RESEARCH ARTICLE

Frequency and comparison among antibiotic resistant Staphylococcus aureus strains in selected hospitals of Peshawar, Pakistan

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Abstract

Objective: To assess and compare the frequency of antibiotic-resistant staphylococcus aureus strains.

Methods: A retrospective study was conducted at the privately-owned Welfare Medical Laboratory, Peshawar, Pakistan, and comprised record related to the period between 07th February 2017 and 23rd March 2018 of patients referred for pus-testing from Khyber Teaching Hospital, Lady Reading Hospital and the Hayatabad Medical Complex. Pus samples were obtained from various parts of body with cotton swabs. The samples were cultured and the isolated staphylococcus aureus strains were analysed against selected antibiotics. The frequency of the isolated strains was tested and compared using Prism 7 software.

Results: Of the 6780 samples, staphylococcus aureus was found in 4315(63.64%). Wild-type staphylococcus aureus strains were 2133(31.46%), followed by 825(12.16%) methicillin-resistant, 792(11.68%) vancomycin intermediate, and 565(8.33%) vancomycin-resistant staphylococcus aureus strains. The isolated strains were significant ($p<0.0001$) for operated wounds, and non-significant ($p=0.8915$) for diabetic foot cases.

Conclusion: The frequency of antibiotic-resistant staphylococcu saureus strains was high.

Keywords: Antibiotic sensitivity, Pakistan, Frequency, S.aureus. (JPMA 70: 1199; 2020)

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Introduction

Over the past few decades, there has been a dramatic increase in the frequency of antibiotic resistant pathogens and strains.[1] The discovery of penicillin in 1940 lowered the occurrence of bacterial infections until Staphylococcus(S.) aureus began to produce beta (β)-lactamase enzyme which destroys the β-lactam ring in penicillin. This increased resistance to penicillin led to the emergence of methicillin-resistant S. aureus (MRSA) and later vancomycin resistant S. aureus (VRSA) strains.[2] Since then, endemics of MRSA and now vancomycin-resistant S. aureus (VRSA) have been occurring occasionally in hospitals and nursing homes worldwide.[3]

S. aureus is a gram-positive, non-spore-forming, non-motile, aerobic/facultative anaerobic bacteria found everywhere in the environment; water, food, domestic animals, human mucosal surfaces, and hospitals.[4] A number of virulence factors, like vanA gene product, heat stable enterotoxins, toxic shock syndrome (TSS) toxins, epidermolysis toxins, pyrogenic exotoxins, and cytolytic toxins, are responsible for pathogenicity and resistance in S. aureus.[5] Similarly, certain cell wall-associated factors, like clumping factors, fibronectin-binding proteins, willebrand factor fibres, are also involved in attachment and colonisation of S. aureus to extracellular matrix proteins, fibrins, platelets, fibrinogen, fibronectin and endothelial cells.[6]

The endemics of hospital-acquired S. aureus infections are most frequent in Asian countries with an estimated proportion of 28% in Hong Kong and Indonesia compared to more than 70% in South Korea. In contrast, the rate of community-acquired MRSA strains in Asia varies considerably, with an estimated range of <5% to >35% in different countries.[7] In 2013, lower frequency of MRSA (31.5%) was reported compared to a 2016 study (36.1%) in Peshawar, Pakistan.[8,9] It has been found that the lack of standard and even basic health facilities may result in increased rate of different health-related problems, especially those caused by pathogens. Similarly, many disease-associated risk factors, like low socio-economic status, poor hygiene and self-medication,also exist in under-developed countries like Pakistan which makes people more vulnerable to antibiotic-resistant micro-organisms, such as MRSA.[10]

It is understood that S. aureus is responsible for a number of asymptomatic infections in humans, ranging from body surface to the deep soft tissue infections, such as meningitis, mastitis and TSS.[11] As S. aureus is a multi-drug resistant bacterium which causes a variety of non-predictable infections in human, it is, therefore, a common practice among researchers to assess S. aureus frequency, prevalence and incidence from time to time. The current study was planned to assess and compare the

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frequency of antibiotic-resistant S. aureus strains.

Materials and Methods

A retrospective study was conducted at the privately-owned Welfare Medical Laboratory (WML), Peshawar, Pakistan, and comprised record related to the period between 07th February 2017 and 23rd March 2018 of patients referred for pus-culture testing from Khyber Teaching Hospital (KTH), Lady Reading Hospital (LRH) and the Hayatabad Medical Complex (HMC).

The pus samples were taken from operated wound (OW), diabetic foot (DF), breast abscess (BA) and miscellaneous (M) body parts of patients under sterile conditions by using cotton swabs. During the study period, history of each patient was taken and only those were selected for further study who were not on any antibiotic therapy. The sampling sites were sterilised with spirit swabs prior to sample collection. The samples were processed for the possible identification and isolation of MRSA, VRSA, vancomycin intermediate S. aureus (VISA) and wild-type S.aureus strains.

Different culture media used included blood agar (Oxoid), MacConkey agar (Oxoid), Mannitol salt agar (Oxoid), and Muller Hinton agar (Oxoid). The blood and MacConkey agar media were used for the initial identification, and Mannitol salt agar for the further confirmation of S. aureus. The media plates were examined for colony appearance after incubation for 24 hrs at 37°C.[12] Biochemical tests, such as coagulase and catalase, were performed to differentiate S. aureus strains from other staphylococcal species.[13]

Similarly, antibiotic sensitivity test against methicillin, oxacillin and vancomycin was performed by using Muller Hinton agar to differentiate isolated S. aureus strains resistant to these antibiotics. After 24-48 hours of incubation at 37°C, the zone diameters were measured around each antibiotic disc based on published guidelines by the Clinical Laboratory Standards Institute.[14]

The prevalence and comparison among different antibiotic-resistant strains of S. aureus were done using Fisher's exact test in Prism 7 statistical software.

Results

Of the 6780 samples, staphylococcus aureus was found in 4315(63.64%) (Table-1).

Wild-type S. aureus strains were 2133(31.46%), followed

Table-1: Overall prevalence of staphylococcus (S.) aureus in Peshawar region.

Total Samples (%)	Positive Cases	Negative Cases	Prevalence
6780	4315	2465	63.63%

Table-2: Comparison of significant pus samples.

Groups	Compared Samples	Positive Cases	Percentage (%) of Positive Cases	Significance $p < 0.05$
MRSA	OW-DF****	278, 150	16.85%, 9.09%	($p < 0.0001$)
	OW-BA**	278, 220	16.85%, 13.33%	($p = 0.0022$)
	OW-M****	278, 177	16.85%, 10.73%	($p < 0.0001$)
	DF-BA****	150, 220	9.09%, 13.33%	($p < 0.0001$)
VRSA	OW-DF****	260, 80	23.01%, 7.08%	($p < 0.0001$)
	OW-BA****	260, 108	23.01%, 9.56%	($p < 0.0001$)
	OW-M****	260, 117	24.30%, 10.93%	($p < 0.0001$)
	DF-M***	80, 117	7.48%, 10.93%	($p < 0.0002$)
VISA	OW-DF****	318, 169	20.08%, 10.67%	($p < 0.0001$)
	OW-BA****	318, 38	20.08%, 2.40%	($p < 0.0001$)
	OW-M**	318, 267	20.08%, 16.86%	($p = 0.0092$)
	DF-BA****	169, 38	10.67%, 2.40%	($p < 0.0001$)
	DF-M****	169, 267	10.67%, 16.86%	($p < 0.0001$)
	BA-M****	38, 267	2.40%, 16.86%	($p < 0.0001$)
Wild Type	OW-DF****	1208. 280	28.32%, 6.56%	($p < 0.0001$)
	OW-BA****	1208, 369	28.32%, 8.65%	($p < 0.0001$)
	OW-M****	1208, 276	28.32%, 6.47%	($p < 0.0001$)
	DF-BA***	280, 369	6.56%, 8.65%	($p < 0.0002$)
	BA-M****	369, 276	8.65%, 6.47%	($p < 0.0001$)

MRSA: Methicillin-resistant Staphylococcus (S.) aureus. VRSA: Vancomycin-resistant S. aureus. VISA: Vancomycin intermediate S. aureus. OW: Operation wound. DF: Diabetic foot. BA: Breast abscess. M: Miscellaneous. The asterisk (*) shows the significance of comparison. The higher number of stars (*) indicate higher significance and vice versa. Comparisons that resulted with the lowest or no significance are not presented.

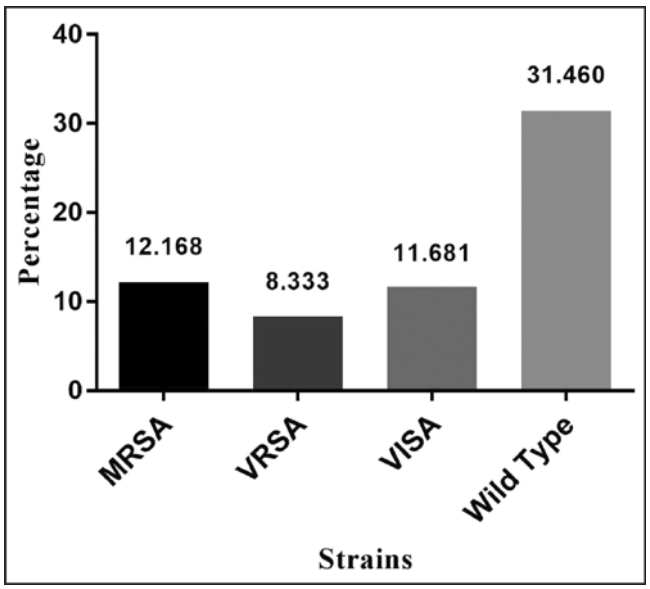

Figure: Prevalence of antibiotic resistant Staphylococcus (S.) aureus strains in Peshawar region.

by 825(12.16%) MRSA, 792(11.68%) VISA, and 565(8.33%) VRSA strains (Figure).

Among MRSA samples, OW-DF,OW-M, and DF-BA were highly significant ($p<0.0001$) compared to OW-BA ($p<0.0022$) and BA-M ($p<0.0155$) that were least significant, and DF-M which was non-significant ($p>0.05$). All the sub-groups of VRSA and VISA were highly significant ($p<0.0001$) except DF-BA ($p< 0.0308$) in VRSA and OW-M ($p<0.0092$) in VISA that were the least significant. DF-M was the only non-significant subgroup in wild-type S. aureus (Table-2).

Only wild-type S. aureus strains showed 31.46% (2133 in number) of maximum resistance to methicillin, oxacillin and vancomycin antibiotics, followed by12.16% (825 in number) resistance in MRSA to methicillin and oxacillin. In contrast, vancomycin effectiveness was observed in 11.68% (792 in number) of VISA and 8.33% (565 in number) of VRSA strains.

Discussion

The main risk factors of S. aureus infections identified Europe and parts of Asia, including Pakistan, were those acquired through either community or hospital. Some of them were ventilation, surgical site, intensive care units (ICUs), diabetes, nasal carriage, invasive devices and antibiotic exposure.[15] The risk factors found in the present study are different except OWs that can be specifically acquired in hospital settings. The frequency of MRSA in Pakistan in 2013 was 31.5%, and in 2016 it was 36.1% in Peshawar.[8,9] The present study, conducted in 2018, found it to be 12.16% in three selected hospitals of Peshawar. Another study conducted in a tertiary care hospital in Karachi reported 72% frequency of S. aureus infections compared to the present study in which overall frequency of S. aureuswas 63.64%.[16]

Wild type S. aureus strains were more common 2133(31.46%) compared to the other strains in the current study. In a study conducted in northeastern Ohio, 280 S. aureus strains were isolated, and only 64(22.85%) were of the wild-type strain.[17] With the emergence of MRSA, vancomycin is used as the main drug of choice for treating MRSA infections, but due to its widespread use, it is also getting sensitive to MRSA strains. According to a study conducted in northern India, only 6 VISA strains were isolated out of 123 oxacillin-resistant S. aureus isolates through minimum inhibitory concentration (MIC) method.[18] Similarly, another study in Kolkata reported only 4(0.56%) VISA strains in 714 positive cases of S. aureus.[19] VRSA are very rare, but their resistance to antibiotics is rapidly increasing as in a 2014 study, their frequency was 28.68% in Egypt.[20] On the other hand, the frequency rates of VISA and VRSA were 792(11.68%) and 565(8.33%) in the present study which is very high.

The current study isolated S. aureus through culturing and antibiotic susceptibility tests alone, which is a limitation. Future studies should focus on molecular techniques as well.

There is a need to control various risk factors, like low socio-economic status and self-medication, that are associated with S. aureus infections. Also, effective infection control strategies, like quorum quenching, phage therapy, immunotherapy, and nanoparticles are needed to be developed and implemented.

Conclusion

There was a high frequency of antibiotic-resistant S. aureus strains in the Peshawar region of Pakistan.

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Conflict of Interest: None.

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