Bangl. J. Vet. Med. (2021).19 (1): 63–68 ISSN: 1729-7893 (Print), 2308-0922 (Online)

Received: 30-05-2021, Accepted: 03-06-2021 DOI:https://doi.org/10.33109/bjvmjj2021vph2

ORIGINAL ARTICLE

Molecular detection and drug resistance pattern of methicillin resistant *Staphylococcus aureus* isolated from hospitalized human patients in Mymensingh

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Abstract

Background: The importance of *Staphylococcus* (*S.*) *aureus* as a persistent nosocomial and community-acquired pathogen has become a global health concern. Methicillin-resistant *S. aureus* (MRSA) is a major challenge to hospitals all over the world due to the emergence and spread of isolates with decreased susceptibilities to several antibiotic classes. The present study was focused to determine the prevalence of MRSA in clinical specimens and investigate the sensitivity pattern of these isolates to various antibiotics used for treating hospitalized patients.

Methods: A total of 50 clinical samples consisting of pus, surgical infections, and wounds in different parts of the body, and diabetic foot ulcers were collected aseptically from Mymensingh Medical College Hospital (MMCH)fromJanuary to May 2016. Isolation and identification of *S. aureus* was performed by cultural, morphological, biochemical characteristics and confirmed by amplification of *nuc* gene by PCR. The antibiotic resistance pattern of the isolates was evaluated by the Kirby-Bauer disk diffusion method using penicillin G, erythromycin, neomycin, ciprofloxacin and oxacillin. Finally, MRSA was detected by amplification of *mecA* gene.

Results: Among the 50 samples, 30% (n=15/50) were confirmed as *S. aureus* and 8 isolates were confirmed as MRSA. The prevalence of MRSA among *S. aureus* isolates was 53.33% in MMCH. The isolated *S. aureus*showed 100% resistant to penicillin G, 66.67% to erythromycin, 60.0% to ciprofloxacin and 26.67% to neomycin.

Conclusion: The increased frequency of MRSA and their extended resistance to several antibiotics were found alarming for the treatment of MRSA infections in humans. None of the five antibiotics is recommended for the treatment of S. aureus infections in humans in MMCH.

Keywords:MRSA, *nuc*gene, *mecA* gene, antibiotic resistance, human patient

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Introduction

Staphylococcus aureus is one of the most important nosocomial human pathogens which are able to emerge after surviving in different environments and can interact successfully with the host (David and Daum, 2010). This outstanding ability allows this bacterium to acquire antibiotic resistance. Staphylococcal infections can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, cellulitis, carbuncles, scalded skin syndrome and abscesses, to life-threatening diseases such as pneumonia, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia and sepsis (Lowy, 1998).

Antibiotic resistance is the most divisive subject in the world. Most of the people in the globe are facing different difficulties in treatment of infections and severe economic losses due to the overuse or misuse of antibiotics in human and animal (Islam et al., 2021). Improper use of antibiotics induce selective pressure to develop antibiotic resistance in animals and humans.

Following the introduction of penicillin in 1940, *S. aureus* resistance appeared, leading to the development of semisynthetic penicillins such as methicillin. In 1960, MRSA was clinically identified. Poor infection control measures and continued indiscriminate exposure to antibiotics in humans and animals lead to MRSA transmission (Lakhundi and Zhang, 2018). In recent years, the prevalence of MRSA is rising. The infection due to the MRSA strains has a higher mortality rate than the infection caused by the methicillin-sensitive *Staphylococcus aureus* (MSSA) strains, which brings great difficulty to treatment (Jokinen et al., 2017). Infections due to *S. aureus*, including the MRSA strains has long been common in Bangladesh. Although some studies have been done in Bangladesh to estimate the burden of MRSA infection with their sensitivity pattern, there is paucity of literature on the prevalence and molecular detection of MRSA in humans in Bangladesh (Yusuf et al., 2013). Hussain *et al*. (2016) reported 15.34 % MRSA prevalent in human sample however Rahman *et al.* (2018) reported MRSA 53.1% and 13.8% in humans and animals, respectively. Current status of the drug resistance among *S. aureus* strains in the local settings needed to be explored (Khan et al., 2016).

Materials and Methods

Study area and sample collection

The present research work was conducted at the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. A total of 50 samples of human cases from pus, surgical infections, wounds in different parts of the body and diabetic foot ulcer were collected from Mymensingh Medical College Hospital (MMCH).

Bacteriological investigation and detection of S aureus

Initially the samples positive for *S. aureus* were screened by inoculation of samples into 5% sheep blood agar (MSA) followed by subculture onto Mannitol salt agar media. The isolates showed beta hemolysis on blood agar and fermented MSA were subjected to different biochemical tests such as sugar fermentation, catalase and coagulase tests according to standard method (Buchanan and Gibbon, 1974). After phenotypic identification the isolates were confirmed as S aureus by amplification of *nuc*gene.

Detection of MRSA and antimicrobial profile

After phenotypic and molecular confirmation of *S aureus,* the isolates were detected as MRSA by disc diffusion tests according to NCCLS guidelines (NCCLS, 2011) on Mueller–Hinton agar using standard discs of Oxacillin (1 µg) following 0.5 McFarland standards. The isolates showed resistance to Oxacillin were confirmed molecularly by amplification of *mecA* gene.

ther than oxacillin standard discs of penicillin G (10 µg), erythromycin (15 µg), Neomycin (30 determine multidrug resistance pattern of the isolated *S. aureus*. Results were recorded as sensitive, intermediately sensitive, or resistant, and the zone of growth inhibition was compared with the zone size provided by the Clinical and Laboratory Standards Institute (CLSI, 2011).

Extraction of genomic DNA and PCR conditions

Genomic DNA of the isolates were extracted by boiling method (Fang et al., 2003). A single colony of each isolate was taken in 100 µl of distilled water, mixed well and then boiled for 10 minutes. Following boiling, the tubes were immediately placed on ice for cold shock and centrifugation was performed at 10,000 rpm for 10 minutes at 4°C. The supernatant was collected which was used as template DNA to perform PCR. *S aureus* specific *nuc* and methicillin resistant *mecA* genes were amplified by PCR using the primers and standard protocol described by Dewanand *et al.* (2007) and Aklilu *et al.* (2010) respectively.

Statistical analysis

All the data were entered in the excel sheet (MS-2013) and transferred to SPSS software (SPSS-22.0, IBM, USA) to compute the frequencies of MRSA.

µg), and ciprofloxacin (5 µg) were also used to

Results

Among the 50 samples analyzed, 15 (30%) were suspected for *Staphylococcus aureus* based on the cultural characteristics and molecular detection of *nuc*gene. The occurrence of *S. aureus* in different samples is presented in Table 1. The overall prevalence of *S. aureus* and MRSA were 30.0% and 42.9% respectively. The highest prevalence of *S. aureus* and MRSA were observed in diabetic foot ulcer samples (100.0%) and surgical infections (66.7%). However, wound samples were found negative for the presence of MRSA.

A total of 15 confirmed *S. aureus* isolates were subjected to disk diffusion test using five commonly used antibiotics to determine their antibiotic profile. All the isolates were found 100 % resistant to penicillin G followed by erythromycin (66.67%), ciprofloxacin (60.0%), oxacillin (53.3%) and neomycin (26.67 %).

Out of 15 *S. aureus* positive isolates 8 (53.33%) showed resistance to oxacillin. However, from 9 (60%) isolates methicillin resistant *mecA*gene were amplified. Sample wise the occurrence of MRSA were 66.67% (2/3) in surgical infections, 55.56% in pus and 53.33% in diabetic ulcer. All types of sample carried MRSA except wound samples (Table 2).

Table 1.Prevalence of *S. aureus* and MRSA isolated from different samples

Sample (n)	Samples positive for *S. aureus* (%)	Prevalence of MRSA among *S. aureus*(%)	Prevalence of MRSA among allsamples (%)
Pus (37)	9 (24.3)	3 (33.3)	8.1
Surgical infections (7)	3 (42.9)	2 (66.7)	14.3
Wounds (4)	1 (25.0)	0	0
Diabetic foot ulcer (2)	2 (100.0)	1 (50.0)	50
Total (50)	15 (30.0)	42.9	12

Table 2.Sample wise antibiogram profile of *Staphylococcus aureus* isolated from hospitalized patients

Number of *S aureus* (n) in different sampl	Resistance pattern of the *S. aureus* to various antibiotics (%)														
	OX			CIP			N			E			P		
	S	IR	R	S	IR	R	S	IR	R	S	IR	R	S	IR	R
Pus (9)	33.33	11.11	55.56	22.22	22.22	55.55	11.11	55.55	33.33	00	11.11	88.89	00	00	100
Surgical infectior	33.33	00	66.67	33.33	00	66.66	66.66	1	00	00	33.33	66.67	00	00	100
Wounds (1)	100	00	00	100	00	00	00	1	00	00	100	00	00	00	100
Diabetic foot ulc	50.00	00	50.00	00	00	100	50.00	00	50.00	00	100	00	00	00	100
Total (15)	40.00	6.67	53.33	26.67	13.33	60.00	26.67	46.67	26.67	00	33.33	66.67	00	00	100

Here: OX- oxacillin, CIP- ciprofloxacin, N- neomycin, E,- erythromycin, P- Penicillin

Discussion

The prevalence of MRSA in different samples of human patients hospitalized in Mymensingh Medical college was determined phenotypically by disc diffusion method and genotypically by amplification of methicillin resistant *mecA* gene. The MRSA is the most pathogenic and superbug organism that readily achieve antibiotic resistance and responsible for death of human due to treatment failure and ineffectiveness of antibiotics

The overall prevalence of *S. aureus* in clinical samples was 30% which was higher than the results of Hussain et al. (2016) but lower than the study of Islam et al. (2011). The prevalence of *S. aureus* was 24.32 % in pus sample, 42.85% in surgical infections, 25% in wound and 100% in diabetic foot ulcer which was similar to the findings of Rahman et al. (2018).

Hussain *et al.* (2016), Rahman *et al.* (2018) and Islam *et al*. (2018) reported the prevalence of MRSA in human as 15.38%, 53.1% and 80.30%,respectively indicating an increasing trend of MRSA in human patients and regionalvariation. In that situation, regular monitoring of the occurrence of MRSA is important to prevent of spread of MRSA and appropriate clinical management of hospital patients. The overall occurrence of MRSA in hospitalized human patients of this study was 53.33% which is similar to the findings of Rahman *et al.* (2018), lower than Islam *et al.* (2018) and higher than Hussain*et al*. (2016) and Islam *et al*. (2015). The *mecA* gene was amplified from 60% isolates of *S. aureus* which was higher than disc diffusion method where 53.3% isolates showed resistance to oxacillin. The isolates which carry *mecA* gene might not express phenotypically and unable to produce β-lactamase which makes the isolates susceptible to methicillin or oxacillin. Further these *mecA* gene carrying isolates might showed resistance to methicillin or oxacillin due to repeated exposure to similar group of antibiotics (van Griethuysen et al., 2005). Besides *mecA* gene, other genes are also responsible for making the *S. aureus* resistant to methicillin (Jonas et al., 2002). The *mecA* gene is located on a mobile genetic element, the staphylococcal chromosome cassette

(SCCmec) and at least 5 SCCmec types (I -V) were detected that vary in genetic base pair construction and size (van Belkum and Verbrugh, 2001).

Infections caused by MRSA in hospitalized patients are increasing worldwide including Bangladesh.The reasons of increasing the rate of MRSA needs to be addressed. The reasons might be irrational use of antibiotics without physician prescription which is common in Bangladesh, transmission of MRSA from human to animal or animal to human, lack of public awareness, use of unsterilized equipment, unhealthy lifestyle and in vivo or invitro formation of biofilm by MRSA.
Due to higher emergence of MRSA and treatment failure by methicillin, vancomycin, daptomycin, linezolid, rifampicin, etc. are largely used (Nguyen and Graber, 2010). There are several major limitations of use of these antibiotics that may potentially contribute to the persistent MRSA bacteremia and other recalcitrant infections. The ideal antibiotic for the treatment of infection caused by MRSA does not yet exist. The high proportion of emergence of MRSA and clinical failures in the treatment of invasive MRSA infections has prompted a re-evaluation of how these infections are managed.

Conclusions

High prevalence of MRSA infection in human and healthcare settings pose a major public health threat. Appropriate action to be needed to improve the infection control programs in healthcare settings. The results of this study provided the baseline for the future surveillance of MRSA in Bangladesh and have the potential to contribute to AMR policy and stewardship.

Acknowledgement

The authors would like to show their gratitude to the Department of Microbiology and Hygiene, Bangladesh Agricultural University, for providing the facility and technical support. The authors would also like to thank Mymensingh Medical College Hospital for providing the samples to conduct the study.

Conflict of Interest

The authors declare no conflict of interest.

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