Original Article

The resistance pattern of Staphylococci against beta-lactam group of antibiotics in Hyderabad, Pakistan

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Objective: To estimate the frequency of antibiotic resistance among the Staphylococci against a set of beta (β) lactam group of antibiotics.

Methodology: A total of 156 cultures of Staphylococci were isolated from clinical specimens and based on the coagulase production were categorized into two groups as; Coagulase Positive Staphylococci (CoPS) and Coagulase Negative Staphylococci (CoNS). The antibiotic resistance was determined using Kirby Baur disc diffusion method.

Results: Out of 156 isolates 57.6% (n=90) isolates were from male patients while 42.4% (n=66) were from female patients. About 62.1 % (n=97) of the isolates were CoPS while 37.9 % (n=59) were CoNS. Among CoPS the highest percentage of resistance (97.9%) was observed against penicillin G whereas among the CoNS the highest resistance was observed against Cefixime (93.2%). The comparative analysis for their potential to resist various members of beta-lactam antibiotics between CoPS and CoNS suggested non-significant differences for majority of the antibiotics used in this study. Comparatively higher resistance against amoxicillin was seen for CoPS (92.7%) than CoNS (79.6%). The odd ratio (OR) and confidence interval (CI 95%) for amoxicillin resistance between CoPS vs CoNS was calculated to be: 3.28 (1.21 – 8.89), while the *p*-values were determined to be 0.015 using chi square test of independence applying 2x2 contingency table. These values suggest a significant association of amoxicillin resistance with CoPS.

Conclusion: In general, beta-lactam antibiotic resistance in Staphylococcal isolates is observed greater in Hyderabad as compared to the previously described resistance in other regions of Pakistan. (Rawal Med J 202;46:18-21).

Keywords: Staphylococcus aureus, Coagulase-Negative Staphylococci (CoNs), beta-lactam antibiotics.

INTRODUCTION

Antibiotic resistance in Staphylococci is of great concern because it colonizes in multiple organs in human being as normal flora and also recognized as the versatile pathogen with various virulent properties. Staphylococci are generally catalase positive and require about a salt concentration up to 10%, for their growth on media.[1] Based on their ability to coagulate plasma, the heterogenous species of Staphylococci are broadly divided into two main groups as, Coagulase Positive Staphylococci (CoPS) and Coagulase Negative Staphylococci (CoNS). *S. aureus* is one of the predominant member of the coagulase positive Staphylococci and the most prevalent source of community and hospital acquired infections.[2,3] The involvement of *S. aureus* and CoNS in various serious life threatening infections is exacerbated by the rapid emergence of antibiotic resistance in these bacteria.[4,5]

Treatment of bacterial infections with β-lactam antibiotics is generally considered safe. The penicillin and cephalosporins derivatives are the members of the β-lactam antibiotics. However, soon after the development of first β-lactam antibiotic, penicillin resistant *S. aureus* emerged.[2,3] The resistance initially began with hospital settings and then spread in community.[4] Resistance is manifested through "Inactivation of β-lactam antibiotics" and "Production of alternate target site for binding to β-lactam drug". Nearly 90% of *S. aureus* species employ beta lactamase (penicillinase) to destroy the penicillin structure. Beta lactamase enzyme is encoded by *blaZ* gene which is located either on main chromosome or transferable plasmid. Expression of altered

penicillin binding protein (PBP2a) generates resistance to penicillinase resistant penicillins (Oxacillin and Methicillin) in *S. aureus*. The prevalence of Methicillin resistance is now at rise and a global problem consequently narrowing the treatment options.

In Pakistan different studies based on the phenotypic determination of penicillin and methicillin resistance have been reported.[5,6] The present study aimed to study the prevalence of *S. aureus* and other species of Staphylococci in different clinical samples and evaluate their antibiotic resistance pattern specifically against beta lactam group of antibiotics.

METHODOLOGY

Analytical grade media and chemical reagents were used for this study. The study was approved by Advances Studies and Research Board and Higher Education Commission funded Molecular Microbiology and Genetics Laboratory at the Institute of Microbiology, University of Sindh, Jamshoro. All the clinical isolates of Staphylococci (n=160) were received from Diagnostic and Research laboratory of Liaquat University of Medical & Health Sciences (LUMHS) Hyderabad from October 2018 to September 2019.

The isolates were collected on Mannitol Salt Agar medium (Oxoid) irrespective of gender, age or ethnicity. A total of 156 reconfirmed Staphylococcus isolates were included in this study. Coagulase test was performed to differentiate the species of Staphylococci as Coagulase Positive Staphylococci (CoPS) and Coagulase Negative Staphylococci (CoNS).

Antibiotic resistance was determined using Kirby-Bauer Disc Diffusion method. The plates were incubated at 37°C for 24 hours. The diameter of the clear zones (zones of inhibition) observed around the antibiotic discs were measured according to the guidelines of Clinical and Laboratory Standard Institute (CLSI).

Statistical Analysis: Statistical analysis was performed using SPSS version 22. A chi-square test used for analysis. $p<0.05$ was considered significant.

RESULTS

The study included 156 Staphylococcal isolates. Majority were from blood (51.2%), Pus (43.5%) and wound (1.9 %). Out of 156, 90 isolates were from male patients while 66 were from females. About 62.1% (n=97) isolates were CoPS.

We used nine different antibiotics; three belonging to penicillin group and six to cephalosporin group. The highest percentage of resistance among CoPS was observed against penicillin G (97.9%) (Table 1) whereas among the CoNS the highest resistance was observed against Cefixime (93.2%) (Table 2).

Table 1. Resistance/sensitivity profile of CoPS and CoNS against Penicillin group of antibiotics.

Antibiotic	Category	Profile	No.	%	% of difference	OR	CI [95%]	*p*-value
Penicillin	CoPs	Resistant	95	97.9	12.4797	7.45	1.52-36.41	0.692
		Sensitive	2	2.1				
	CoNs	Resistant	51	86.4				
		Sensitive	8	15.6				
Amoxicillin	CoPs	Resistant	90	92.7	15.206	3.28	1.21-8.89	0.015
		Sensitive	7	7.3				
	CoNs	Resistant	47	79.6				
		Sensitive	12	20.4				
Oxacillin	CoPs	Resistant	76	78.3	1.64661	0.92	0.42-2.05	0.846
		Sensitive	21	21.7				
	CoNs	Resistant	47	79.6				
		Sensitive	12	20.4				

Table 2. Resistance/sensitivity profile of CoPS and CoNS against Cephalosporin group of antibiotics.

Antibiotic	Source	Profile	No.	%	% of difference	OR	CI [95%]	*p*-value
Cephradine (CE) 1st G	CoPs	Resistant	86	88.6	4.85549	1.23	0.46-3.25	0.681
		Sensitive	11	13.4				
	CoNs	Resistant	51	84.4				
		Sensitive	8	15.6				
Cefoxitin (FOX) 2nd G	CoPs	Resistant	76	78.3	2.71845	1.13	0.52-2.43	0.763
		Sensitive	21	21.7				
	CoNs	Resistant	45	76.2				
		Sensitive	14	23.8				
Cefuroxime (CXM) 2nd G	CoPs	Resistant	58	59.7	0.672269	1.02	0.53-1.97	0.954
		Sensitive	39	40.3				
	CoNs	Resistant	35	59.3				
		Sensitive	24	40.7				
Cefixime (CFM) 3rd G	CoPs	Resistant	91	93.8	0.641711	1.1	0.3-4.08	0.883
		Sensitive	6	6.2				
	CoNs	Resistant	55	93.2				
		Sensitive	4	6.8				
Ceftriaxone (CRO) 3rd G	CoPs	Resistant	81	83.5	11.3924	1.73	0.78-3.82	0.175
		Sensitive	16	16.5				
	CoNs	Resistant	44	74.5				
		Sensitive	15	25.5				
Cefepime (FEP) 4th G	CoPs	Resistant	80	82.4	1.46699	1.0	0.47-2.49	0.86
		Sensitive	17	17.6				
	CoNs	Resistant	48	81.2				
		Sensitive	11	18.7				

The analysis against Penicillin, Oxacillin, as well as 1st, 2nd, 3rd and 4th generations of cephalosporins suggested non-significant differences. However; significantly different pattern against amoxicillin was seen. About 15.2% of difference was observed in the resistance potential between CoPS and CoNS against amoxicillin. The OR and CI (95%) was calculated as: 3.28 and (1.21 – 8.89) respectively, while *p*-value was 0.015. Amongst the Cephalosporin's group, a slight variation is seen for the 3rd Generation representative, Ceftriaxone (CRO) between CoPS and CoNS indicating a non-significant but a substantial susceptibility of CoNS (~25 %) to this antibiotic (Table 2).

DISCUSSION

Soon after the discovery of Penicillin in 1944, more than half of the *S. aureus* were reported resistant to the Penicillin and other penicillinase resistant Penicillins.[3,7] Globally, Staphylococcal infections are one of the most common contagions in both the community and health settings.[8] The *S. epidermis* group of CoNS is well documented for blood stream infections, endocarditis, peritonitis and meningitis specifically through foreign body related devices in hospital settings.

The shunt associated isolation frequency of CoNS from CSF and ascitic fluid is reported 11% and 67%, respectively.[3,7] However, in the present study due to the limited size of CSF and ascitic fluid samples and lack of previous history of patients, we are unable to speculate about the comparative prevalence of CoNS-associated peritonitis or meningitis infections. Penicillin resistance in Staphylococci from clinical

samples is reported above 80-90%.[9,10] In our study, nearly 98% S. *aureus* were resistant to penicillin whereas in CoNS, ~86% isolates were found resistant to penicillin. However, it is interesting to note that resistant to Oxacillin is nearly equivalent in both the CoPS and CoNS (~78 and 79%) isolates, which is significantly greater to the data obtained in other studies in Pakistan.[11,14] The Oxacillin resistant Staphylococci are shown to be resistant to multiple antibiotics including Cephalosporins.[15,16]

A notable reduction in the resistance was observed against the cefuroxime, a member of the 2nd Generation in both the CoPS and CoNS. Nearly similar level of resistance or sensitivity profile was observed between CoPS and CoNS against the all five members of cephalosporins except ceftriaxone, a 3rd generation member of cephalosporin group, showing > 10 percent of difference. In one study conducted in Pakistan, Cephradine resistance in *S. aureus* is described about 60%.[17] It is interesting to observe that the isolated CoPS and CoNS' species showed minimum resistance (59%) to 2nd G- Cefuroxime, perhaps due to the lack of selective pressure of this antibiotic in the particular surroundings.

CONCLUSION

In general, beta-lactam antibiotic resistance in Staphylococcal isolates is observed greater in Hyderabad as compared to the previously described resistance in other regions of Pakistan.

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