

Prevalence of coagulase-positive methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in dogs in Bangladesh

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Abstract

Background: The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) have a significant health impact on people with direct or supportive occupations in veterinary medicine including veterinarians, animal handlers, laboratory personnel and pet owners.

Objectives: This cross-sectional survey was conducted to determine the prevalence of and risk factors for *S. aureus*, *S. pseudintermedius*, MRSA and MRSP in dogs in Bangladesh.

Methods: A total of 358 swab samples were collected from different body sites of 150 dogs attending a university teaching veterinary hospital between January and June 2018. Standard bacteriological procedures were followed to isolate *Staphylococcus*, and identification was confirmed to the species level by PCR to detect the *nuc* gene. MRSA and MRSP were confirmed by the presence of the *mecA* gene.

Results: The prevalence of coagulase-positive *S. aureus* and *S. pseudintermedius* in dogs were 16% and 45.3%, respectively. *S. aureus* and *S. pseudintermedius* isolates displayed the highest resistance against nalidixic acid (95.2% and 91%, respectively) and erythromycin (89.3% and 84.7%, respectively). Notably, all the staphylococcal isolates showed resistance to ≥ 3 antimicrobial classes. The prevalence of MRSA and MRSP in dogs was 8.7% and 6%, respectively. Multivariable logistic regression analysis identified the following variables as risk factors for MRSA colonisation in dogs: dogs with dermatitis (odds ratio [OR], 12.24, 95% CI: 3.12–57.33; $p < 0.001$) and history of antibiotic use (OR 8.73, 95% CI: 2.23–43.10; $p < 0.001$). Presence of otitis (OR 14.22; 95% CI: 1.64–103.58; $p = 0.008$) and oral lesions (OR 9.48, 95% CI: 1.14–64.82; $p = 0.002$) were identified as the significant risk factors for the carriage of MRSP.

Conclusions: The circulation of multidrug-resistant *S. aureus* and *S. pseudintermedius* is a serious concern to dogs and humans. To our knowledge, this is the first report of *S. pseudintermedius* and MRSP affecting dogs in Bangladesh.

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KEYWORDS

carriage, dogs, MRSA, MRSP, risk factors

1 | INTRODUCTION

Staphylococci represent significant opportunistic bacterial pathogens in humans and animals. The most common species associated with companion animal infections are coagulase-positive *Staphylococcus aureus* and *Staphylococcus pseudintermedius* (Weese & van Duijkeren, 2010). *S. aureus* causes many clinical conditions in humans and animals, ranging from mild skin infections to life-threatening bacteremia (Kong et al., 2016; O'Gara, 2017). *S. pseudintermedius*, a skin commensal, is frequently isolated from dogs with cutaneous and wound infections (Weese & van Duijkeren, 2010). Humans are not permanently colonised with *S. pseudintermedius*, but can become transient carriers if they come in close contact with infected dogs. Therefore, zoonotic transmission of *S. pseudintermedius* from dogs to humans is a public health concern (Paul et al., 2011). Moreover, humans colonised with *S. pseudintermedius* may act as a vehicle for transmission between animals which is also an important concern.

Since the inception of antibacterial drugs into the practice of modern medicine, resistant staphylococci have evolved in response to antibiotic selective pressure. Many staphylococcal species exhibit some degree of antimicrobial resistance (Schwarz et al., 2017). Moreover, a number of reports on companion animals colonised or infected with multiple drug-resistant organisms, such as methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *S. pseudintermedius* (MRSP) have been published (Algammal et al., 2020; Murphy et al., 2009). Over the past decade, there has been significant concern about antimicrobial resistance that accrete considerable public health attention, namely the emergence and spread of MRSA and MRSP in humans and animals (Guardabassi et al., 2013). In human medicine, methicillin resistance in *S. aureus* strains have contributed to the scope of multidrug resistance since the early 1960s (Barber, 1961). Infection with MRSA in small animals, particularly in dogs, has been recorded in many countries, with wound infections, surgical site infections, pyoderma, pyogenic endocarditis, suppurative pneumonia, osteomyelitis, septic arthritis, otitis and urinary tract infections (Algammal et al., 2020; Weese & van Duijkeren, 2010). Over the past few years, *S. pseudintermedius* has gained importance due to the increasing rate of resistance to methicillin and non- β -lactam antibiotics. There are several reports published on *S. aureus* and *S. pseudintermedius* isolates showing resistance to many antimicrobials authorised for use in veterinary medicine (Perreten et al., 2010; Weese & van Duijkeren, 2010). Both MRSA and MRSP infections have been shown to occur in humans and animals that have high zoonotic and zoonoanthropotic potential. Similarly, pets are increasingly considered potential reservoirs of MRSA and MRSP in cases of refractory or recurrent human infections (Loeffler & Lloyd, 2010).

Dogs are regarded as one of the best ancient companion animals and remain in close contact with humans. Like other parts of the

world, the tendency towards rearing dogs is increasing nowadays in Bangladesh. Thus human–animal behavioural relationships are becoming more intimate which may create a potential chance to transmit zoonotic pathogens like *S. aureus* and *S. pseudintermedius* from dogs to humans. However, information on the magnitude of *S. aureus* and *S. pseudintermedius* infection in dogs in Bangladesh and their antimicrobial resistance pattern is limited, if not absent. As these organisms are usually resistant to a wide range of antimicrobial agents (Algammal et al., 2020), clinical management of animals infected with MRSA and MRSP represents a great challenge to the veterinary profession. The objectives of this study were to determine the prevalence of *S. aureus* and *S. pseudintermedius* in dogs in Bangladesh, their antimicrobial resistance pattern, and identify the risk factors associated with MRSA and MRSP colonisation in dogs.

2 | MATERIALS AND METHODS

2.1 | Collection and preparation of samples

To determine coagulase-positive *S. aureus* and *S. pseudintermedius* in dogs, we conducted a cross-sectional survey on the dogs admitted to a Teaching Veterinary Hospital (TVH) from January to July 2018. The average number of dogs admitted daily to the TVH is approximately 10. All the dogs were registered to the hospital for the purpose of treatment or vaccination or for general health check-up. The health status of the dogs was determined based on clinical examinations by the veterinarian on duty. Immediately upon admission swab samples were taken from the perineum and mouth from each healthy dog. One additional swab from each of the infection sites was collected if there were any skin wounds, dermatitis, abscess or ear infections. A questionnaire was used to collect animal demographic and clinical data from the dog owners by interviewing directly. The first author (EAR) conducted the interviews and collected specimens with a prior consent from the dog owners. A sterile cotton swab was rotated several times against the oral mucosa, the surface of the perineal area and/or the infection site to collect a sample from a particular site. Swabs from a body site were placed individually in 5 ml Mueller Hinton broth (MHB) (Oxoid Ltd., Basingstoke, UK) supplemented with 6.5% NaCl and stored at 4°C until processing. All procedures were carried out under an approval of the Ethics Committee of CVASU [Approval no. CVASU/Dir (R&E) EC/2019/39 (2/8)].

2.2 | Isolation and identification of *S. aureus* and *S. pseudintermedius*

The swabs placed in MHB were incubated overnight at 37°C for primary selective enrichment. From this broth culture, 10 μ l was

streaked onto 5% bovine blood agar and incubated at 37°C for 24 h. Three to five colonies on blood agar plates displaying the characteristic appearance of staphylococci (medium-sized, smooth, pigmented or non-pigmented, raised and haemolytic) were further subcultured onto Mannitol salt agar (Oxoid Ltd., Basingstoke, UK) and incubated at 37°C for 24 h (Weese & van Duijkeren, 2010). The presumptive colonies of staphylococci on Mannitol salt agar were further tested by Gram's staining, catalase and tube coagulase tests. Before conducting the coagulase test all suspected staphylococci isolates were further sub-cultured on blood agar plates at 37°C for 24 h. All coagulase-positive isolates were investigated for the confirmation of *S. aureus* and *S. pseudintermedius* by PCR targeting the *nuc* gene as described previously (Sasaki et al., 2010). Bacterial genomic DNA was extracted from the freshly grown cultures on blood agar plate using boiling lysis method (Millar et al., 2000). All PCR-confirmed *S. aureus* and *S. pseudintermedius* were grown in 5 ml brain heart infusion broth (BHIB) (Oxoid Ltd., Basingstoke, UK) and stored at -80°C for further analysis.

2.3 | Antimicrobial susceptibility testing of *S. aureus* and *S. pseudintermedius*

All *S. aureus* and *S. pseudintermedius* isolates were screened for antimicrobial susceptibility against 14 antimicrobials representing 8 different classes using agar disk diffusion method. The following antimicrobials (Oxoid Ltd., Basingstoke, UK) were used: amoxicillin + clavulanic acid (30 µg), ampicillin (10 µg), cefaclor (30 µg), cefoxitin (10 µg), ciprofloxacin (10 µg), erythromycin (15 µg), gentamicin (30 µg), nalidixic acid (10 µg), oxacillin (5 µg), penicillin (10 IU), streptomycin (100 µg), sulfamethoxazole-trimethoprim (1.25 + 23.75 µg), tetracycline (30 µg) and vancomycin (30 µg). The zone of inhibition around each disk was measured and interpreted as susceptible (S), intermediate (I) or resistant (R) according to Clinical and Laboratory Standards Institute (CLSI) guidelines for veterinary pathogens (CLSI, 2008). In the case of nalidixic acid, the interpretation was made based on earlier study described by Vaez et al. (2011). Methicillin resistance was determined by measuring zone diameter around oxacillin and cefoxitin disks (Schissler et al., 2009). *S. aureus* and *S. pseudintermedius* isolates showing resistance against ≥3 antimicrobial classes were defined as multidrug resistant (MDR) (Magiorakos et al., 2012).

2.4 | Detection of the *mecA* gene

All oxacillin- and cefoxitin-resistant *S. aureus* and *S. pseudintermedius* isolates were further tested for the presence of the *mecA* gene by PCR as described earlier (Larsen et al., 2008). Nuclease-free water and an in-house MRSA strain were used as negative and positive control, respectively.

2.5 | Statistical analysis

A dog was considered positive for *S. aureus* or *S. pseudintermedius* when samples from at least one of the different body sites tested positive for the organism. The prevalence was calculated considering the number of positive dogs as the numerator divided by the number of dogs sampled as the denominator. Data were analysed using 'R' Program (version 3.5.1) (R CoreTeam, 2016). All possible risk factors were analysed for four target outcomes: the presence of *S. aureus*, *S. pseudintermedius*, MRSA and MRSP. First, univariable analysis was performed to identify possible risk factors for the four outcomes mentioned. Any factor having a *p* value of ≤0.20 was entered into multivariable logistic regression model. Forward stepwise selection approach was used to build the model. Variables with *p* value of 0.05 were considered significant and kept in the final model. The logistic regression analysis was performed using the *glmer* function from the lme4 package (Bates et al., 2014) in R version 3.5.1 (R CoreTeam, 2016). The 95% confidence interval of the prevalence values was calculated by the modified Wald method using the Graph Pad Quick Calcs online tool (www.graphpad.com/quickcalcs/).

3 | RESULTS

3.1 | Distribution of *S. aureus*, *S. pseudintermedius*, MRSA and MRSP

A total of 358 samples were collected from 150 dogs. Among them, 146 were from 73 healthy dogs, and 212 from 77 clinically sick dogs. Of the total samples obtained, 300 were from oral and perineal regions and the rest were from clinical cases of dermatitis (*n* = 28), skin wound (*n* = 22) and otitis (*n* = 8). An overview of the samples collected from different body sites, isolation frequency of *S. aureus* and *S. pseudintermedius* from the samples and the distribution of MRSA and MRSP is shown in Table 1. Out of the 150 dogs, 24 (16%; 95% CI: 10.9%–22.8%) and 68 (45.3%; 95% CI: 37.6%–53.3%) were positive for *S. aureus* and *S. pseudintermedius*, respectively. Overall, coagulase-positive staphylococci were isolated from 142 (39.7%; 95% CI: 34.7%–44.8%) out of the 358 samples (Table 1). A total of 28 and 111 isolates were confirmed as *S. aureus* and *S. pseudintermedius*, respectively. However, the remaining three isolates were not confirmed by PCR either *S. aureus* or *S. pseudintermedius*. Of the *S. aureus* and *S. pseudintermedius* isolates, 13 (46.4%; 95% CI: 29.5%–64.2%) and 9 (8.1%; 95% CI: 4.1%–14.9%) were positive for the *mecA* gene, and thus classified as MRSA and MRSP, respectively. The overall prevalence of MRSA in dogs was 8.7% (95% CI: 5.0%–14.4%) and MRSP was 6% (95% CI: 3%–11.2%).

3.2 | Antimicrobial susceptibility profiles

All *S. aureus* and *S. pseudintermedius* isolates were found to be MDR (Figures 1b and 1d, 2 and 3). The highest resistance in *S. aureus* and *S.*

TABLE 1 Distribution of *S. aureus*, *S. pseudintermedius*, methicillin-resistant *S. aureus* and methicillin-resistant *S. pseudintermedius* in different body sites of clinically healthy and sick dogs

Body sites	No. sample ^a	No. coagulase-positive <i>Staphylococcus</i> (%; 95% CI)	No. <i>S. pseudintermedius</i>			
			No. <i>S. aureus</i> (%; 95% CI)	(%; 95% CI)	No. MRSA(%; 95% CI)	No. MRSP(%; 95% CI)
Perineal	150	61 ^b (40.7, 33.1–48.7)	9 (6.0)(3.0–11.2)	51 (34.0)(26.9–41.9)	3 (33.3)(11.7–64.9)	3 (5.9) (1.4–16.5)
Oral	150	47 ^b (31.3)(24.4–39.2)	3 (2.0)(0.4–6.0)	43 (28.7)(22.0–36.4)	2 (66.7) (20.2–94.4)	1 (2.3) (0.0–13.2)
Dermatitis	28	16 ^b (57.1)(39.1–73.5)	8 (28.6)(15.1–47.2)	7 (25.0)(12.4–43.6)	5 (62.5) (30.4–86.5)	2 (28.6) (7.6–64.8)
Skin Wound	22	13 (59.1)(38.7–76.8)	7 (31.8)(16.2–52.9)	6 (27.3)(12.9–48.4)	2 (28.6) (7.6–64.8)	1 (16.7) (1.1–58.2)
Otitis	8	5 (62.5)(30.4–86.5)	1 (12.5)(0.1–49.2)	4 (50.0)(21.5–78.5)	1 (100) (16.8–100.0)	2 (50.0) (15.0–85.0)
Total	358	142 (39.7) (34.7–44.8)	28 (7.8)(5.4–11.1)	111 (31.0)(26.4–36.0)	13 (46.4) (29.5–64.2)	9 (8.1) (4.1–14.9)

Abbreviations: CI, confidence interval; MRSA, methicillin-resistant *Staphylococcus aureus*; MRSP, methicillin-resistant *Staphylococcus pseudintermedius*.

^aConsidered as denominator to calculate prevalence for the specific organism or its specific antibiotic-resistant type (MRSA and MRSP).

^bThree staphylococci isolates were not confirmed by PCR as *S. aureus* or *S. pseudintermedius*.

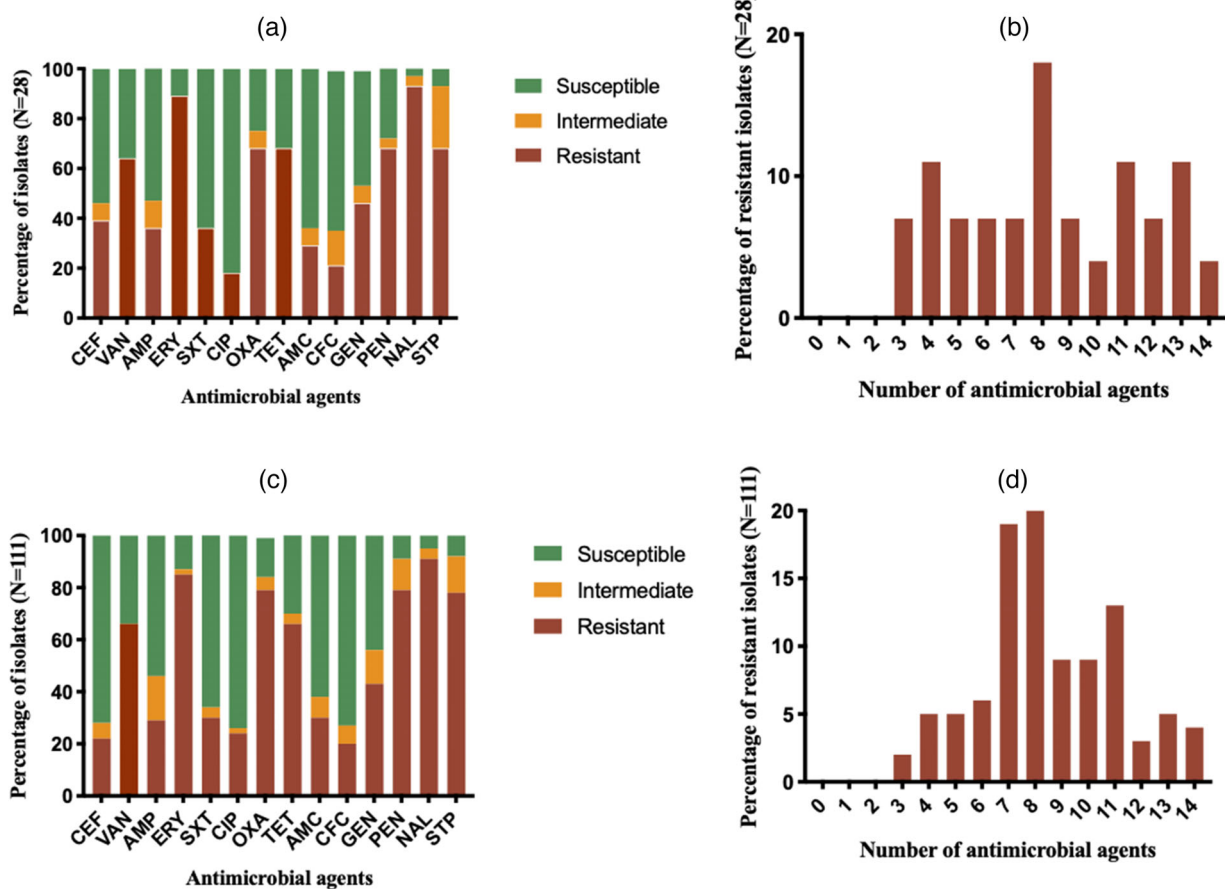


FIGURE 1 Antimicrobial resistance profile of *S. aureus* (a, b) and *S. pseudintermedius* (c, d). CEF: cefoxitin; VAN: vancomycin; AMP: ampicillin; ERY: erythromycin; SXT: sulfamethoxazole-trimethoprim; CIP: ciprofloxacin; OXA: oxacillin; TET: tetracycline; AMC: amoxicillin + clavulanic acid; CFC: cefaclor; GEN: gentamicin; NAL: nalidixic acid; PEN: penicillin; STP: streptomycin.

pseudintermedius was observed against nalidixic acid (95.2% and 91.0%, respectively) followed by erythromycin (89.3% and 84.7%, respectively) (Figure 1a and 1c). Resistance against oxacillin was detected in 72.5% in *S. aureus* and 79.3% in *S. pseudintermedius*. Similarly, in both

cases, majority of the isolates displayed resistance against tetracycline and vancomycin (Figure 1a and 1c). The antibiogram profiles of methicillin-resistant and methicillin-sensitive isolates are displayed in Figures 2 and 3, respectively.

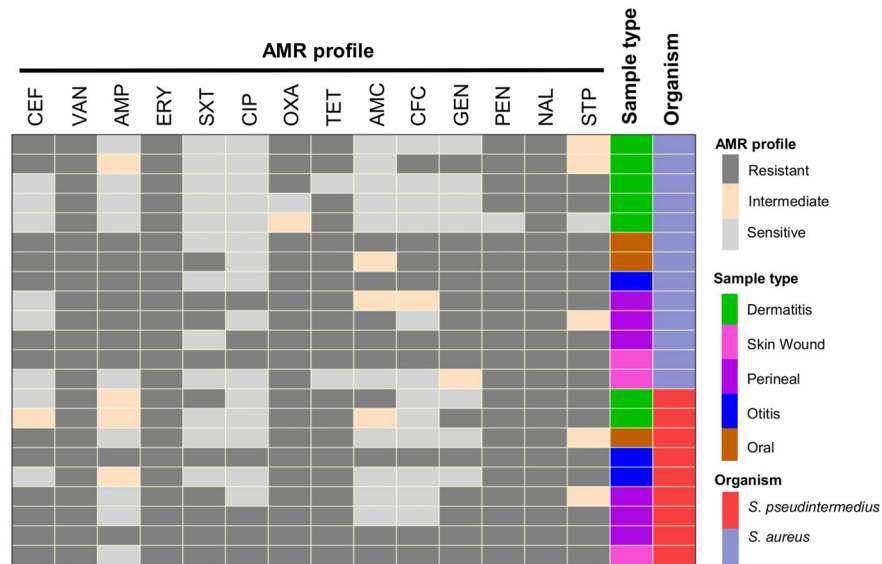


FIGURE 2 Heat map showing distribution of antimicrobial resistance profile of methicillin-resistant *S. aureus* and methicillin-resistant *S. pseudintermedius* isolates (*mecA* gene positive). Each row represents one isolate. CEF: ceftiofur; VAN: vancomycin; AMP: ampicillin; ERY: erythromycin; SXT: sulfamethoxazole-trimethoprim; CIP: ciprofloxacin; OXA: oxacillin; TET: tetracycline; AMC: amoxicillin + clavulanic acid; CFC: cefaclor; GEN: gentamicin; NAL: nalidixic acid; PEN: penicillin; STP: streptomycin.

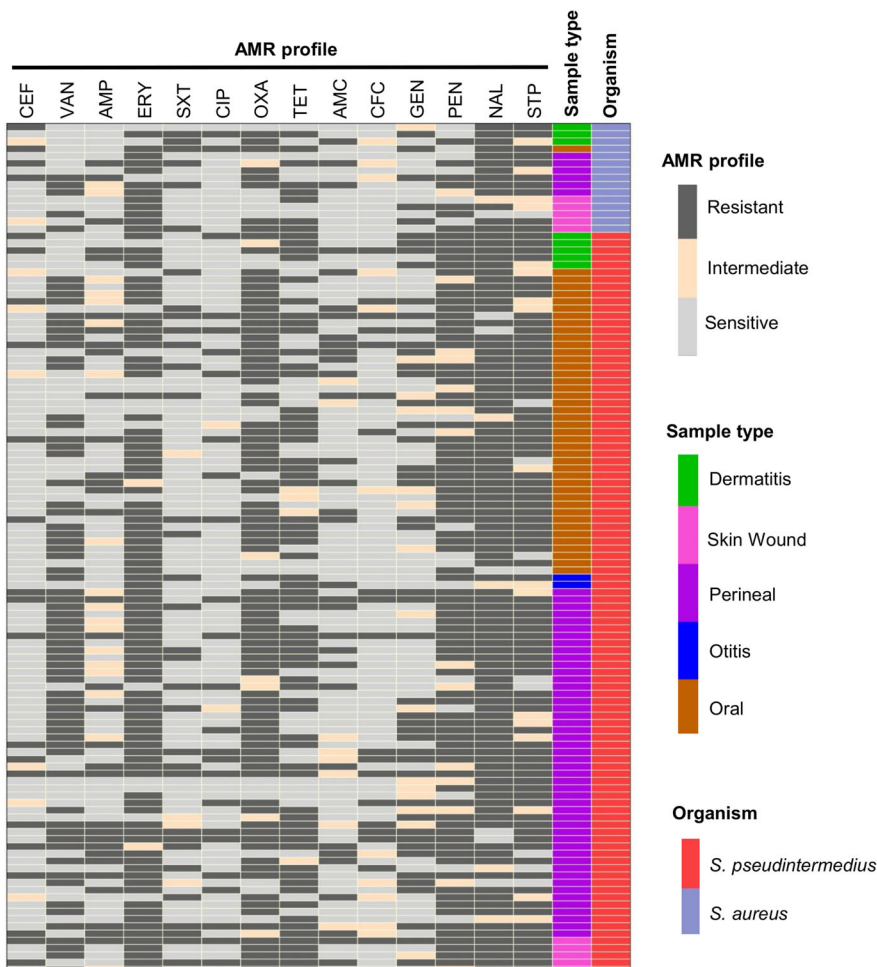


FIGURE 3 Heat map showing distribution of antimicrobial resistance profile of methicillin-sensitive *S. aureus* and methicillin-sensitive *S. pseudintermedius* isolates (*mecA* gene negative). Each row represents one isolate. CEF: ceftiofur; VAN: vancomycin; AMP: ampicillin; ERY: erythromycin; SXT: sulfamethoxazole-trimethoprim; CIP: ciprofloxacin; OXA: oxacillin; TET: tetracycline; AMC: amoxicillin + clavulanic acid; CFC: cefaclor; GEN: gentamicin; NAL: nalidixic acid; PEN: penicillin; STP: streptomycin.

TABLE 2 Multivariable logistic regression model for assessing the risk factors independently associated with the *S. aureus* and methicillin-resistant *S. aureus* (MRSA) from different body sites of both clinically healthy and sick dogs

Outcome variable	Explanatory variable	Description	OR (95% CI)	p Value
<i>S. aureus</i>	Dermatitis	Yes	10.07 (3.42–32.77)	<0.001
		No	1	Reference
	Antibiotic use	Yes	4.50 (1.54–14.07)	<0.001
		No	1	Reference
	Skin wound	Yes	3.46 (1.06–11.15)	0.006
		No	1	Reference
MRSA	Dermatitis	Yes	12.24 (3.12–57.33)	<0.001
		No	1	Reference
	Antibiotic use	Yes	8.73 (2.23–43.10)	<0.001
		No	1	Reference

Abbreviation: OR, odds ratio.

3.3 | Risk factors associated with the carriage of *S. aureus* and MRSA in dogs

The univariable analysis identified five potential risk factors ($p \leq 0.20$) associated with the carriage of *S. aureus* in dogs (Supplementary Table S1). In the subsequent multivariable analysis, three variables were identified as significant risk factors associated with the carriage of *S. aureus*. The significantly associated variables were 'presence of dermatitis' (OR 10.07, 95% CI 3.42–32.77, $p < 0.001$), 'history of antibiotic use in the past one month' (OR 4.50, 95% CI 1.54–14.07, $p < 0.001$) and 'presence of skin wound' (OR 3.46, 95% CI 1.06–11.15, $p = 0.006$) (Table 2).

Seven out of the fourteen variables were identified in the univariable analysis as the potential risk factors for the carriage of MRSA in dogs (Supplementary Table S2). Two variables, 'presence of dermatitis' (OR 12.24, 95% CI 3.12–57.33, $p < 0.001$) and 'history of antibiotic use' (OR 8.73, 95% CI 2.23–43.10, $p < 0.001$) were retained significant in the final model (Table 2).

3.4 | Risk factors associated with the carriage of *S. pseudintermedius* and MRSP in dogs

Of the fourteen variables tested in the univariable analysis (Supplementary Tables S3 and S4), six and five were eligible ($p < 0.20$) for multivariable analysis for the carriage of *S. pseudintermedius* and MRSP, respectively. In multivariable analysis for *S. pseudintermedius* carriage, two were retained in the final model: 'presence of dermatitis' (OR 3.16, 95% CI 1.33–7.91, $p = 0.011$) and 'presence of skin wound' (OR 3.02, 95% CI 1.16–8.54, $p = 0.027$) (Table 3). Similarly, in the multivariable analysis for MRSP, two factors were found to be significantly associated: 'presence of otitis' (OR 14.22, 95% CI 1.64–103.58, $p = 0.008$) and 'presence of oral lesions' (OR 9.48, 95% CI 1.14–64.82, $p = 0.002$) (Table 3).

4 | DISCUSSION

This cross-sectional study reveals a very high prevalence of MDR *S. aureus* and *S. pseudintermedius* along with MRSA and MRSP in dogs. Although the prevalence of MRSA has been reported in few studies (Afroz et al., 2008; Habibullah et al., 2017), the prevalence of MRSP has seemingly never been reported in dogs in Bangladesh. It is important to know the prevalence of multidrug-resistant bacteria in pets because it contributes to clinical management of diseased dogs. Moreover, the zoonotic potential of MRSP is well known (Guardabassi et al., 2013; Paul et al., 2011) and it helps increase pet owner awareness.

The results of this study show that coagulase-positive staphylococci were isolated from 39.7% of samples collected from different body sites of 150 dogs. The proportion of coagulase-positive isolates in this study is comparatively lower than an earlier report of the United States (70%) (Griffeth et al., 2008). However, Sasaki et al. (2007) reported 52.6% coagulase-positive *S. aureus* in dogs in Japan, which is nearly similar to our findings. As staphylococci are predominant commensal pathogens of dogs, the prevalence estimates may be influenced by many factors such as species, breed, age, sex, managements, clinical condition, geographical location etc.

The overall frequency of *S. aureus* (16%) carriage in dogs was in close agreement with previously reported studies in the United States where they sampled dogs at veterinary hospitals (Griffeth et al., 2008; Iverson et al., 2015). Also, Hanselman et al. (2009) reported 14% prevalence in household dogs in Canada. The overall prevalence of *S. pseudintermedius* (45.3%) in dogs is somewhat similar to the previously reported prevalence in Brazil (38.4%) (Penna et al., 2010), the United States (53%) (Iverson et al., 2015) and Tunisia (55%) (Gharsa et al., 2013). On the contrary, very high prevalence of *S. pseudintermedius* in dogs was reported in Japan (89.50%) (Kawakami et al., 2010), Poland (87.6%) (Garbacz et al., 2013), the United Kingdom (87.5%) (Fazakerley et al., 2009), Canada (87.4%) (Rubin et al., 2011) and Korea (61.15%) (Yoon et al., 2010). As a skin commensal, *S. aureus* and *S. pseudintermedius* are

TABLE 3 Multivariable logistic regression model for assessing the risk factors independently associated with the *S. pseudintermedius* and methicillin-resistant *S. pseudintermedius* (MRSP) from different body sites of both clinically healthy and sick dogs

Outcome variable	Explanatory variable	Description	OR (95% CI)	p Value
<i>S. pseudintermedius</i>	Dermatitis	Yes	3.16 (1.33–7.91)	0.011
		No	1	Reference
	Skin wound	Yes	3.02 (1.16–8.54)	0.027
		No	1	Reference
MRSP	Otitis	Yes	14.22 (1.64–103.58)	0.008
		No	1	Reference
	Oral lesions	Yes	9.48 (1.14–64.82)	0.002
		No	1	Reference

Abbreviation: OR, odds ratio.

frequently isolated from the nares, mouth, pharynx, forehead, groin and anus of healthy dogs and cats (Beck et al., 2012; Garbacz et al., 2013). The isolation rate of *S. pseudintermedius* from perineum and oral cavity/mucosa/mouth were 51% and 43%, respectively, in our study. Moreover, these two body sites are recognised as the most common colonisation sites for *S. pseudintermedius* in dogs reported in different studies (Garbacz et al., 2013; Paul et al., 2011; van Duijkeren et al., 2011). The carriage frequency of *S. pseudintermedius* in different body sites found in this study is consistent with the previous findings of Hanselman et al. (2009) and Beck et al. (2012) who reported that the nose and rectum of the healthy dogs generally carried 46% and 47.6% *S. pseudintermedius*, respectively. The variation of carriage percentages among different studies might be due to the variations in sample size, sample collection technique, culture methods, breeds of dogs, health status, environments, management as well as geographical location. The prevalence of *S. aureus* and *S. pseudintermedius* in dogs with dermatitis were 28.6% and 25% in this study, which is comparatively lower than the previous report (45%) (Beck et al., 2012).

Both *S. aureus* and *S. pseudintermedius* exhibited great diversity of resistance against the 14 antimicrobials tested in this study. This type of diverse antimicrobial resistance profile of these two organisms was previously reported by a number of studies (Algammal et al., 2020; Couto et al., 2011; Davis et al., 2014; Garbacz et al., 2013; Kern & Perreten, 2013; Perkins et al., 2020; Perreten et al., 2010).

Most of the *S. aureus* isolates in this study showed resistance to erythromycin (89.3%) and nalidixic acid (95.2%). High resistance frequency of *S. aureus* against erythromycin was reported in several previous studies (Davis et al., 2014; Garbacz et al., 2013; Morris et al., 2006; Schmitz et al., 2000). It may be due to repeated exposure to the same antimicrobial(s) and/or lower doses of the drug made the organism more tolerant against these antimicrobials. Moreover, the use of similar groups of antimicrobials that have similar modes of action against a particular organism may encourage development of more resistance. In this study, the *S. aureus* displayed high proportion of susceptibility to ciprofloxacin (82%) which is similar to the reports of Raviglione et al. (1990). The high rate of susceptibility to ciprofloxacin may be due to less frequent use of these antimicrobials in dogs. Surprisingly, *S. aureus*

and *S. pseudintermedius* exhibited higher resistance to tetracycline compared with previous studies (Morris et al., 2006; Gharsa et al., 2013; Garbacz et al., 2013). This higher resistance profile of this antibiotic is probably due to either cross-transmission of resistant organisms or acquisition of resistance genes. Nalidixic acid, erythromycin and tetracycline are less frequently used in pet animal practices in Bangladesh but the resistances of these antimicrobials are alarming for future treatment guidelines. However, vancomycin is reserved as last resort drugs for MRSA and MRSP infected patients in both pets and human clinics (Guardabassi & Prescott, 2015; Wunderink et al., 2003).

In this study, the prevalence of MRSA was found to be 8.7%. Similar prevalence (8%) was also reported at pet hospitals in the United States (Iverson et al., 2015). Depending on the study area and sample size, high proportion of MRSA (>50%) was reported in the United States, some Asian countries and Malta (Stefani et al., 2012). Whereas, intermediate frequency of MRSA (25–50%) was reported in African countries, China and some part of Europe (Mejia et al., 2010; Stefani et al., 2012; Vincze et al., 2014). Moreover, the global trend of MRSA prevalence was comparatively higher than our present study. This variation in reported prevalence studies from different geographical locations might be due to sample size of conducted study, breed characteristics, frequency of disease condition, repeated exposure of antimicrobials, different human–animal behavioural relationships, accessibility and availability of veterinary care as well as diverse antimicrobial stewardship practices in different countries.

The prevalence of MRSP (6%) in dogs in this study is close to the prevalence reported by several previous studies (Ishihara et al., 2010; Beck et al., 2012; Gold et al., 2014). Kawakami et al. (2010) and Perreten et al. (2010) reported the prevalence of MRSP was 66.5% in Japan and 72.8% in Europe in different clinically diseased dogs, which is much higher than our observation in Bangladesh. There have been higher prevalence reports in dogs presenting with pyoderma to dermatology referral clinics. Beck et al. (2012) reported that the prevalence of MRSP was 45.2% when dogs suffer from pyoderma and others disease condition. The percentage is higher in diseased dogs because concurrent infection can occur when a dog is infected with a resistant strain. Moreover, the dog may spread the infection if it comes in

close contact with other dogs. So, long-term treatment of chronic disease condition using topical or systemic antimicrobials may encourage the development of MRSP very rapidly. On the contrary, several studies revealed that the prevalence of MRSP in healthy dogs was 4.5% (Canada) (Hanselman et al., 2009), 4.0% (USA) (Abraham et al., 2007), 3.9% (Denmark) (Paul et al., 2011), 2.1% (Canada) (Hanselman et al., 2008) and 1% (USA) (Iverson et al., 2015) at veterinary hospital which is comparatively lower than our study. However, administration of broad-spectrum antimicrobials, particularly, concurrent use of β -lactams and fluoroquinolones in pet animals might play significant roles in the emergence of MRSA and MRSP in dogs (Guardabassi et al., 2013). Any *mecA* gene-encoding bacteria have the ability to produce β -lactamase enzymes which are the main trigger to inhibit the function of β -lactam antimicrobials (Bush & Bradford, 2020). Overuse of fluoroquinolones may encourage the mutation of nucleotide resulting the emergence of resistant strains (Bakken, 2004).

We identified dermatitis and antibiotic use as the risk factors associated with higher prevalence of MRSA in dogs. Long-time use of antimicrobials in chronic infections might be a reason behind this association (Ventrella et al., 2017). On the other hand, selection pressure exerted by the use of antimicrobial therapy is a well-documented risk factor for MRSA (Weber et al., 2003; Eckholm et al., 2013; Gnanamani et al., 2017). Presence of otitis and oral lesions in dogs were found as risk factors for the carriage of MRSP. However, some studies reported that dogs with chronic skin and ear diseases that visited veterinary clinics more frequently and received topical or systemic antimicrobial medication or glucocorticoids were at higher risk of MRSP infection (van Duiker et al., 2011; Weese et al., 2012; Lehner et al., 2014). The imprudent use of broad-spectrum antimicrobials in the treatment of MRSA and MRSP infection must be unsanctioned, and a standard treatment guideline should be based on the laboratory reports of the antimicrobial susceptibility testing. However, during an emergency and urgent situation, veterinarians do not always get the chance to perform culture and sensitivity testing during choosing antimicrobials. Therefore, keeping a cumulative hospital antibiogram would be highly beneficial to guide empiric antimicrobial therapeutic decisions (Fowler et al., 2016).

Potential transmission of MRSA and MRSP from pet animals to owners (Hanselman et al., 2009), suggests their high presence in companion animals. Moreover, the veterinary care providers may act as a vehicle for transmission between patients via contaminated hands or clothes. There is also an increased risk of environmental contamination posing risk of exposure to animal patients while in the hospital. Furthermore, the veterinary healthcare providers may behave as transitory carriers, bringing MDR organism's home with them and exposing their own pets.

The study has some limitations. We sampled the dogs that were registered to the hospital during the study period without considering the statistical sample size calculation. Additionally, we could not perform detailed genotyping of the isolates due to resource constraints. It would be very valuable to know the sequence types of *S. aureus* and *S. pseudintermedius* circulating in dogs. Furthermore, this study was conducted in a particular region of the country although the prevalence of staphylococci may vary according to geographical locations. These limitations should be addressed in future research.

5 | CONCLUSION

The carriage rate of *S. aureus* and *S. pseudintermedius* in pet dogs in Bangladesh appear to be 16% and 45.3%, respectively. The overwhelming majority of the strains belonging to both the species are not only MDR but a significant section of them also carrying the *mecA* gene, a marker for the methicillin resistance. The prevalence of MRSA in dogs with dermatitis and with the history of antibiotic use might be higher while the presence of otitis and oral lesions seem to be positively associated a higher prevalence for MRSP.

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AUTHOR CONTRIBUTIONS

Eaftekhhar Ahmed Rana: conceptualisation; data curation; formal analysis; investigation; methodology; project administration; writing – original draft. Md Zohorul Islam: conceptualization; data curation; formal analysis; supervision; visualisation; writing – review & editing. Tridip Das: data curation; visualisation; writing – review & editing. Avijit Dutta: writing – review & editing. Abdul Ahad: project administration; writing – review & editing. Paritosh Kumar Biswas: formal analysis; writing – review & editing. Himel Barua: conceptualisation; data curation; formal analysis; methodology; project administration; supervision; writing – review & editing.

ETHICAL STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. All procedures were carried out under an approval of the Ethics Committee of CVASU (Approval no. CVASU/Dir (R&E) EC/2019/39 (2/8)).

PLACE OF STUDY

The study was conducted at Sahedul Alam Quaderi Teaching Veterinary Hospital (SAQ-TVH) at Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. The microbiological investigation was conducted at microbiology laboratory of the Department of Microbiology and Veterinary Public Health, CVASU, Bangladesh.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available within the article and its supplementary materials

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/vms3.701>.

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