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Frequency of resistance to methicillin and other antimicrobial agents among *Staphylococcus aureus* strains isolated from pigs and their human handlers in Trinidad

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged recently worldwide in production animals, particularly pigs and veal calves, which act as reservoirs for MRSA strains for human infection. The study determined the prevalence of MRSA and other resistant strains of *S. aureus* isolated from the anterior nares of pigs and human handlers on pig farms in Trinidad.
Methods: Isolation of S. aureus was done by concurrently inoculating Baird-Parker agar (BPA) and Chromagar MRSA (CHROM) with swab samples and isolates were identified using standard methods. Suspect MRSA isolates from Chromagar and BPA were subjected to confirmatory test using Oxoid PBP2 latex agglutination test. The disc diffusion method was used to determine resistance to antimicrobial agents.
Results: The frequency of isolation of MRSA was 2.1% (15 of 723) for pigs but 0.0% (0 of 72) for humans. Generally, for isolates of *S. aureus* from humans there was a high frequency of resistance compared with those from pigs, which had moderate resistance to the following antimicrobials: penicillin G (54.5%, 51.5%), ampicillin (59.1%, 49.5%), and streptomycin (59.1%, 37.1%), respectively. There was moderate resistance to tetracycline (36.4%, 41.2%) and gentamycin (27.2%, 23.7%) for human and pig *S. aureus* isolates, respectively, and low resistance to sulfamethoxazole-trimethoprim (4.5%, 6.2%) and norfloxacin (9.1%, 12.4%), respectively. The frequency of resistance to oxacillin by the disc method was 36.4 and 34.0% from *S. aureus* isolates from humans and pigs, respectively. Out of a total of 78 isolates of *S. aureus* from both human and pig sources that were resistant to oxacillin by the disc diffusion method, only 15 (19.2%) were confirmed as MRSA by the PBP'2 latex test kit.
Conclusions: The detection of MRSA strains in pigs, albeit at a low frequency, coupled with a high frequency of resistance to commonly used antimicrobial agents in pig and humans could have zoonotic and therapeutic implications. Finally, the diagnostic limitation of using CHROMagar and testing for oxacillin resistance by the disc diffusion method alone to determine MRSA strains without performing confirmatory tests cannot be overemphasized because the possibility of overdiagnosis of MRSA infections cannot be ignored.

Keywords: Staphylococcus aureus; *MRSA*; *methicillin*; *pigs*; *resistance*; *Trinidad*

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Annually, methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for over 2 million nosocomial infections worldwide (1–3). In the United States alone, MRSA is the tenth leading cause of death in humans, as well as the most common antimicrobial drug-resistant pathogen in hospitals and healthcare facilities, whereas in the United Kingdom it was estimated that MRSA was responsible for deaths of over 5,000 per year (4). These infections in humans include food poisoning, soft tissue infection, purulent pneumonia, upper respiratory infections, subcutaneous abscesses, toxic shock syndrome, post-operative infections and bacteremia (5–7).

Infections, particularly those that are nosocomial in nature, have been demonstrated in pet animals (cats and dogs) and horses (8, 9). Severe diseases have also been reported to be caused by MRSA in livestock (10–12). The zoonotic nature of MRSA is well documented in the literature with human owners of pet animals and

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veterinarians and other personnel associated with veterinary hospitals having either acquired or transmitted infections and diseases to or from companion animals (11, 13). Recent studies have shown that livestock, particularly pigs and veal calves, act as a reservoir for MRSA sequence type (ST) 398 or clonal complex (CC) 398 and are a potential source of human acquisition (14, 15). Although these transmissions are primarily between animals, similar isolates have been found in humans that come in contact with these pigs (10). The spread of MRSA strains from pigs to humans has been well documented in The Netherlands and Denmark (14, 16, 17).

Resistance of *S. aureus* strains to methicillin and to other antimicrobial agents have been attributed to result from the substantial misuse or abuse of antimicrobial agents, *S. aureus* has acquired resistance mechanisms (18, 19). It has been reported that *S. aureus* resistance develops either through mutations and re-arrangements within the staphylococcal genome, or by the acquisition of resistance determinants (20). *S. aureus* does this primarily through the production of altered penicillin-binding-protein (PBP) (plasmid mediated), which rendered all currently available β-lactams ineffective (21).

In Trinidad and Tobago, Adesiyun et al. (22) in a study conducted in 1992–1993, reported the frequency of resistance to oxacillin among strains of *S. aureus* from human clinical and non-clinical sources to be 0.7%. In a later study, a considerably higher prevalence (12.8%) of MRSA was detected in humans in 2000–2001 in specimens obtained from three hospitals (23). Data are unavailable on the prevalence of community-associated MRSA in the country to add to the rather old existing information from hospitalized patients. To date, there is no published report on the occurrence of MRSA strains in pigs or their human handlers. The current study was therefore conducted to determine the prevalence of MRSA and resistance to other antimicrobial agents among *S. aureus* strains recovered from pigs and their human handlers across pig farms in Trinidad.

Materials and methods

Sources of samples

This cross-sectional study was conducted between June 2010 and December 2010, when a total of 723 pigs from three age groups: nursery pigs, <10 weeks; growers/finishers, 11–22 weeks; breeders, >22 weeks; and 72 persons were sampled. The samples originated from 34 farms across the seven counties of Trinidad (Fig. 1). The distribution of farms from where samples were collected in the seven counties is shown in Fig. 2. St. Andrew County contributed the highest proportion of samples (47.1%) while St. David County provided the lowest (5.9%).

Samples were taken from pig farms from all seven counties across Trinidad. These farms were classified based on the number of pigs reared, into the following groups: small (less than 100 pigs), medium (over 100 pigs to 1,000 pigs), and large (higher than 1,000 pigs). All farms were contacted in advance to enquire about their willingness to participate in the project before being recruited into the study and all willing participants were made part of the study. No compensation or incentives were offered to the farm, farm managers, or participating handlers. The investigators however agreed and provided results relevant to the respective farmers on completion of the study.

Fig. 1. Location of farms sampled in the counties of Trinidad.

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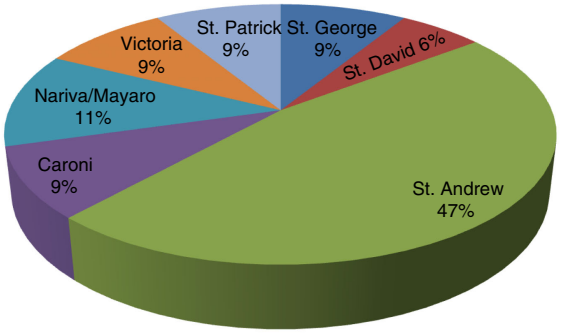

Fig. 2. Distribution of farms sampled from the seven counties.

Determination of sample size

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

$$n = [t^2 \times p(1-p)]/m^2(24),$$

where n = required sample size, t = confidence level at 95% (standard value of 1.96), p = estimated prevalence of MRSA (15), and m = margin of error.

For the study, the sample size was determined to be 600 and 264 for pigs and humans, respectively. Thereafter, the calculated sample size was proportionally distributed among the small and medium farms based on the animal population, for farmers who agreed to participate in the study.

Questionnaire survey

Different questionnaires were distributed to the farm owners and the farm employees from whom samples were to be taken. Questionnaires administered to the farmers were used to acquire demographic data, as well as to elucidate information on pigs which included their breed, age, sex, previous use of antibiotics or surgery, deworming programs on the farm, type of interaction persons had with the pigs, present illness (skin lesions, respiratory problems, etc.), length of time persons have spent working on the farm, and so on. In cases where the owner of the farm is also the individual in contact with the pigs, only one questionnaire (owner) was administered.

Each farmer who agreed to participate in the study also completed a consent form, and they were assured that the data were to be treated with the strictest confidentiality.

Sampling protocol

A sampling protocol of 90 pigs from large farms, 20 out of every 100 pigs for medium farms and small farms, a proportionate formula was used based on the total number of pigs on farm and total number pigs in the county, that is

$$n = \frac{\text{No. of pigs on farm}}{\text{Total no. of pigs in country}} \times \text{Target sample size}$$

For farms with less than 10 pigs, all were sampled.

For the study, a total of 34 farms (Fig. 1) comprising 26 small, 6 medium, and 2 large farmers were sampled for the study.

Collection of samples

On the farm, pigs were selected through convenience sampling and evenly distributed among all the pens present on the farm. Farm owners or employed farm hands assisted in the restraint, in addition to the use of a pig snare for the collection of nasal swabs.

Sterile swabs were used to collect samples from the anterior nares of both the pigs and human individuals who participated in the study. These samples were collected using sterile Transport Swabs (COPAN Innovation, USA) for human participants and sterile swabs with Amies transport media (Oxoid Ltd, Basingstoke, Hampshire, England) in test tubes were used for the sampling of pigs. All samples collected were transported to the laboratory ice-cooled. All precautionary measures were taken when collecting (latex gloves, face mask and for sampling of large farms, hair nets, and disposable gowns), storing, and handling samples given its zoonotic potential.

Isolation of MRSA and resistance of S. aureus *to antimicrobial agents*

All swabs collected were inoculated concurrently onto CHROMagar MRSA (BBL, USA), which is a selective and differential medium for the detection of MRSA strains and Baird–Parker agar (BPA) (Oxoid Ltd, Basingstoke, Hampshire, England) and plated for isolation. Each batch of samples and agar were plated along with a positive MRSA control isolate. All inoculated CHROMagar MRSA plates were incubated for 24 hours at 37°C. Isolates that appeared as mauve colonies were tentatively considered MRSA as recommended by the manufacturer of the media and as earlier reported (25). Selected colonies were then streaked onto blood agar plates and incubated aerobically at 37°C overnight. Inoculated BPA plates were incubated aerobically at 37°C for 48 hours. Colonies with typical appearance (black, black with halos and grey colonies) of staphylococci were tentatively selected as staphylococci as earlier described (11, 26) and plated onto blood agar plates. All typical colonies selected from both CHROMagar and BPA plates were Gram stained and subjected to biochemical tests using standard methods (26). All isolates confirmed to be *S. aureus* were each inoculated into 0.5 ml of brain heart infusion (BHI) broth and incubated at 37°C for 24 hours followed by the addition of 0.5 ml of sterile 50% glycerol and frozen at −80°C for later use.

The disc diffusion method (27) was used to assess the resistance of *S. aureus* strains to methicillin and other antimicrobial agents. The antimicrobial agents tested and their concentrations are as follows: ampicillin (10 μg), penicillin G (10 units), streptomycin (10 μg), tetracycline

(30 μg), sulfamethoxazole/trimethoprim (25 μg), norfloxacin (10 μg), and gentamycin (10 μg). Oxacillin discs (1 μg) were used to assess methicillin resistance due to the unstable nature of methicillin disc as earlier reported (23, 28).

All oxacillin-resistant isolates, assumed to be methicillin resistant, were subjected to a confirmatory test for MRSA using the OXOID (PBP'2) latex agglutination test Oxoid Limited, Basingstoke, Hampshire, England (29), using positive controls for each batch of testing.

Statistical analyses

The frequency of isolation of *S. aureus* strains (including MRSA strains) from different sources (pigs and human handlers) were compared using the Statistical Package for Social Sciences (SPSS) version 16 to analyze the data generated in the study. All statistical analyses were two-tailed and interpreted at the 0.05 level of significance. The Chi-square and regression analyses were done on the data.

Approval by ethics committee

The study was approved by the Ethics Committee of the Faculty of Medical Sciences prior to the commencement of the study.

Results

Comparison of the frequency of isolation of S. aureus *by BPA and CHROMagar*

The efficiency of both BPA and CHROMagar plates in isolating *S. aureus* from the samples is shown in Table 1. For 72 human samples tested on CHROMagar, 7 showed typical mauve appearance of which 5 (71.4%) were identified as staphylococci but none (0.0%) was *S. aureus.* For growths on BPA, of the 72 human samples tested which yielded 150 isolates with typical appearance, 132 (88.0%) were identified as staphylococci but only 22 (16.7%) of these were confirmed as *S. aureus.* The prevalence of *S. aureus* in human handlers' anterior nares, as determined by the BPA was 30.6% (22 of 72). The frequency of recovery of *S. aureus* from staphylococcal isolates and colonies with typical appearance on BPA was 16.7% (22 of 132) and 14.7% (22 of 150), respectively.

For samples collected from pigs, of 723 plated on CHROMagar, a total of 547 (75.7%) displayed typical mauve appearance, 485 (88.7%) were identified as staphylococci of which only 33 (6.0%) of these were confirmed to be *S. aureus.* For colonies recovered on BPA, of the 1,892 isolates with typical appearance, 1,462 (77.3%) were identified as staphylococci of which only 64 (3.4%) were confirmed as *S. aureus.* The prevalence of *S. aureus* in the anterior nares of pigs sampled was 8.9% (64 of 723). Frequency of detection of *S. aureus* from staphylococci and from isolates, which appeared typical on BPA, was 4.4% (64 of 1,462) and 3.4% (64 of 1,892), respectively.

Based on the isolation and confirmation of staphylococci and *S. aureus*, both CHROMagar and BPA each had a specificity of 100%, an indication that both were able to correctly identify isolates that were non-*S. aureus.* However, the sensitivity of CHROMagar was greater at 22.4% compared with BPA which had a sensitivity of 13.3%, that is, CHROMagar was better able to correctly identify isolates that were *S. aureus.* The differences were statistically significant ($P < 0.05$; χ^2).

Overall, the frequency of detection of *S. aureus* in human swabs following the use of confirmatory tests was 22.2% (16 of 72) compared with that of swabs from pigs which was 8.9% (64 of 723) and 4.6% (33 of 723) for isolates from BPA and CHROMagar, respectively (Table 1). The difference was statistically significantly different ($P < 0.05$; χ^2).

With the use of the Oxoid PBP'2 test kit to confirm MRSA strains from *S. aureus* strains that grew on CHROMagar, the prevalence of MRSA in the human swabs sampled was 0.0% (0 of 72) and 2.1% (15 of 723) in the pig swabs. The difference was statistically significantly different ($P < 0.05$; χ^2). For the isolates of *S. aureus* recovered from CHROMagar, the frequency of detection of MRSA strains was 0.0% (0 of 0) and 44.5% (15 of 33) for human and pig samples, respectively.

Of the 15 confirmed MRSA strains recovered from pigs, 10 (66.7%) originated from the two large farms sampled in the study.

Frequency of S. aureus *by county*

The distribution of isolation of *S. aureus* across farms in the seven counties is shown in Fig. 3. For humans,

Table 1. Frequency of detection of staphylococci and *S. aureus* on CHROMagar and BPA from human and pig swabs

Source	Media	No. of samples tested	No. of isolates with typical appearance	No. (%) of isolates positive for:	
				Staphylococcus spp.	*S. aureus*
Humans	CHROMagar	72	7	5 (71.4)	0 (0.0)
	BPA	72	150	132 (88.0)	22 (14.7)
Pigs	CHROMagar	723	547	485 (88.7)	33 (6.0)
	BPA	723	1,892	1,462 (77.3)	64 (3.4)

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Victoria county and St. David county had comparatively high frequency of isolation of *S. aureus*, 60.0% and 40.0%, respectively; while, for pig samples the frequency of isolation was generally low across counties with the highest rate (22.8%) detected in St. George County. Interestingly the county which had the highest carriage of *S. aureus* among humans, Victoria County, had the lowest among pigs (0%).

Isolation of S. aureus *by risk factors*

For humans, contact with pigs affected the carriage of *S. aureus* where persons with greater frequency of contact with pigs had a significantly higher ($p = 0.024$) carriage of *S. aureus*. For pigs, production affected the carriage rate of *S. aureus* ($p = 0.004$); where farms that had mixed production or multiple age groups had greater carriage of *S. aureus*. Farm size also played a significant role in the carriage of *S. aureus* ($p = 0.00$); with medium and large farms having a significantly higher carriage rate than small farms. Carriage rate of *S. aureus* was also significantly ($p = 0.04$) affected by age with older pigs, growers and breeders, having a greater carriage than piglets in nurseries. Previous use of antimicrobial agents did not significantly ($p = 0.111$) affect the carriage rate of *S. aureus*.

Frequency of resistance of S. aureus *to antimicrobial agents*

Table 2 shows the frequency of resistance to the antimicrobial agents tested among *S. aureus* isolates recovered from both human and pig populations. There was a high frequency of resistance among human isolates while a moderate frequency was detected among pig isolates to penicillin G (54.5%, 51.5%), ampicillin (59.1%, 49.5%) and streptomycin (59.1%, 37.1%), respectively. Moderate frequency of resistance was observed in both human and pig populations to tetracycline (36.4%, 41.2%) and gentamycin (27.2%, 23.7%), respectively. However, there was low resistance to sulfamethoxazole-trimethoprim (SXT) (4.5%, 6.2%) and norfloxacin (9.1%, 12.4%) for human and pig isolates, respectively. Resistance to oxacillin, which indicates methicillin-resistance, was comparable for human isolates, 36.4% (8 of 22) but low for pig isolates 34.0% (33 of 97). The frequency of resistance to streptomycin was significantly ($P < 0.05$; χ^2) higher among human isolates compared with pig isolates.

Overall, of the 72 humans sampled, 16 (12.2%) were carriers of *S. aureus* strains which exhibited resistance to one or more antimicrobial agents compared with a prevalence of 11.9% (86 of 723) for pig isolates.

Of the 34 farms sampled, 30 (88.2%) had isolates which were resistant to oxacillin.

The resistance patterns of *S. aureus* strains from pigs and human handlers are displayed in Table 3. For *S. aureus* isolates from humans, resistance was moderate to the penicillin group (68.2%) but high for pig isolates (83.2%). The frequency of resistance to the aminoglycoside group was moderate for both human (45.5%) and pig (49.5%) isolates. For the 'Others' group which comprised norfloxacin, tetracycline and SXT, both human and pig isolates showed a low frequency of resistance at 9.1 and 9.9% respectively. The frequency of resistance to the penicillins groups of antimicrobial agents among *S. aureus* isolates from pigs was statistically significantly ($P < 0.05$; χ^2) higher than detected for human isolates.

Frequency of resistance of S. aureus *to methicillin resistance confirmed by PBP'2*

A comparison was made between the oxacillin-resistant isolates from both pigs and human samples, detected by the conventional disc diffusion method and those confirmed by PBP'2 testing. Overall, of the 117 isolates tested, 41 (35%) exhibited resistance to oxacillin by the disc diffusion method. However, only 15 (36.6%) of the 41 oxacillin-resistant isolates were confirmed to be methicillin-resistant. The difference was statistically significant ($P < 0.05$; χ^2).

None (0.0%) of the isolates from human samples tested positive for PBP'2.

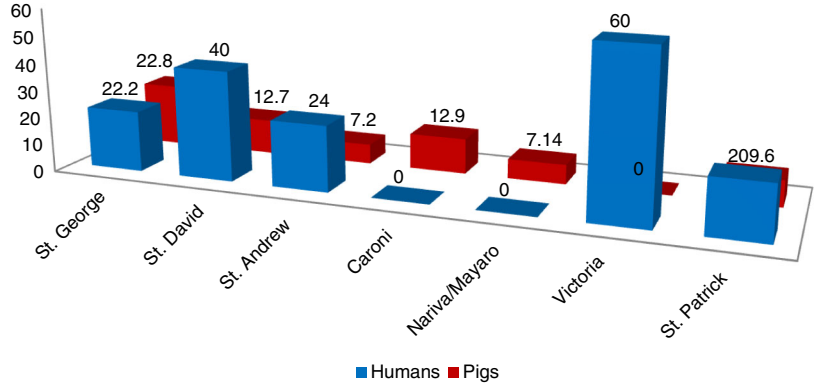

Fig. 3. Frequency of isolation of *S. aureus* from pigs and their human handlers by county in Trinidad.

Table 2. Frequency of resistance to antimicrobial agents among *S. aureus* strains from humans and pigs

Source	Number tested	No. (%) with resistant isolates	No. of *S. aureus* isolates tested	No. (%) of *S. aureus* isolates resistant[a]	No. (%) of isolates[c] resistant to:							
					Ox[b]	P	A	S	TE	CN	SXT	NOR
Human	72	16 (22.2)	22	21 (95.5)	8 (36.4)	12 (54.5)	13 (59.1)	13 (59.1)	8 (36.4)	6 (27.2)	1 (4.5)	2 (9.1)
Pigs	723	86 (11.9)	97	96 (99.0)	33 (34.0)	50 (51.5)	48 (49.5)	36 (37.1)	40 (41.2)	23 (23.7)	6 (6.2)	12 (12.4)
Total	795	102 (12.8)	119	117 (98.3)	41 (34.5)	62 (52.1)	61 (51.2)	49 (41.2)	48 (40.3)	29 (24.4)	7 (5.9)	14 (11.8)

[a]Resistant to one or more antimicrobial agents.
[b]Ox, oxacillin; P, penicillin G; A, ampicillin; S, streptomycin; TE, tetracycline; CN, gentamycin; SXT, sulfamethoxazole/trimethoprim; NOR, norfloxacillin.
[c]The predominant multi-resistance observed were as follows: OX–P–SXT (10 isolates), A–TE (8 isolates) using the total row, that is, pooled for human and pig isolates.

Discussion

Although the CHROMagar is a differential media primarily designed to screen for MRSA strains in samples, it is of interest that when it was inoculated concurrently with swabs from the anterior nares of human handlers along with BPA, for the 72 swabs samples only seven colonies displayed characteristic mauve appearance and of these, none (0.0%) was confirmed as *S. aureus* strains. This finding has both diagnostic and therapeutic implications because CHROMagar is used to screen clinical specimens for the presence of MRSA, that is, all mauve colonies are presumed to be MRSA until being further subjected to confirmatory testing, Therefore, the implication is that the occurrence of MRSA among clinical samples may be overdiagnosed and treatment regimens may be implemented inappropriately for patients (30, 31), if phenotypic features on CHROMagar alone are used as a criterion for intervention without subjecting them to confirmatory tests. This risk is definitely higher in developing countries because of costs, where the CHROMagar may be used as a screening test for MRSA without follow-up confirmatory tests such as the PBP'2 latex test which detects the *mecA* gene responsible for methicillin resistance. For this reason, it has been recommended that with the use of MRSA-selective chromogenic agar such as CHROMagar, positive samples should be confirmed rapidly by latex agglutination with antibodies directed against PBP 2a (31–33). The 0.0% prevalence for MRSA using CHROMagar in the current study may be a true reflection of the MRSA carriage by the humans sampled because CHROMagar used in the current study and other chromogenic agar were reported to have high sensitivity ($>95\%$) (32). Malhotra-Kumar et al. (34) reported that CHROMagar media have a positive predictive value for MRSA of approximately 84%. Failure to detect MRSA in personnel associated with pigs in Trinidad is considerably lower than the carriage reported in US swine workers, 45% (15), in Danish and Belgian veterinarians, 7.5% (35), in Danish food animal veterinarians, 3.9% (16) and in health care personnel in contact with pigs and veal calves in The Netherlands, 1.7% (36). However, similarly low prevalence of MRSA, 0.24% (1 of 423) was reported for apparently healthy humans with animal contact in Tunisia (37). To date, there is no published report on the prevalence of MRSA in humans associated with pig farms or other livestock farms in the country, although rates of 0.7 and 12.8% have been reported in human clinical and non-clinical specimens (22, 23).

In comparison, with the use of CHROMagar on pig anterior nares samples, the prevalence of *S. aureus* strains was 4.6% (33 of 723) of which a total of 15 strains were confirmed to be MRSA by the Oxoid PBP'2 latex test kit. The prevalence of 2.1% (15 of 723) for MRSA in pigs detected in the current study is higher than the 0% found

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Table 3. Frequency of multi-resistance of *S. aureus* to antimicrobial agents among human and pig isolates

Antimicrobial class/group	Antimicrobial agents	No. (%) of isolates resistant from:	
		Humans ($n = 22$)	Pigs ($n = 101$)
Penicillins	Penicillin G and ampicillin	15 (68.2)	84 (83.2)
Aminoglycosides	Gentamycin and streptomycin	10 (45.5)	50 (49.5)
Others	Norfloxacin, tetracycline and SXT	2 (9.1)	10 (9.9)

for human handlers on the pig farms but much lower than the prevalent rates reported elsewhere, for example, 49% in the United States (15), 8% in pigs sampled at fairs in the United States (38), 49–70.8% in slaughter pigs at abattoirs in Germany (39), and 28% in Iberian pigs (40). This is considered the first documentation of MRSA strains on pig farms in Trinidad. The rather low prevalence of MRSA detected in pigs is an indication that MRSA strains may not fully established on pig farms in the country and may be responsible, in part, for the failure to isolate the strain from the human handlers sampled. The possibility of management playing some role in the lack of transmission from pigs to man cannot be ignored. However, it is pertinent to mention that sampling from the anterior nares of pigs alone may have been a limitation since it was reported that the skin behind the ears was the anatomical site with the highest relative sensitivity (91.4%) for MRSA detection compared to perineum and anterior nares, with a relative sensitivity of 76.5 and 75.3%, respectively (33). Pigs have been reported to be an important reservoir of zoonotic ST 398 and CC 398 (14, 15) and human infections acquired through contact with pigs are also well documented in the literature (14, 16, 17, 35, 36, 41). The public health risk posed by the detection of MRSA in pigs in the current study, albeit at a low frequency, to humans either on the farms or to abattoir workers during slaughter can therefore not be completely ignored.

The BPA, a well-known selective media for the isolation of staphylococci (26), which was used to culture the same swab samples plated on CHROMagar detected a significantly higher prevalence of *S. aureus* in human handlers (30.6%) than found in pigs (8.9%). This is in agreement with published reports which have documented higher carriage rates of *S. aureus* in the anterior nares of apparently healthy human beings (42, 43). In the current study, the carriage rate (8.9%) detected in pigs is higher than that reported for pigs in the United States, 15.9% (38), while the rate (30.6%) found in humans is higher than the carriage rates of 23.4 and 28% reported in apparently healthy humans in Malaysia (44) and Australia (42), respectively. The fact that the prevalence for *S. aureus* in human and pig samples was 0.0% (0 of 72) and 4.6% (33 of 723), respectively, with the use of CHROMagar is lower than that found by the use of BPA is surprising because a study which compared the ability of CHROMagar and conventional media for isolating *S. aureus* had reported that the efficiency was similar (45).

The location of farms by county and the number of samples (human and pigs) tested did not significantly affect the carriage rate for *S. aureus* as reflected by the fact that Victoria county which represented the lowest proportion (9%) of farms sampled recorded the highest carriage rate (52%) for *S. aureus* compared with St. Andrew county which provided 47% of the farms but the carriage rate detected for *S. aureus* was 31% and the differences were statistically significant. This is an indication that the location and number of farms may not be important in affecting the carriage rate of *S. aureus* but other factors such as management practices may be more relevant. It is pertinent to mention that the degree of contact of human handlers with the pigs on the sampled farms significantly increased the carriage rate for *S. aureus* in the current study.

In comparison to a study in the United States conducted by Smith et al. (15), where the prevalence of carriage of *S. aureus* was highest among piglets, in this study the highest carriage was among older pigs such as growers and breeders. This difference may be due, in part, to the fact that in Trinidad and Tobago, farmers have free access to antimicrobial agents and their use or over use. Older pigs present on farms may therefore have been exposed to antimicrobial agents (including oxacillin) for considerably longer periods of time than younger pigs or piglets. It is also pertinent to mention that Weese et al. (46) in a Canadian study reported that the overall pre-weaning prevalence for MRSA was 34.5% compared with a post-weaning prevalence of 85%. The same study further reported that there was a significant association between sow and piglet colonization, an indication that age alone may be responsible for colonization on pig farms.

It is of therapeutic relevance to have detected widespread resistance to a number of antimicrobial agents used for treatment of humans and animals on livestock farms in the country. The overall prevalence of resistance by *S. aureus* strains to one or more of the eight antimicrobial agents tested is high for both human (95.5%) and pig (99.0%) isolates tested. The prevalence of resistance detected in the current study is very high

compared to published reports by others (47, 48). In a study conducted on *S. aureus* isolated from dairy cows and milk, Adesiyun et al. (49) reported the prevalence of resistance to nine antimicrobial agents tested to be 18.7 and 12.9% among bulk milk and composite milk isolates of *S. aureus,* respectively, compared to 49.3 and 69.5% among isolates from human anterior nares and hand swabs, respectively. Resistance to antimicrobial agents has been attributed to misuse or abuse of antimicrobial agents in human and veterinary practice (18, 19, 50). The findings in the current study did not come as a surprise since livestock farmers have access to antimicrobial agents at local feed stores without the input of veterinarians, a practice common in most developing countries.

Also of therapeutic relevance is the finding that of the eight antimicrobial agents tested, in five of these (oxacillin, penicillin, ampicillin, streptomycin, and gentamycin), human isolates had higher prevalence of resistance than pig isolates. Again, this may also reflect the uncontrolled access and use of antimicrobial agents in humans and animals in the country. It has been documented that there could be an exchange of resistant strains of bacteria between humans and animals (pets and livestock) (11, 13).

The rather high prevalence of resistance displayed to penicillin, ampicillin, streptomycin and tetracycline both in prevalence either to individual agents or as multi-resistance was again not unexpected because they are commonly used in medical and veterinary practices in the country. The high prevalence of resistance exhibited by *S. aureus* against these three antimicrobial agents is higher than that reported in other countries by others (48, 51, 52). The comparatively lower prevalence of resistance expressed by *S. aureus* strains from both human and pig isolates to norfloxacin, gentamycin, and SXT reflect less frequent use on the farms due to cost and availability. The low prevalence of resistance to the three antimicrobial agents has been reported by others elsewhere (48, 51), although another study reported a prevalence of 33.6% to gentamycin (52).

For both human and pig isolates of *S. aureus*, the prevalence of resistance to oxacillin was 34.52% (41 of 119) by the disc diffusion method. This is important because it is used to assay for resistance to methicillin, which is considered unstable while oxacillin is better at maintaining its activity when stored (28, 53). Furthermore, it is a common practice to determine methicillin resistance by the disc method in most local diagnostic laboratories and the emanating results used as a basis for therapy. Of concern is the fact that only 36.6% (15 of 41) of the isolates of *S. aureus* determined to be oxacillin resistant by the disc method were confirmed to be MRSA by the PBP'2 diagnostic test. By extension, it means that $>80\%$ of the isolates were wrongly classified as MRSA by the disc diffusion method which may lead to inappropriate therapeutic intervention, particularly in humans. Another possibility or explanation is that methicillin resistance may be brought about by other novel genes (for example mecC genes, etc.) different from the mecA gene detected by the PBP'2 test. Reports have been variable in the estimates of the oxacillin in detecting MRSA, 77.3% to 96.4% for the sensitivity and 84.6% to $<100\%$ for the specificity (28, 53), rates which are considerably higher than found in the current study. A number of limitations have been associated with the detection of MRSA which include a difficulty due to the presence of subpopulations within a given culture of staphylococci where one is susceptible and the other is resistant. It has been stated that all cultured cells have the genetic ability to express resistance; however, only some populations express that resistance in vitro in a phenomenon referred to as heteroresistance (54). Heteroresistance is reported to be associated with staphylococci that are resistant to penicillinase-stable penicillins, such as oxacillin, and because these cell populations grow at a slower rate than oxacillin-susceptible populations, has led the Clinical and Laboratory Standards Institute (CLSI) to recommend that plates be incubated for a full 24 hours at 33–35°C (55).

It is concluded that MRSA strains exist on pig farms in Trinidad albeit at a low prevalence of 2.1%, with all isolates recovered from swabs of anterior nares of pigs sampled while all human samples were negative for the organism. The use of CHROMagar alone to detect MRSA resulted in a false-positive rate of 9.7% (7 of 72) and 73.6% (532 of 723) in human and pig isolates respectively based on typical phenotypic appearance on the media. The use of the PBP'2 latex test to confirm MRSA identified only 19.2% of 76 isolates determined to be oxacillin-resistant strains by the disc diffusion method as MRSA. Failure to employ diagnostic tests after screening samples with CHROMagar and the use of the oxacillin disc diffusion method is most likely to results in false-positive resistance and therefore an over-estimation of MRSA and unnecessary chemotherapy. This is of particular relevance in developing countries where cost of diagnostic tests may be unaffordable and not readily available. Easy and uncontrolled access of pig farmers and members of the human population in the country to antimicrobial agents may be responsible, in part, to the relatively high resistance to penicillin, ampicillin, streptomycin and tetracycline detected among *S. aureus* strains from both human and pig sources.

It is therefore recommended that there is need for stricter control of the use of antimicrobial agents in both the medical and veterinary profession in the country to reduce the apparent abuse or overuse of antimicrobial agents. Second, although the CHROMagar is a known screening media for the detection of MRSA strains,

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therapeutic intervention should only take place after instituting confirmatory tests on *S. aureus* strains, which exhibited typical phenotypic appearance on the agar. Third, although oxacillin is used to assess methicillin resistance among *S. aureus* by the disc diffusion method, it is recommended that all oxacillin-resistant strains are subjected to confirmatory test before treatment is instituted. Finally, the 15 strains of MRSA strains recovered from pigs should be subjected to molecular typing to determine their sequence types, particularly ST 398, which has never been documented in pigs or other animal species in the Caribbean region.

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