farmers in the country. The frequencies of resistance or susceptibility exhibited by the MRSA isolates to other antimicrobial agents in our study also agree with published reports [39,40].

The prevalence (2.3%) of MRSA in abattoir workers in Trinidad and Tobago was slightly higher than the 0.0% in Switzerland [41] but considerably lower than the 7.3% to 21.1% reported in other countries [42-44]. It is noteworthy to mention that MRSA was not isolated from pig farm workers in an earlier study in the country [16]. The low prevalence of MRSA in slaughterhouse workers should not be ignored since LA-MRSA can cause the same types of infections as HA-MRSA strains [45].

The single MRSA isolate from an abattoir worker was resistant to oxacillin, cefoxitin, penicillin, ampicillin and vancomycin, a finding in the reports of exhibited resistance to β-lactam and other antimicrobial agents [4]. A high frequency of resistance by MRSA strains to oxacillin, cefoxitin, penicillin and ampicillin has also been reported in human hospital settings in Trinidad and Tobago [17], as well as to other antimicrobial agents [46].

The overall frequency of *S. aureus* from pigs at the abattoirs in Trinidad and Tobago was 5.6% and the isolation of *S. aureus* at a higher frequency (4.0%) from the anterior nares of pigs than from the skin behind the ear (2.9%) agrees with the report of Linhares *et al.* [47] who reported a higher frequency of isolation of *S. aureus* from the anterior nares than from any other anatomical site, including the skin. Our findings also support the recommendation of Linhares *et al.* [47] who encouraged the sampling of more than one anatomical site to avoid underestimating the prevalence of *S. aureus* in pigs.

It is of therapeutic relevance to have detected such a high frequency of resistance (100.0%) to antimicrobial agents by *S. aureus* strains isolated from pigs at the abattoir. A similarly high frequency of resistance (99.0%) was reported by Gordon *et al.* [16] for *S. aureus* isolates from pigs on farms in Trinidad. However, a lower prevalence of resistance (73.0%) for *S. aureus* strains from pigs has been reported in Switzerland by Reisen and Perreten [48].

The highest frequency of resistance by *S. aureus* isolated from pigs in the current study was exhibited to penicillin and ampicillin and this has been attributed to the presence of the *blaZ* gene which encodes β-lactamase and thus renders the β-lactam inactive [49]. The high frequency of resistance of *S. aureus* to penicillin and ampicillin is consistent with reports by Park *et al.* [50] in Canada and Gordon *et al.* [16] in Trinidad.

The lowest frequency of resistance by *S. aureus* was observed to sulfamethoxazole/trimethoprim (5.2%) in the current study which is consistent with the 6.2% [16] reported for pigs on farms in Trinidad and the 0.0% reported by Park *et al*. [50] in Canada. This was no surprise since it is known that SXT is active against the majority of *S. aureus.* The finding that all isolates of *S. aureus* tested in our study were sensitive to vancomycin is in agreement with the report of Opplinger *et al*. [51] in a study conducted in Switzerland where all 83 *S. aureus* isolates from 41 pig farms were also sensitive to the antibiotic. This is primarily because vancomycin is not commonly used on livestock.

It is also of therapeutic significance to have detected that as high as 88.3% of the isolates of *S. aureus* from the pigs and workers studied exhibited multidrug resistance, most frequently to antimicrobial classes β-lactams, aminoglycosides, fluoroquinolones, tetracyclines, macrolides and lincosamides. The occurrence of multidrug resistance in *S. aureus* strains has also been reported in *S. aureus* isolates from abattoir workers in the USA [52], pig farmers in Switzerland [51] and in Trinidad and Tobago [16]. Multidrug resistant *S. aureus* should not be taken lightly because their infections are associated with higher expenditure and worse health outcomes [53].

The high frequency of resistance to antimicrobial agents by *S. aureus* and MRSA strains from pigs cannot be ignored since foodborne or zoonotic pathogens can be transferred from food producing animals to humans by direct contact with the animals [3,4], direct contact with contaminated carcasses, or indirectly through food or the environment [54]. It is also established that humans can transmit bacteria that originated from farms to other humans through direct contact, food contamination during processing, or contamination of shared environments [55].

Conclusions

Our study has demonstrated the occurrence of MRSA and multidrug resistant *S. aureus* in slaughtered pigs and abattoir workers in Trinidad and Tobago. The therapeutic implications of antibiotic resistant *S. aureus* in pigs and humans in contact with pigs cannot be over-emphasized.

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