Antibiotic resistance patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from livestock and associated farmers in Anuradhapura, Sri Lanka

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Abstract

Introduction **The animal husbandry comes to play an important role according to new economic reforms of the rural economy in South Asia including Sri Lanka, and the rural farming community has a poor knowledge about hygienic issues of animal husbandry, which can lead to spread of pathogenic bacterial strains from animals to humans. Our study was conducted to evaluate methicillin susceptible *Staphylococcus aureus* (MRSA) colonization and its antimicrobial resistance pattern among livestock (n=188) and related farmers (n=94) in Anuradhapura District, Sri Lanka.**

Methods ***S. aureus* isolates were identified using mannitol salt agar, coagulase test and DNAase test. The agar plate dilution method was conducted to determine the minimum inhibitory concentration (MIC) of oxacillin against *S. aureus.* Antimicrobial susceptibility testing for other antibiotics was performed against MRSA isolates using antibiotic containing discs. To assess the MRSA transmission from livestock to humans, we have grouped MRSA strains according to antimicrobial susceptibility patterns against the tested antibiotics.**

Results **Among MRSA isolates, 14 different groups with similar MIC and antibiotic susceptibility patterns were identified. Of those, 2 groups amongst pigs and pig farmers showed a significant relationship (p=0.031). The other groups did not show any significant relationship between animals and the farmers. The percentages of MRSA prevalence in pigs and pig farmers were 26.6% each, in poultry and poultry farmers 8.3% and 13.3% respectively, in cattle and cattle farmers 8.3% and 3%. Compared to human MRSA isolates, animal isolates were significantly more resistant to ciprofloxacin (p=0.031), gentamicin (p=0.010) and clindamycin (p=0.011). Similarly, animal methicillin susceptible *Staphylococcus aureus* (MSSA) isolates were significantly more resistant to ciprofloxacin (p=0.022) and doxycycline (p=0.012).**

Conclusion **Pig farming showed a higher prevalence and 2.4 times higher risk (OR=2.4, CI95%: 1.2-4.8) of likely transmission of MRSA between animals and humans than cattle and poultry farming. Overall, 65% of MRSA and MSSA animal isolates were multidrug resistant.**

Keywords **Animal husbandry, MRSA, cross transmission, antimicrobial resistance and hygiene**

Introduction

Staphylococcus aureus (SA) is a human pathogen which can cause a wide array of diseases, including skin and soft tissue infections, osteomyelitis, infective endocarditis, pneumonia and sepsis. Owning to the emergence of "superbugs", methicillin-resistant *Staphylococcus aureus* (MRSA) represents a global therapeutic

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challenge because many of these MRSA isolates are multidrug resistant.[1]

According to the source of origin, the most prevalent categories of MRSA are hospital acquired MRSA (HA-MRSA) and community acquired MRSA (CA-MRSA).[1,2] However, in 2005, the existence of a MRSA clone distinct from HA-MRSA and CA-MRSA has been reported and the clone was observed to be associated with livestock, including pigs, cattle, chicken and turkey.[2] This new clone was identified as livestock-associated MRSA (LA-MRSA).[3] LA-MRSA differs from CA-MRSA, because it lacks the Panton Valentine Leukocidin (PVL) gene and has broader, variable patterns of antimicrobial susceptibility. Furthermore, recent detection of a *mecA* homologue, designated *mecC*, was detected among wild animals and water samples with a wide geographical distribution in Europe.[4]

The pattern of antimicrobial susceptibility varies according to the degree and duration of livestock exposure to antimicrobials.[5] Since antimicrobials are commonly used in livestock as growth promoters, such exposure is threatening because it facilitates the evolution of antimicrobial resistance. If antimicrobials are used in sub-therapeutic concentrations, the tendency to develop antimicrobial resistance is high.[6] In animal gut these resistance genes can be horizontally transferred and animal-emerging antibiotic resistance genes can finally be acquired by human pathogens by resistant food contaminants that originate from animals and are consumed or handled by humans. In addition, MRSA cross contamination can occur following entry into and transmission via healthcare facilities or spread via environmental routes including air, water, or manure.[7,8]

However, recently, the United States of America and some European countries including the Netherlands, Denmark and Norway, have implemented the necessary legislation to minimize antibiotic use in livestock.[9] During the past decades, the food habits of the people have dramatically changed from plant to animal origin with rising trends in consumption of foods of animal origin in the South Asian region.[10] According to new economic reforms, traditional animal husbandry for cattle has been expanded. The small-scale farming of poultry and piggery was introduced to face the increased demand of egg and meat products. Therefore, the animal husbandry plays an important role in the rural economy in South Asia, including Sri Lanka. However, the rural farming community has a poor knowledge about hygienic issues of animal husbandry.[11] Thus poor hygiene can lead to spread of pathogenic bacterial strains from animals to humans.[12] A French study has described that pig farming is a risk factor for increased nasal *Staphylococcus aureus* colonization in humans.[13]

In Sri Lanka, animal health is regulated by the department of animal products and the attention has been focused on bovine tuberculosis, livestock associated coliforms and avian influenza.[14] Although MRSA was detected in healthcare settings and the community for decades, in Sri Lanka, the livestock related MRSA prevalence has not been studied yet.[15] Hence, we conducted a study to evaluate MRSA and SA colonization and its antimicrobial resistance patterns among livestock and related farmers in Anuradhapura District, Sri Lanka.

Methods

Study population

This is a descriptive cross-sectional study which was conducted among the livestock farming community in Anuradhapura District of Sri Lanka in January-March 2017. Demographical data were collected using an interviewer based questionnaire. The farmers who were hospitalized within the previous 12 months or had used antibiotics within the previous 30 days were excluded from the study.

MRSA isolation

After explaining the procedure, nasal and axillary swabs were collected from livestock farmers using moistened sterile cotton swabs. A total of 188 (94 nasal and 94 axillary) swabs were obtained from farmers. Two animals from each farm were randomly selected and 188 (62 pigs, 64 chicks and 62 cattle) nasal or perineal or peri-anal swabs were collected from animals. The swabs were incubated overnight in brain heart infusion (BHI HiMedia, Mumbaim India) broth containing 7.5% NaCl at 37°C in ambient air incubator. All the swabs were sub-cultured into blood agar (HiMedia) and MacConkey agar (HiMedia). Blood agar plates showing a growth were again sub-cultured to mannitol salt agar to select SA. Selected SA cultures were confirmed further, using DNAase and tube coagulase tests. The isolated SA strains were assayed for methicillin resistance using the agar plate dilution method. A dilution series was prepared using oxacillin (Sigma, St. Louis, MO, USA) compounds in the concentration range 0.03–128 μg/mL. SA was suspended in water using a cotton swab until the visual density was equal to McFarland 0.5 standard. The agar plates which contained oxacillin compounds were inoculated using SA suspension. The plates were incubated for 24 hours at 37°C and were observed for white colored spots indicative of growth of the organism. NCTC 6571 SA was used for quality control of the dilution series.[16] The isolates having a minimum inhibitory concentration (MIC) ≥ 4 μg/mL against oxacillin were identified as MRSA. Oxacillin resistance was cross-confirmed using cefoxitin disc diffusion test.[17,18]

Antibiotic susceptibility tests

Antimicrobial susceptibility testing (disk diffusion test) was performed against MRSA isolates using teicoplanin, vancomycin, linezolid, doxycycline, penicillin, ciprofloxacin, clindamycin, erythromycin, gentamicin, sulfamethoxazole-trimethoprim, chloramphenicol, fusidic acid (Sigma) and for MSSA isolates, in addition to the above, ampicillin, cefuroxime and amoxicillin/clavulanic acid were used. The disc diffusion test was conducted according to Clinical and Laboratory Standards Institute (CLSI M100-S27) guidelines and CLSI breakpoints were taken to determine the antimicrobial resistance and susceptibility.[18]

Antibiogram typing has for many years been and still is in the field of clinical microbiology a first-line method to identify possible cases of bacterial cross-transmission in healthcare institutions.[19] This phenomenon was employed to assess the MRSA transmission from livestock to humans. We have grouped MRSA strains according to the antimicrobial susceptibility pattern (ASP) of each strain against the tested antibiotics. Strains having exact similarity in antimicrobial susceptibility were taken as similar, indicating a likely cross transmission between livestock and humans. Furthermore, oxacillin MIC was also taken in to match those strains. The data were double checked and transported to SAS 9.1 (2005, New Jersey, USA) for statistical analysis.[20] Demographic data were expressed as central tendency. MRSA and MSSA colonization percentages were analyzed using Chi-squared/Fisher's exact test for statistical significance. Logistic regression was performed to assess the risk of transmission of MRSA and MSSA strains between humans and livestock. Risk was expressed as odds ratio with 95% confidence interval.

Results

In our study cohort, males were significantly more engaged in livestock farming than females (p=0.021). The cattle farmers were traditionally doing farming over a period of 20±2.5 years (p=0.021) while piggery and poultry farmers were recently introduced in farming, and had been engaged over a period of 5±2 years.

Prevalence of MSSA and MRSA among farmers and livestock

Fifteen MRSA isolates (15.9%) were identified from farmers and 26 isolates (13.8%) were identified from livestock. Twenty-five MSSA (26.6%) isolates were identified from farmers and 46 (24.4%) isolates were identified from livestock. Significantly higher percentages of MRSA ($\chi(1)=7.8$, p=0.003) and MSSA ($\chi(1)=8.8$, p=0.004) carriage were observed among pigs and pig farmers than in other livestock and related farmers (Table 1). Further, among livestock farmers nasal MRSA carriage was significantly higher (100%) compared to axillary carriage (56%) (p=0.021).

Antibiotic susceptibility patterns of MRSA and MSSA in farmers and livestock

The MRSA isolates were divided into groups according to their MIC against oxacillin and their antibiotic susceptibility pattern against

Table 1. Prevalence of MRSA and MSSA among livestock and related farmers

Isolate	Livestock			Farmers			χ(df)=value and p value
	Pigs n=62	Poultry n=64	Cattle n=62	Piggery n=31	Poultry n=32	Cattle n=31	
MRSA	16 (25.8%)*	6 (9.3%)	4 (6.2%)	8 (25.8%)*	4 (12.5%)	3 (9.6%)	(1)=7.8 0.003*
MSSA	20 (32.2%)**	14 (21.8%)	12 (19.3%)	16 (51.6%)**	5 (15.6%)	4 (12.9%)	(1)=8.8 0.004**

MRSA – methicillin resistant *Staphylococcus aureus*; **MSSA** – methicillin susceptible *Staphylococcus aureus*.
*MRSA percentage in pigs and piggery farmers; **MSSA percentage in pigs and piggery farmers.

other tested antibiotics. The MRSA strains having the same MIC against oxacillin and a similar antibiotic sensitivity pattern against the assayed antibiotics were put into one group. Among MRSA isolates, 14 groups with similar MIC and antibiotic susceptibility pattern were identified. Of those, 2 groups [the MIC > 128 μg/mL group (χ(1)=11.4, p=0.031) and the MIC = 64 μg/mL group (χ(1)=710.4, p=0.031)] between pigs and pig farmers showed significant relationship (Table 2). Exact MIC against oxacillin and ASP between human and livestock strains indicated the likely transmission between animals and human. The isolates from chick and poultry farmers and from cattle and cattle farmers did not show significant similarities in terms of antibiotic susceptibility patterns. MRSA isolates among 8 groups (5 human-pig; 2 human-cattle and 1 human-chick) were resistant to ciprofloxacin, clindamycin, erythromycin and gentamicin and all groups were susceptible to vancomycin, teicoplanin, linezolid and fusidic acid (Table 2).

A significant similarity of susceptibility percentages between human and animal isolates was observed for clindamycin, ciprofloxacin and gentamicin. All the MRSA and MSSA strains isolated from livestock were resistant to clindamycin and all the MRSA isolates were resistant to gentamicin and ciprofloxacin. None of the strains isolated from livestock farmers showed total resistance (100%) against any of the antibiotics assayed (Table 3).

Based on ASP and MIC compatibility among MRSA isolates, there was a 2.4 times higher risk of transmission between pigs to piggery farmers (OR=2.4, 95%CI: 1.2-4.8) while there was no significant risk of transmission from cattle to humans (OR=0.7, 95% CI: 0.1-0.8) and from chick to humans (OR=0.8, 95%CI: 0.2-1.3).

Discussion

The prevalence of SA has rapidly changed over last few decades over the globe, thus, novel data are needed. In developing countries, the demand for processed meat products is significantly increased because of lifestyle changes due to rapid urbanization. In Sri Lanka, the production of poultry products has been increased significantly during the last five years.[21] This situation is quite common in other South Asian countries including India. In this study, we assessed colonization with MRSA among Sri Lankan livestock and related farmers. The study showed that the MRSA prevalence among livestock was 13.8% (pigs: 25.8%, poultry: 9.3% and cattle: 6.2%) which is lower when compared with European countries, where the percentage in Germany and Netherlands was >35%[22,23] and in Poland 20.6%.[24] However, some Asian countries exhibit lower carriage of MRSA in livestock as is the case in China: 11.4%,[25] Korea: 3.2%,[22] Malaysia: 1.4%,[24] Japan: 0.9%,[29] and Taiwan: 14.4%.[26] In our study, MRSA carriage among livestock farmers was 15.9% (piggery: 25.8% poultry: 12.5% and cattle: 9.6%). Nasal MRSA carriage was significantly higher compared to axillary carriage. A French study in 2005 showed nasal MSSA carriage was predominant in piggery farmers.[13] Similar to livestock, the livestock farmer's MRSA carriage also showed low prevalence compared to Europe and the USA.[21-26] But the Sri Lankan prevalence is higher than that of other Asian countries, which report

Table 2. Groups of MRSA isolates among farmers and livestock; antimicrobial susceptibility patterns of MRSA with oxacillin minimal inhibitory concentration (µg/mL)

Group number according to ASP and MIC	Oxacillin MIC (µg/mlL)	Antimicrobial susceptibility pattern							Number of strains from livestock			Number of strains from farmers			χ(df)= value and p value
		Doxycycline	Penicillin	Ciprofloxacin	Clindamycin	Erythromycin,	Gentamicin	Trimethoprim-sulfamethoxazole	Pigs	Chicks	Cattle	Pig farmers	Poultry farmers	Cattle farmers	
1	>128	S	R	R	R	R	R	S	4			2			(1)=11.4 0.031
2	64	R	R	R	R	R	R	S	8*	2		4*	1		(1)=10.4 0.031*
3	128	S	R	S	S	R	S	S	-			1			-
4	>128	R	R	S	S	S	R	S	-			1			-
5	32	R	R	R	R	R	R	R	1			-			-
6	64	R	R	R	R	R	R	S	2						-
7	64	S	R	R	R	R	R	S	1						
8	128	R	R	S	S	R	S	S					1		
9	>128	R	R	S	S	S	S	S					1		
10	32	R	R	R	R	R	R	S		3					
11	32	S	R	R	R	R	R	S			2			2	0.06
12	>128	R	R	R	R	R	R	S			1			1	0.06
13	32	S	R	R	R	S	R	S			1				
14	32	R	R	R	R	S	S	S			1				

ASP – antimicrobial susceptibility pattern; **R** – resistant; **S** – susceptible.
All MRSA isolates were susceptible to vancomycin, teicoplanin, linezolid and fusidic acid. P value <0.05 was taken as significant.

rates of 5.5% in Malaysia,[23,24] 13% in Taiwan[25] and 1.7% in China.[26]

This wide variability between West and East still needs to be explained. Perhaps this could correlate with the scale of farming and the use of antibiotics and antibiotic analogues as feed additives. In Sri Lanka, use of sub-therapeutic concentrations of antimicrobials is well regulated and only the following are allowed for use: bacitracin, virginiamycin, flavomycin and avilamycin.[27] Further, the methods deployed for MRSA sampling and isolation can also vary among studies. In our study, MSSA carriage among livestock was 26.6% and 24.4% among farmers, whereas both values stood at the middle range in the difference between Eastern and Western countries. Doxycycline resistance was almost similar in human (66.6%) and livestock MRSA (61.5%) isolates. In Sri Lanka, in the period prior to 1996, doxycycline-based growth promoters were used extensively but they are currently banned. However, the use of doxycycline needs to be further investigated as the persistence of such resistance might indicate continued use in sub-therapeutic concentrations.[27,28] All isolates were susceptible to vancomycin, teicoplanin and linezolid. Further, most of the MRSA isolates were susceptible to

Table 3. Comparison of antimicrobial resistance patterns of MSSA and MRSA isolates among farmers and livestock in the study cohort

Antibiotic	Number and percentage (%) of resistant isolates in animals (n=188)		Number and percentage (%) of resistant isolates in farmers (n=94)		Relationship of animal and farmer susceptibility percentage (χ(df)=value and p value)	
	MSSA (n=46)	MRSA (n=26)	MSSA (n=25)	MRSA (n=15)	MSSA	MRSA
Doxycycline	29 (62.5%)	17 (61.5%)	4 (16.6%)	10 (66.6%)	(1)=9.2 0.012	- >0.05
Trimethoprim-sulfamethoxazole	3 (6.6%)	1 (3.2%)	1 (3.6%)	1 (3.6%)	- >0.05	- >0.05
Clindamycin	46 (100%)	26 (100%)	21 (85%)	11 (73.3%)	- >0.05	(1)=8.9 0.011
Ciprofloxacin	28 (60%)	26 (100%)	3 (13.3%)	10 (73.3%)	(1)=10.4 0.022	(1)=9.5 0.031
Gentamicin	20 (44.3%)	26 (100%)	5 (32.3%)	10 (73.3%)	- >0.05	(1)=10.3 0.010
Chloramphenicol	3 (7.2%)	1 (4.2%)	2 (8.6%)	1 (3.6%)	- >0.05	- >0.05
Ampicillin	40 (88%)	-	19 (76%)	-	- >0.05	-
Cefuroxime	16 (34%)	-	8 (32%)	-	>0.05	-
Amoxicillin-clavulanate	7 (16%)	-	3 (12%)	-	- >0.05	-

MRSA – methicillin resistant *Staphylococcus aureus*; **MSSA** – methicillin susceptible *Staphylococcus aureus*.
All MRSA and MSSA isolates were susceptible to vancomycin, teicoplanin, linezolid and fusidic acid. P<0.05 was considered as significant.

chloramphenicol and co-trimoxazole, thus reflecting little relative exposure.[29] MRSA carriage among cattle (6.2%) and cattle farmers (9.6%) was the lowest among the livestock and farming community. Sri Lanka has 25,000 water bodies, consisting of reservoirs and man-made lakes,[30] and cattle farming has been traditionally performed here for centuries. the The emergence and transmission of antibiotic resistant bacteria related to cattle farming appears to be minimal compared to piggery and poultry. This may be due to the fact that cattle live in open environments near reservoirs, and are fed with natural green grass which grows in those areas.

A significantly higher prevalence and similar antibiotic susceptibility patterns of MRSA and MSSA between pigs and pig farmers was observed. This association can be explained as MRSA and MSSA carriage is high among pigs, animals with a relatively long life span up to their slaughter age (6 months), and which require high contact time and demand a maximum effort of handling compared to chicks (life span 28-42 days). When compared to cattle (with a mean life span of 3-5 years), pigs have shorter life spans but the effort of handling and the contact time is higher. In our cohort, another important factor is that pigs live in packed rooms with low mobility.

The greater risk of acquiring MRSA in pig farming was also observed in several other countries. The prevalence of MRSA in the general community in the Netherlands is among the lowest in the Europe (<1%). However, MRSA was isolated in rates of 30-45% among pig farmers in the Netherlands. In Denmark, it has been observed that 46% of pigs were positive for MRSA harboring the CC398 clonal complex. From a large-scale study in Germany it has been found that 70% of swine farms are positive for MRSA.[31] In the Asian region, Malaysia exhibits a MRSA prevalence of 1.38% among pigs and 5.5% among pig farmers.[32] In the USA, the percentages are 49% among pigs and 45% among pig related workers.[33] Japan has reported the presence of livestock associated gene segments (ST398, ST9, ST5, ST97 and ST705) among pig farmers.[34]

The lack of typing data is a drawback of our study. Compared to genotyping and multilocus sequence typing, antibiogram based phenotypic methods have a low sensitivity and specificity.[35] Therefore, genotypic comparisons would have ensured a stronger relationship for data on human and livestock transmission compared to the one reported in our current results.

MRSA and SA are suitable for phenotyping since they are metabolically diverse within their species.[35] However, the data presented about antibiotic susceptibility patterns and colonization patterns indicate that the carriage and transmission of MRSA in pig farming could be significant in Sri Lanka.

Conclusions

Pig farming showed a higher prevalence and 2.4 times higher risk of likely transmission of MRSA between animals and humans than cattle and poultry farming. Overall, 65% of MRSA and MSSA animal isolates were multidrug resistant. The use of doxycycline needs to be further investigated as the persistence of such resistance might indicate continued use in sub-therapeutic concentrations. Furthermore, health education related to hygienic measures in livestock needs to be performed regularly.

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