ORIGINAL ARTICLE

NASAL CARRIAGE RATE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS,* ANTIMICROBIAL SUSCEPTIBILITY PATTERN AND ASSOCIATED RISK FACTORS AMONG PREGNANT WOMEN AT THE UNIVERSITY OF GONDAR COMPREHENSIVE SPECIALIZED HOSPITAL NORTHWEST, ETHIOPIA

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ABSTRACT

Background: *Methicillin-resistant S. aureus (MRSA) infection has become a significant burden worldwide that poses a formidable clinical threat, with persistently high morbidity and mortality and successful treatment remains challenging. Pregnant mothers are at increased risk of acquiring MRSA during their frequent checkups at health institutions. Indeed, there is a paucity of information on the nasal carriage rate of MRSA among pregnant women. The aim of this study was to determine the nasal carriage rate of MRSA, antimicrobial susceptibility patterns, and associated risk factors among pregnant women attending the University of Gondar Compressive Specialized Hospital.*

Method*: A hospital-based cross-sectional study was carried out involving a total of 423 pregnant women from February-April 2020. A systematic random sampling technique was used to recruit the study participants. Nasal swabs were collected and inoculated on Mannitol salt agar and blood agar then incubated at 35°C for 24hrs. Gram stain, catalase and coagulase tests were performed. Antimicrobial susceptibility tests were performed on Muller Hinton agar. Methicillin resistance was detected using a cefoxitin disc. Staphylococcus aureus ATCC 25923 was used as quality control. Data were entered using Epi-Info version 7 and analyzed using SPSS version 20 statistical software. The association between outcome and risk factors was done using the Pearson Chi-square test. P-value < 0.05 was considered to be statistically significant.*

Result*: S. aureus and MRSA were isolated in 60/423 (14.2%) and 8/423 (1.9%) of the study participants, respectively. The highest isolation rates of total S. aureus and MRSA isolate were found in the age group 31-40 years and in urban dwellers. One hundred percent of the MRSA strains were sensitive to erythromycin and clindamycin. Nasal carriage of MRSA was significantly associated with age, history of having a respiratory infection, history of hospitalization, history of contact with a person with skin infection, and history of sharing personal items such as towels or razors.*

Conclusion: *The result of this study indicated that a considerable amount of MRSA nasal colonization is observed. Hence, routine screening of pregnant women is important to prevent endogenous infections and the spread of MRSA.*

Keywords: *MRSA, Nasal carriage, pregnant women, Gondar*

INTRODUCTION

Staphylococcus aureus is an important human pathogen responsible for a variety of nosocomial and community-acquired infections. It is one of the bacterial commensal floras in healthy individuals, which mainly colonize the skin and anterior nares (1). Methicillin-resistant *Staphylococcus aureus* (MRSA) remains one of the major multiple antibiotic-resistant bacterial pathogens causing serious community and healthcare-associated infections (2). Methicillin-resistant *Staphylococcus aureus* colonization increases the risk of infection, Recently, the rate of infections due to MRSA strains has increased globally, with marked variations in different regions. Peo-

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ple with *MRSA* infections are 64% more likely to die than people with drug-sensitive infections (3). Methicillin-resistant *Staphylococcus aureus* colonization in pregnant and postpartum women ranges from 5-73% (4,5). Carriage of MRSA is a prerequisite for most MRSA infections and plays an important role in the dissemination of this organism within health care facilities and into the community (6). Hence, carriage of MRSA during pregnancy or later could be a source of serious infection to neonates (7).

MRSA is usually spread by contact with the skin or hands of infected individuals, transient carriage from the hands of health care workers, contaminated objects, or the environment (8). Nasal decolonization of pregnant women during their hospital admission reduces the risk of infection among antenatal and postpartum women (9). The finding of a study conducted in Uganda indicated that older reproductive age is one of the risk factors for MRSA colonization (10).

Knowledge of MRSA carriage rate and its antimicrobial susceptibility pattern is very important for the appropriate selection of antimicrobial agents to prevent subsequent infection due to MRSA in mothers and their infants. Data on the nasal carriage of MRSA of a pregnant woman may also be important before admission for delivery. Although carriage rates and associated factors of MRSA in pregnant women were reported in developed countries, there is a lack of such data in Ethiopia. Therefore, this study aimed to assess the nasal carriage rate, antimicrobial susceptibility pattern, and associated risk factors of MRSA from nasal swabs of pregnant women attending antenatal care at the University of Gondar Compressive Specialized Hospital.

METHOD

Study area, design, and period: A hospital-based cross-sectional study was conducted from February 1, 2020, to April 30, 2020, at the University of Gondar Compressive Specialized Hospital. This hospital provides various outpatient and inpatient services for about 5 million inhabitants of the surrounding areas (11). According to the 2019 health management information systems (HMIS) report, there were about 11,300 antenatal care follow-ups, 3600 deliveries and 9100 prevention of mother-to-child transmission of HIV (PMTCT) services in the hospital.

Inclusion and exclusion criteria

Inclusion criteria: All pregnant women, who visited ANC at the University of Gondar compressive specialized hospital during the study period, were invited to participate in the study.

Exclusion criteria: Pregnant women who had recent nasal bleeding or a nasal health condition, including a history of recent nasal surgery or infection, use of nasal medications at the time of data collection, and/or antibiotic treatment within two weeks of their hospital visit, were excluded from the study.

Sample size and sampling technique: The sample size was calculated using a single population proportion statistical formula $N = Z\alpha/2\ p\ (1-p)/d2$. Where n= number of study participants that were enrolled in the study, Z = test statistic which allows us to calculate our results with 95% confidence, P = the proportion to be used on estimates from previous work (50%, since there is no previous study on the carriage rate of MRSA among pregnant women in the area) and d = the level of precision (0.05). To minimize errors arising from the likelihood of non-compliance,

ten percent of the sample size was added giving a final sample size of 423 (12).

A systematic random sampling technique was used to recruit the study participants. According to 2019 records of the hospital, about 50 pregnant women visit the antenatal care unit on each working day. In brief, 50 women x 20 working days per month for three months give a total of 3000 women. The sampling interval was calculated as 3000/423=7. The first woman on the first day of sample collection was selected by a lottery method. Thereafter, every 7th woman was invited to participate and provide her informed consent until the required sample size was achieved.

Data collection and Laboratory processing

Questionnaire: Socio-demographic and clinical data were collected using a pretested standardized questionnaire. The questionnaire was translated to Amharic, the local language, and re-translated back to English to ensure the reliability of the instrument.

Specimen Collection: Nasal swabs were taken from the anterior nares using a sterile cotton-tipped applicator stick moistened with normal saline. The specimens were transported to the school of Biomedical and Laboratory Sciences, Biomedical complex laboratory of Bacteriology section, within two hours of collection for microbiological analysis (13).

Isolation and identification of *S. aureus:* Each swab sample was inoculated onto Mannitol salt agar (MSA) and blood agar plates (BAP) (Pune India) and incubated aerobically at 35 °C for 24 hours. After the incubation, the culture plates were examined for growth, size, color, and morphology of the colonies. Preliminary identification of *S. aureus* was performed by observing golden-yellow colonies on MSA and beta-hemolysis on sheep blood agar. Further confirmation of the isolates was carried out by performing Gram-stain, catalase, and coagulase tests (14, 15).

Antibiotic susceptibility testing: The antibiotic susceptibility patterns of *S. aureus* isolates were determined by using the Kirby-Bauer disc diffusion method. Penicillin (10U), erythromycin (15µg), clindamycin (2µg), cotrimoxazole (30µg), doxycycline (30µg), ciprofloxacillin (5µg), chloramphenicol (30µg) and gentamicin (10µg) were used (16). The antibiotic discs were applied on the surfaces of the inoculated Mueller-Hinton agar and incubated at 35 ° C for 24 hr. After incubation, the diameter of the zone of inhibition produced by each antibiotic disc was measured and interpreted using the Clinical Laboratory Sciences Institute (CLSI) guideline. Thereafter, the results were interpreted as resistant, intermediate, and susceptible (17).

Identification of MRSA: Methicillin resistance S. *aureus* was detected by using the Cefoxitin disk. Cefoxitin disk (30µg) was applied on the surface of the inoculated plate and incubated at 35 °C for 16-18 hrs. After incubation, the diameter of the zone of inhibition was measured and interpreted as resistance (MRSA), if the zone diameter was less than or equal to 21mm (17).

Data quality control: Standard operating procedures (SOPs) were strictly followed for each activity in the laboratory. *Staphylococcus aureus* (ATCC 25923) was used as quality control for checking the performance of the culture media and antimicrobial discs. The sterility of culture media was checked by incubating 5% of the batch at 35 ^{0}C overnight.

Data Analysis and interpretation: Data were entered using Epi-Info version 7 and transferred to SPSS version 20 software for analysis. Descriptive analysis using the Pearson Chi-square test ($\chi 2$) was used. P-value < 0.05 was considered to be statistically significant.

Ethical Considerations: Ethical approval was obtained from the Ethical Review Committee of the school of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar with a reference number SBMLS/2445/2020 and an official letter of support was obtained from the University of Gondar Compressive Specialized Hospital prior to the data collection. Informed written consent was obtained from each participant after explaining the objectives of the study. Information obtained from study participants was kept confidential. The attending physician was informed about mothers who were positive for MRSA.

RESULT

Socio-demographic characteristics of study participants: A total of 423 pregnant women were enrolled in the present study. The ages of these women ranged from 18 to 40 years with a mean age of 26.13 ± 4.34. Most 375/ 423 (88.7%) of the pregnant women were in the age group of 18-30 years. The majority, 396/423 (93.6%) were from urban areas, while the remaining 27/423 (6.4%) were from rural areas. Many of the pregnant women, 258/423 (61%) were housewives.

Prevalence and distribution of *Staphylococcus aureus* and MRSA: The overall colonization rates of S. *aureus* and MRSA were 60/423 (14.2%) [95% CI: 10.9 -17.7] and 8/423 (1.9%) [95% CI: 0.7-3.3] , respectively. Of the total S. *aureus* (60) isolate, 8/60 (13.33%) [95% CI: 6.7-21.7] were MRSA strains. The highest isolation rates of *S. aureus* (27.1%, 13/48) and MRSA (6.25%, 3/48) were found in the age group 31-40 (Table 1).

Factors associated with MRSA colonization: In this study, the Pearson Chi-square test was carried out to show the association of MRSA with possible risk factors. Age (P=0.019), having a history of respiratory infection in the past 1 year (P=0.000), history of hospitalization (P=0.000), history of contact to a person with skin infection (P=0.000), and history of sharing personal items such as towels or razors (P=0.001) were significantly associated with MRSA nasal colonization. The rest variables were not significantly associated with MRSA nasal colonization (Table 1).

Antimicrobial susceptibility patterns: All MRSA isolates were resistant to penicillin G (100%) and ciprofloxacin 7 (87.5%). On the other hand, these isolates were sensitive to clindamycin 8 (100%), erythromycin 8 (100%), doxycycline 7 (87.5%), gentamicin 7 (87.5%) and chloramphenicol 7 (87.5%). Out of the 60 *S. aureus* isolates, more than 75% were sensitive to gentamycin (78.3%), sulphamethoxazole-trimethoprim (86.7%), doxycycline (83.3%), clindamycin (100%), erythromycin (100%), and chloramphenicol (80%). On the contrary, 47 (78.3%) and 25 (41.7%) of the isolates were resistant to penicillin and ciprofloxacin (Table 2).

Of the 60 isolates of *S. aureus*, 15 (25 %) of them were multidrug-resistant. Fourteen (93%) were resistant to three antimicrobial classes and 1(7%) were resistant to four antimicrobial classes.

Table 1: Socio-demographic characteristics, distribution and possible risk factors of MRSA nasal colonization among pregnant women at the University of Gondar Compressive Specialized Hospital, 2020.

Characteristics		***S.aureus* growth**			**P value (Total *S.aureus*)**	**P value (MRSA)**
		MSSA N (%)	**MRSA N (%)**	**Negative N (%)**		
Age group (years)	18-30 (n=375)	42 (11.2)	5(1.3)	328 (87.5)	0.008	0.019*
	31-40 (n=48)	10 (20.8)	3 (6.2)	35 (72.9)		
Educational status	Illiterate (n=91)	15 (16.5)	4 (4.4)	72 (79.1)		0.094
	Primary school (n=176)	22 (12.5)	1 (0.6)	153 (86.9)	0.112	
	Secondary school and above (n=156)	15 (9.6)	3 (1.9)	138 (88.5)		
Occupation	Merchant (n=61)	6 (9.8)	1 (1.6)	54 (88.5)	0.035	0.349
	House wife(n=258)	36 (14.0)	4 (1.6)	218 (84.5)		
	Daily labor (n=11)	4 (36.4)	1 (9.1)	6 (54.5)		
	Civil servant and others (n=93)	6 (6.5)	2 (2.2)	85 (91.4)		
Previous pregnancy	Yes (n=191)	32 (16.8)	5 (2.6)	154 (80.6)	0.021	0.320
	No (n=232)	20 (8.6)	3 (1.3)	209 (90.1)		
Mode of previous delivery	Vaginal (n=167)	29 (17.4)	3 (1.8)	135 (80.8)	0.023	0.101
	Cesarean (n=28)	3 (10.7)	2 (7.1)	23 (82.1)		
	No delivery (n=228)	20 (8.8)	3 (1.3)	205 (89.9)		
Gestational age	First trimester (n=65)	6 (9.2)	1 (1.5)	58 (89.2)	0.041	0.357
	2^{nd} trimester (n=117)	7 (6.0)	4 (3.4)	106 (90.6)		
	3^{rd} trimester (n=241)	39 (16.2)	3 (1.2)	199 (82.6)		
Number of follow up during this pregnancy	Once (n=85)	8 (9.4)	1 (1.2)	76 (89.4)	0.152	0.913
	Two times (n=72)	3 (4.2)	1 (1.4)	68 (94.4)		
	Three times (n=47)	5 (10.6)	1 (2.1)	41 (87.2)		
	Four times (n= 219)	36 (16.4)	5 (2.3)	178 (81.3)		
History of respiratory infection in the past 1 year	Yes (n= 11)	2 (18.2)	4 (36.4)	5 (45.5)	0.000	0.000*
	No (n= 412)	50 (12.1)	4 (1.0)	358 (86.9)		
History of hospitalization	Yes (n= 17)	6 (35.3)	3 (17.6)	8 (47.1)	0.000	0.000*
	No (n=406)	46 (11.3)	5 (1.2)	355 (87.4)		
History of contact with a person with skin infection	Yes (n= 8)	2 (25.0)	2 (25.0)	4 (50.0)	0.000	0.000*
	No (n= 415)	50 (12.0)	6 (1.4)	359 (86.5)		
History of sharing personal items such as towels or razors	Yes (n= 4)	0 (0.0)	1 (25.0)	3 (75.0)	N/A	0.001*
	No (n= 419)	52 (12.4)	7 (1.7)	360 (85.9)		

Abbreviations: *MSSA, Methicillin Susceptible S.aureus; MRSA, Methicillin Resistant S.aureus.*

Table 2: Antimicrobial susceptibility patterns of MRSA and MSSA among pregnant women at the University of Gondar Compressive Specialized Hospital in 2020.

Antibiotics		***S.aureus*** **(n=60) N (%)**	**MRSA (n= 8) N (%)**	**MSSA (n=52) N (%)**
Gentamycin	Sensitive	47 (78.3)	7 (87.5)	40 (77)
	Resistance	13 (21.7)	1 (12 .5)	12 (23)
Sulphamethoxa-	Sensitive	52 (86.7)	5 (62.5)	47 (90.3)
zole-trimethoprim	Resistance	8 (13.3)	3 (37.5)	5 (9.7)
Doxycycline	Sensitive	50 (83.3)	7 (87.5)	43 (83)
	Resistance	10 (16.7)	1 (12.5)	9 (17)
Clindamycin	Sensitive	60 (100)	8 (100)	52 (100)
	Resistance	0	0	0
Erythromycin	Sensitive	60 (100)	8 (100)	100
	Resistance	0	0	0
Penicillin	Sensitive	13(21.7)	0	13 (25)
	Resistance	47 (78.3)	8(100)	39 (75)
Ciprofloxacin	Sensitive	35 (58.3)	1(12.5)	34 (65)
	Resistance	25 (41.7)	7 (87.5)	18 (35)
Chloroamphicol	Sensitive	48(80)	7 (87.5)	41(79)
	Resistance	12(20)	1 (12.5)	11(21)

DISCUSSION

MRSA infections are globally emerging as life-threatening infections in the community and can no longer be considered healthcare-associated infections only. Therefore, hospital-based infection control measures alone are not enough to fight the increasing MRSA infections in the community (18). The present study assessed the nasal carriage rate of *S. aureus* and MRSA among pregnant women in Northwest Ethiopia and shows about one-seventh of the nasal swabs from pregnant women were positive. The overall carriage rate of *S. aureus* and MRSA was 14.2% [95% CI: 10.9 -17.7] and 1.9% [95% CI: 0.7-3.3], respectively. Due to the lack of other similar reports in Ethiopia, the differences and similarities of the carriage rates obtained in this study are not compared to other local studies. In comparison with international reports, our findings were similar to a report in Las Vegas, Nevada 16% and 0.3%*S. aureus* and MRSA, respectively (19), but lower than a report in Colombia 31% vs 9% (1) and China 25.7% vs 5.6% (4). The difference in the carriage rate might be due to better diagnostic facilities used or poor infection prevention and control practices in previous studies. In this study, the nasal carriage rate of MRSA (13.33%) is within the 2014 estimated range of WHO for the African region (12-80%) (20).

In the current study, the highest isolation rate of MRSA nasal colonization(6.25%, 3/48) was observed in the age group 31-40 compared to women aged 18-30 years (1.3%, 5/375) which is in line with a study conducted in Uganda (10).

In the antimicrobial susceptibility pattern, *S.aureus* isolates were resistant to penicillin (78.3%). One hundred percent of the *S. aureus* strains were sensitive to clindamycin which was similar to a study conducted in China (4). The high sensitivity in these studies might be due to the infrequent use of this antibiotic in the study area.

Several studies in different populations showed that socio-demographic characteristics and other risk factors contribute substantially to the nasal carriage of MRSA (21-23). Similarly, in the current study, age (P=0.019), having a history of respiratory infection in the past 1 year (P=0.000), history of hospitalization (P=0.000), history of contact with a person with skin infection (P=0.000), and history of sharing personal items such as towels or razors (P=0.001) were significantly associated with MRSA nasal colonization. The reason for this association might be due to the repeated visits to the hospital, which is a highly contagious area, particularly for drug-resistant pathogens.

Limitations: This study was limited to MRSA detection and the minimum inhibitory concentration for some important antibiotics such as vancomycin. Another limitation is the lack of follow-up to assess the persistence of MRSA carriage.

CONCLUSION

In this study, a considerable amount of MRSA nasal colonization was observed among pregnant women. The findings of this study suggest the need for screening pregnant women for prevention and control of MRSA colonization and associated infections. Erythromycin and clindamycin are highly effective for the treatment of MRSA. Health institutions are recommended to develop guidelines for the prevention and control of MRSA.

Abbreviations: MRSA-Methicillin-resistant *Staphylococcus aureus;* **MSSA-**Methicillin-sensitive *Staphylococcus aureus*

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