Original Article

Evaluation and Susceptibility Pattern of Staphylococci Isolated From Clinical Specimens in POF Hospital, Wah Cantt.

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Abstract

Objective: To determine the frequency and association of the antibiotic susceptibility pattern between methicillin-resistant and sensitive Staphylococci and find out the association between age, gender, the outcome of patients, and type of specimens with methicillin resistance in methicillin-resistant and sensitive Staphylococcal isolates

Materials & Methods: This cross-sectional study was carried out in the Microbiology Department of POF Hospital, Wah from January 2019 to September 2020. One hundred and eighty-four staphylococci isolated from clinical specimens were processed as per standard methodology.

Results: Out of 184, methicillin-resistant S. aureus and methicillin-resistant coagulase-negative staphylococci were 38.04% and 13.04% respectively. Infections caused by Methicillin-resistant staphylococcal isolates were higher among the age group 31-40 years (71.4%, OR=2.68). Out of thirty expired patients, 33.3% had been infected with methicillin-resistant staphylococcus aureus and 20% with methicillin-resistant coagulase-negative species. The methicillin-resistant staphylococci were most frequent in the miscellaneous category of clinical specimens (80.0%, OR=4.63). The susceptibility analysis revealed that methicillin-resistant staphylococci are 100% resistant to penicillin, meropenem, and amoxicillin-clavulanate (p=0.000). A significant association of methicillin resistance was also noticed against amikacin (p=0.002), ciprofloxacin (p=0.001), clindamycin (p=0.005), and erythromycin (p=0.000). Moxifloxacin, linezolid, and vancomycin are the most effective choices for infections caused by methicillin-resistant staphylococci.

Conclusions: The methicillin-resistant staphylococci are highly resistant to commonly used oral as well as injectable used antibiotics. The establishment and implementation of infection control policies are required to combat the grave situation of increasing antibiotic resistance.

Keywords: Antibiotic susceptibility, Methicillin-resistant staphylococci, ciprofloxacin, clindamycin.

Introduction

Staphylococci are Gram-positive spherical bacteria that rapidly develop resistance to many antimicrobials resulting in therapeutic failures. High-throughput species identification of Staphylococci is possible by PCR coupled to electrospray ionization-mass spectrometry in research laboratories.[1,2] Although the aforementioned methods have revolutionized diagnostic modalities but routine laboratories in developing countries have limited access to advanced technologies due to their high costs and technical expertise. Despite limitations, biochemical tests like coagulase activity are used for presumptive identification of clinical isolates of Staphylococcus aureus, especially in laboratories with constrained resources.[3] The coagulase of staphylococci is the virulent protein that binds prothrombin to form a complex with thrombin and leads to fibrin polymerization.[4] The detection of coagulase in staphylococci obtained from human specimens is usually equated with the species identification of S. aureus and clinically, common species of staphylococci other than S.aureus are referred to as coagulase-negative staphylococci.[5]

Among all species of Staphylococci, S.aureus is considered to be the most pathogenic, being responsible for a variety of infections, ranging in severity from food intoxication or boil to septicaemia. S.aureus and the coagulase-negative staphylococci are members of normal human microbiota which colonize the skin and mucous membranes but may become pathogenic following breaks in the cutaneous epithelial barrier through trauma or medical interventions. Advancements in the fields of medicine, surgery, and bioengineering have paved the way for the increased use of prosthetic implants and medical devices. The most frequently isolated coagulase-negative S.epidermidis is associated with implanted appliances and devices, especially in patients of extreme age and immunocompromised conditions.[5] The significance of staphylococcal isolated from a specimen requires clinical correlation to determine whether it's a contaminant, colonizer, or pathogen.

Multidrug-resistant strains of staphylococci particularly methicillin-resistant staphylococci are well-documented etiological agents of nosocomial infections and are associated with increased morbidity and mortality. The literature review indicates that the frequency of methicillin-resistant staphylococci is heterogenous within the country as well as across the country.[6]

The resistance against beta-lactams developed because of the adaption of S. aureus to the exogenously acquired SCCmec, deletion, and mutation of genes implicated in general metabolism and general stress response and the adjustment of metabolic networks resulted in an increase of β-lactams minimal inhibitory concentration. Multiple studies based on whole genome sequencing technologies indicated that mecA developed from a harmless core gene (mecA1) encoding the penicillin-binding protein D (PbpD) from staphylococcal species of animal origin (S.sciuri group). The emergence of the resistance determinant involved a distortion of the PbpD active site, an increase in mecA1 expression, the addition of regulators (mecR1, mecI), and integration into a mobile genetic element (SCCmec). SCCmec was then transferred into species of coagulase-negative staphylococci (CoNS) that is transferred to S.aureus of human origin.[7] Colonization of skin and mucous membranes of the inpatient by multidrug-resistant CoNS strain and its transmission by hands of health care workers is a critical step in the making CoNS a successful nosocomial pathogen.

The dynamic antimicrobial resistance phenomenon renders the antibiotics susceptibility profile of a specific region at a specified period inapplicable to other regions or in another time period.[8] The emerging antimicrobial resistance has been declared as one of the top ten global public health threats of the modern century.[9] It's an established fact that antimicrobial susceptibility data from any given regional, national, or international surveillance study cannot reliably predict the drug resistance profiles of pathogens isolated from an individual patient. The local susceptibility profiles serve to rationalize the empirical treatment resulting in evidence-based practices and better outcomes in terms of enhanced recovery from infections, shorter duration of hospital stay, and cost-effectiveness.

The current study was planned to

i. Determine the frequency and association of the antibiotic susceptibility pattern between methicillin-resistant and sensitive Staphylococci isolated from clinical specimens as a result of culture and sensitivity.
ii. Find out the association of age, gender, the outcome of patients, and type of specimens with methicillin resistance in methicillin-resistant and sensitive Staphylococcal isolates.

Materials and Methods

It was a descriptive cross-sectional study that was conducted at the department of Microbiology, POF Hospital, Wah from January 2020 to March 2021. The sampling technique was non-probability, consecutive sampling. One hundred and eighty-four clinical specimens of either gender, of all ages, yielding growth of staphylococci were included in the study. Duplicate samples of the same patient from the same site were not included.

The specimens were inoculated on appropriate culture mediums like blood agar, MacConkey agar, and cysteine lactose electrolyte deficient agar (urine). These were incubated at 35-37˚C under aerobic conditions for 24 hours. After overnight incubation, the agar plates were examined for the growth of bacteria and their colonial morphology. The isolates were stained by the Gram method. The Gram-positive cocci with positive catalase reaction were considered Staphylococci. Further differentiation of Staphylococci into coagulase-negative staphylococci and Staphylococcus aureus was done based on coagulase test and DNase tests.[1] All staphylococci were screened for methicillin susceptibility by using a 30 µg cefoxitin disc. The antimicrobial susceptibility tests were performed by the disc diffusion method according to clinical laboratory standards institute recommendations. The bacterial suspensions of isolates equivalent to 0.5 McFarland standard turbidity were placed on Mueller-Hinton agar (Oxoid, Basingstoke, UK). The discs of penicillin (10 IU), amoxicillin-clavulanate (20/10 µg), trimethoprim-sulfamethoxazole (1.25/ 23.75 µg), cephradine (30 µg), cefoxitin (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), meropenem (10 µg) and linezolid (30 µg) were placed on the Mueller–Hinton agar followed by incubation at 37 °C for overnight. After overnight incubation, the diameter of each zone of inhibition around the antimicrobial disc was measured. The susceptibility testing results were interpreted according to recommendations of CLSI as sensitive, intermediate, and resistant. Concurrent quality control testing was performed with Staphylococcus aureus ATCC 25923.[10]

Vancomycin MIC by Epsilometer Test (E-test) was determined by following the manufacturer's instructions. The 0.5 McFarland standard suspension of the isolate was applied on the Mueller-Hinton agar plate. The vancomycin E-test strip (Liofilchem, Italy) was applied over the plate with the help of an applicator within 5 min of lawn culture. Plates were incubated at 37 °C for 24 h. A teardrop zone of inhibition was observed. The zone edge intersecting the graded strip at the minimum concentration of the antibiotic was interpreted as the MIC. The isolates of S.aureus were reported sensitive to Vancomycin with MICs of ≤2 µg/ml, intermediate susceptible with MICs of 4-8 µg/ml, and resistant with MIC ≥8 µg/ml. The isolates other than S.aureus were interpreted as sensitive to vancomycin with MICs of ≤4 µg/ml, intermediate susceptible with MICs 8-16 µg/ml, and resistant with MIC ≥32 µg/ml.[10]

The data was entered and analyzed using SPSS version 23. The numerical data were analyzed by chi-square test, odd ratio, and 95% confidence interval to determine the statistical difference in the age, gender, and specimens. The association of antibiotic susceptibility patterns for methicillin resistance and methicillin-sensitive S.aureus as well as methicillin resistance and methicillin-sensitive coagulase-negative staphylococci were determined by the chi-square test. A p-value less than 0.05 was considered significant.

Results

A total of one hundred and eighty-four staphylococci were analyzed. Out of which, methicillin-resistant S.aureus and methicillin-resistant coagulase-negative staphylococci were 38.04% and 13.04% respectively. (Figure 1)

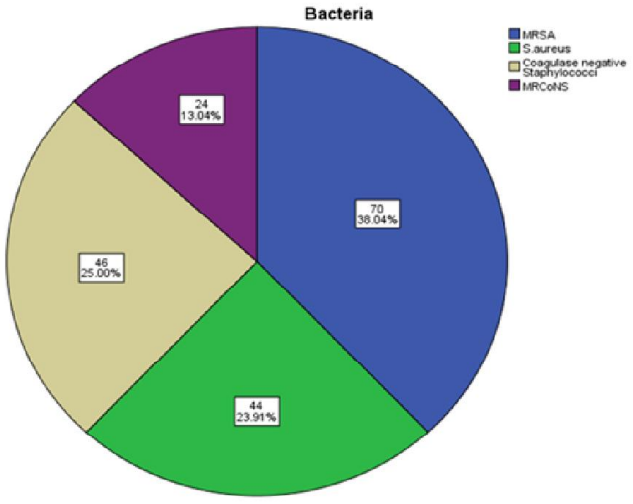

Figure 1: Staphylococci isolated from clinical specimens in POF Hospital (n=184)

The frequency of male patients was found to be 49.1% and 50.9% for methicillin-resistant staphylococci and methicillin-sensitive staphylococci respectively.

Similarly, the frequency of female patients was 51.4% and 48.6% for methicillin-resistant staphylococci and methicillin-sensitive staphylococci respectively. (Table 1)

Infections caused by Methicillin-resistant staphylococcal isolates were higher among the age group 31-40 years (71.4%, OR=2.68) followed by the age group 11-20 and 21-30 years. (66.7%, OR=0.365) Infections caused by Methicillin-sensitive staphylococci were higher among the age group 51-60 years (66.6%, OR=0.48) followed by neonates (65.7%, OR=0.35). (Table 1)

Out of thirty expired patients, 53.3% had been infected with methicillin-resistant staphylococcal infections and 49.4% with methicillin-sensitive staphylococcal infections. (OR= 1.17)

The specimen-wise distribution showed that methicillin-resistant staphylococci were most frequent in the miscellaneous category (80.0%, OR=4.63) followed by pus (53.8%, OR=1.30). On the other hand, the methicillin-sensitive staphylococci were most frequent in the blood (60.5%, OR=0.45) followed by pus (46.2%, OR=1.30).

The susceptibility analysis revealed that methicillin-resistant staphylococci are 100% resistant to penicillin (p=0.000) followed by meropenem (93.9%, p=0.000) and amoxicillin-clavulanate (74.2%, p=0.000). A significant association of methicillin resistance was noticed for amikacin, ciprofloxacin, clindamycin, and erythromycin. (Table 2)

Table 1: Methicillin resistance of staphylococci isolated from clinical specimens in POF Hospital (n=184)

Variables	*Staphylococcal isolates*	*Methicillin-resistant n(%)*	*Methicillin sensitive n(%)*	*X2*	*OR*	*95% confidence interval*		*P value*
						Lower limit	Upper limit	
GENDER								
Male	110	54(49.1%)	56(50.9%)	0.090	0.91	0507	1.647	0.764
Female	74	38(51.4%)	36(48.6%)					
AGE (GROUPS) IN YEARS								
Neonates	70	24(34.3%)	46(65.7%)	11.16	0.35	0.190	0.656	0.001
<10	80	30(37.5%)	50(62.5%)	8.846	0.40	0.223	0.740	0.003
11-20	6	4(66.7%)	2(33.3%)	0.689	2.04	0.365	11.453	0.406
21-30	6	4(66.7%)	2(33.3%)	0.689	2.04	0.365	11.453	0.406
31-40	14	10(71.4%)	4(28.6%)	2.783	2.68	0.810	8.889	0.095
41-50	10	6(60.0%)	4(40.0%)	0.423	1.53	0.418	5.630	0.515
51-60	6	2(33.3%)	4(66.6%)	0.689	0.48	0.087	2.737	0.406
>60	26	16(61.5%)	10(38.5%)	1.612	1.72	0.738	4.037	0.204
OUTCOME OF PATIENTS								
Expiry	30	16(53.3%)	14(46.7%)	0.159	1.17	0.536	2.568	0.690
Shift out	154	76(49.4%)	78(50.6%)					
SPECIMENS								
Blood	86	34(39.5%)	52(60.5%)	7.074	0.45	0.250	0.814	0.008
Pus	78	42(53.8%)	36(46.2%)	0.801	1.30	0.727	2.348	0.371
Miscellaneous	20	16(80.0%)	4(20.0%)	5.947	4.632	1.484	14.450	0.004

X2 = Chi square, OR = odd ratio, n = Total number of Staphylococci isolated

Table 2: Comparison of Antibiotics susceptibility pattern between methicillin-susceptible and resistant staphylococci isolated from clinical specimens

Antibiotics	*Drug susceptibility*	*Methicillin-resistant Staphylococci*	*Methicillin sensitive Staphylococci*	*P value*
Penicillin	Susceptible	0 (0 %)	24 (26.1%)	0.000
	Resistant	92(100%)	68 (73.9%)	
Amoxycillin-clavulanate	Susceptible	0 (0 %)	58(100%)	0.000
	Resistant	92(74.2%)	34(27.0%)	
Amikacin	Susceptible	52 (41.9%)	72 (58.1%)	0.002
	Resistant	40 (66.7%)	20 (33.3%)	

Ciprofloxacin	Susceptible	25(34.2 %)	48(65.8%)	0.001
	Resistant	67(60.4%)	44(39.6%)	
Clindamycin	Susceptible	43(41.0%)	62(59.0%)	0.005
	Resistant	49(62.0%)	30(38.0%)	
Clarithromycin	Susceptible	44(43.6 %)	57(56.4%)	0.054
	Resistant	48(57.8%)	35(42.2%)	
Cotrimoxazole	Susceptible	28(45.2 %)	34(54.8%)	0.349
	Resistant	64(52.5%)	58(47.5%)	
Doxycycline	Susceptible	42(43.8 %)	54(56.3%)	0.077
	Resistant	50(56.8%)	38(43.2%)	
Erythromycin	Susceptible	16(27.6%)	42(72.4%)	0.000
	Resistant	76(60.3%)	50(39.7%)	
Gentamicin	Susceptible	41(44.1 %)	52(55.9%)	0.105
	Resistant	51(56.0%)	40(44.0%)	
Linezolid	Susceptible	90(50.0 %)	90(50.0 %)	1.000
	Resistant	2(50.0 %)	2(50.0 %)	
Meropenem	Susceptible	0(0 %)	86(100 %)	0.000
	Resistant	92(93.9%)	6(6.1 %)	
Moxifloxacin	Susceptible	64(50%)	64(50%)	1.000
	Resistant	28(50%)	28(50%)	
Vancomycin	Susceptible	88(49.7%)	89(50.3 %)	1.000
	Resistant	4(57.1%)	3(42.9 %)	

Discussion

Methicillin-resistant staphylococci are the most frequent etiological agents of nosocomial and devices related infections. Existing literature on MRSA has demonstrated that there is a significant geographical variation in the frequency of staphylococci within and between countries. The incidence of MRSA is 66.7% as reported in a study conducted by Hussain et al in Pakistan.[11] Another local study showed isolation of MRSA from 76% of clinical isolates.[12] These results are in contrast to data of our study which revealed a relatively less frequency of MRSA which is 38.04%.

In Pakistan, the data regarding the susceptibility pattern of coagulase-negative staphylococci are scarce. A study published in 2013 which was conducted in Karachi revealed that 70% of CoNS was methicillin-resistant and resistance to other commonly prescribed ciprofloxacin, erythromycin and doxycycline was 35.2%, 58.3%, and 24.7% respectively.[13] Study conducted by Latif et al at Rawalpindi concluded a rise in methicillin resistance rate in CoNS from 22.7% to 59.6% in three consecutive years.[14] The contrasting results of our study may be explained by differences in hospital catchment areas and surgical interventions including biological implants and prostheses fabrications. The hospital environment and the normal human microbiota of patients and health care personnel play crucial roles as the reservoirs and vectors for the spread of antibiotic-resistant bugs. The methicillin-resistant Staphylococci are resistant to beta-lactam antimicrobials including penicillins, β lactam combination agents, cephems except for ceftaroline and carbapenems.[15] Limited treatment options are left behind for the treatment of methicillin-resistant staphylococcal isolates, thus rendering the situation more cumbersome. A significant association of methicillin-resistant staphylococcal isolates is found with the resistance pattern of beta-lactam drugs including penicillin, amoxicillin-clavulanate, and meropenem.

Our study concluded an insignificant association of gender with methicillin resistance in staphylococci. Humphrey et al reviewed the epidemiology of MRSA which shows a male predominance in cases of bloodstream infections and poorer outcomes for female patients. The multifactorial reasons including comorbidities behavioral and physiological factors explain the difference.[16] In a recent study conducted by Aratani et al, methicillin resistance was not significantly associated with mortality of patients with S.aureus bacteremia.[17] Our study also concluded the same. The limitations of our study are that the history of antibiotics use and underlying diseases of patients were not available.

Some strains of the staphylococci which harbour genetic elements for methicillin resistance also carry the genes that confer resistance to non-beta lactam antimicrobials.[18] Analysis of our data revealed a

significant association of resistance of methicillin-resistant staphylococci against amikacin, ciprofloxacin, and erythromycin which are commonly used non-beta lactam antibiotics in our setup. This finding is in concordance with those reported by Shah et al.[19] Similar resistance patterns have been reported in other studies from Islamabad[20] and Nepal.[21]

This situation is entirely different when compared to the frequency and susceptibility pattern of methicillin-resistant staphylococcal isolates prevailing in England as published in the English surveillance programme for antimicrobial utilization and resistance (ESPAUR) report, 2019.[22]

The contrasting results provide evidence of injudicious and imprudent use of antibiotics in our settings.

According to the literature review, linezolid and vancomycin are the most convenient and effective choices for methicillin-resistant staphylococci owing to their high susceptibility patterns.[23,24] Our data supports the fact that moxifloxacin, linezolid, and vancomycin are the most effective therapeutic options for staphylococcal infections in our setup. There is an insignificant association between methicillin-resistant staphylococcal isolates with resistance patterns of moxifloxacin, linezolid, and vancomycin. These results of susceptibility of staphylococcal isolates against linezolid and vancomycin are closer to findings as reported by Rosatto et al.[25] Judicious use of these antibiotics should help physicians treat patients with multidrug-resistant infections. Strict infection control measures and antibiotic stewardship may prevent the development of resistance to these last-resort antibiotics. More in-depth studies and research on the dynamics of resistance development are required in our setup to consolidate the current treatment measures for a better health outcome.

Conclusion

The methicillin-resistant staphylococci are highly resistant to commonly prescribed oral as well as injectable antibiotics. The establishment and implementation of infection control policies are required to combat the grave situation of increasing antibiotic resistance.

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