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Aminoglycoside Resistance Pattern in *Staphylococcus aureus* at Hyderabad, Pakistan

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Abstract: A total of 118 *Staphylococcusaureus* isolates from various clinical and non-clinical sources were processed for the determination of antibiotic resistance against a set of aminoglycoside group of antibiotics. In this study,44% (n=52) isolates were from various clinical specimens i.e. pus, blood and nose, while 56% (n=66) were recovered from the skin surfaces of healthy volunteers representing the non-clinical isolates. The highest percentage of resistance among both clinical and non-clinical isolates was observed against Neomycin (i.e. clinical = 86.5%, non-clinical = 39.3%). The lowest percentage of resistance among both clinical and non-clinical isolates was observed against Gentamycin (i.e. clinical = 34.6%, non-clinical = 4.5%). The percentage of difference for Amikacin, Gentamycin and To bramycin resistance between clinical non-clinical isolates was observed to be more than 100. The Odd Ratio for Amikacin, Gentamycin and Tobramycin resistance between clinical and non-clinical isolates was 17.75, 11.12, and 20.59, respectively while the *p*-values were determined as 0.0001 in all the three cases, suggesting a significant association of Amikacin, Gentamycin and Tobramycin resistance with clinical *S.aureus*isolates.

Keywords: *S.aureus*, Aminoglycosides, Antibiotic Resistance, Hyderabad.

1. INTRODUCTION

Staphylococcusaureus (*S.aureus*) is one of the communal pathogen associated with both community and hospital acquired infections (Ahmed *et al.,* 2002). The emerging resistance to various antibiotics is quite wide spread in *S.aureus* now and is a major public health concern (Atif *et al.,* 2018).(Bayer and Murray. 2009) (Resistance to Penicillin was witnessed in this organism soon after few years of the discovery of antibiotics (Chambers, and Deleo. 2009) and continued further to new antibiotics with the passage of time A (Cameron, *et al.,* 2011). wide spread resistance to beta-lactam antibiotics is reported for *S.aureus* both in community and hospital acquired infections (Khosravi, *et al.,* 2017). One of the notable characteristic of *S.aureus* is its asymptomatic association with human being as a normal flora (Foster, 2017). (Gillespie, *et al.,* 1985) Nearly 20-80 % of the human population harbors *S aureus* in their nares and possibly at high risk of acquiring and spreading S.*aureus* infections specially in health compromised states (Gillespie, *et al.,* 1985). Antibiotics that inhibit the protein synthesis in bacterial cells comprise a large number of compounds such as Chloramphenicol, Erythromycin, Lincomycin, Aminoglycosides and Tetracycline. These exert a broad-spectrum activity against a wide variety of Gram –ve and Gram +ve bacteria (Hamilton. *et al.,* 2017) *S.aureus* resistance against various commonly used protein synthesis inhibiting antibiotics has been witnessed (Hamilton. *et al.,* 2017) 10). (Sohail, and Latif. 2017)

The current study is however focused on the Aminoglycoside group of antibiotics. Aminoglycosides are the antibiotics that disturb the bacterial growth by affecting the protein synthesis. Their bactericidal effect is exerted by binding to the 30S subunit of ribosomes, ultimately impeding the translation process in bacteria. Aminoglycosides are commonly used to treat Staphylococcal infections either alone or in combination with beta-lactam antibiotics to provide synergistic effect in severe infections like endocarditis and bacteremia (Otter and French. (2011). Resistance to various Aminoglycosides is common in *S. aureus*, and is encoded by either plasmid or chromosomal genes (Patoli, *et al.,* 2017). Staphylococci usually exhibit resistance to aminoglycosides by inactivating the antibiotics through different types of Aminoglycoside-Modifying Enzymes (AMEs). For example, Staphylococci mediate the resistance to Tobramycin, Gentamycin and Kanamycin by Aminoglycoside Phosphotransferases (APHs) and Aminoglycoside Acetyl transferases (AACs) enzymes. The other aminoglycosides like Amikacin and Neomycin are inactivated by different category of enzyme termed as Aminoglycoside-Nucleotidyl Transferases (ANTs) (Foster, 2017) (Rahimi 2016). After the enzymatic modifications (acetylation, phosphorylation or adenylation), the Aminoglycoside antibiotics are unable to bind the ribosomal subunit, hence fail to inhibit the synthesis of proteins in bacteria (Schito, 2006).

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2. MATERIALS AND METHODS

Analytical grade media were used for this study. The Nutrient Broth, Mannitol Salt Agar, Muller Hinton agar were all purchased from Oxoid. The antibiotic discs were also purchased from Oxoid. The cotton swabs were prepared manually and sterilized as per the standard protocol.

Clinical Isolates

A total of 44 identified *S. aureus* were collected during September (2018) to February (2019) from Diagnostic and Research Laboratory, LUMHS at Hyderabad. All of these were specifically isolated from clinical specimens i.e. blood, pus and nose.

Non-clinical Isolates

The non-clinical *S. aureus*were isolated from the skin surfaces of healthy volunteers. The isolation and identification of the *S. aureus* was performed in the HEC funded Molecular Microbiology and Genetics Laboratory at the Institute of Microbiology, University of Sindh, Jamshoro. For this a total of 100 healthy volunteers were approached. The sterile cotton swabs soaked in sterile normal saline were rubbed on the skin surfaces of the volunteers. The swabs were immediately inoculated on the Mannitol Salt Agar. After 24 hours of incubation at 37°C, the suspected *S. aureus*colonies were further identified and confirmed through microscopic examination and biochemical tests such as catalase and coagulase production as per the standard protocol.

Determination of antibiotic Resistance

The determination of antibiotic resistance against aminoglycoside group of antibiotics was performed by using *Kirby-Bauer* Disc Diffusion method. The overnight liquid culture was diluted to OD600 = 0.5 to achieve the McFarland's standard prerequisite for disc diffusion method. The diluted culture was then inoculated and spread evenly on Muller Hinton Agar using sterile cotton swab. The antibiotic discs (Oxoid) were placed on the agar surface. A flat contact between the disc and agar surface was then achieved by applying a gentle pressure on the discs. 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 Laboratory Standard Institute (CLSI).

Statistical Analysis

The data was analyzed using IBM SPSS version 20. Aminoglycoside resistance and Clinical and non-clinical *S. aureus*isolates were the variables of the interest. The Odds Ratios (ORs) and Confidence intervals (CI 95%) were calculated manually and by a statistics calculator. *p*-values were calculated using Fisher's Exact test employing 2x2 contingency table.

3. RESULTS

One hundred and eighteen(118) *S. aureus*isolates from various clinical and non-clinical origin were processed for the determination of antibiotic resistance against a set of Aminoglycoside group of antibiotics. Among the clinical category of samples, 31.3% (n=37) were from male group of patients and 12.7% (n=15) were from female group of patients, whereas; among the non-clinical category of samples 20.4% (n=24) were from male group and 35.6% (n=42) were from female group **(Fig. 1).** The clinical isolates were recovered from various clinical specimens i.e. pus, blood and nose while non-clinical were recovered from the skin surfaces of healthy volunteers. The total percentages of clinical and non-clinical isolates were calculated to be 44% (n=52) and 56% (n=66), respectively **(Fig. 1).**

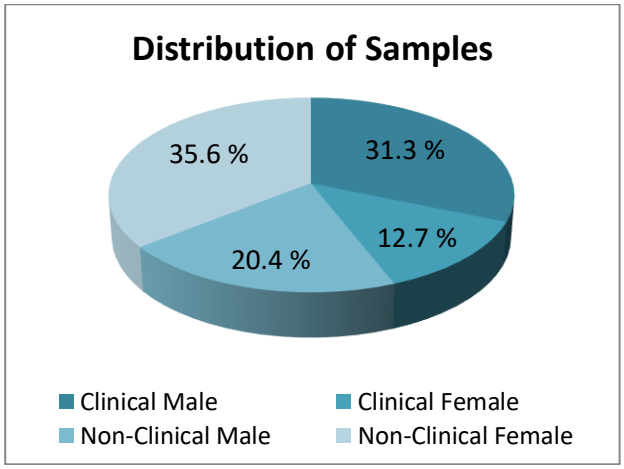

Fig. 1Pie chart displaying the percentages of sample distribution for this study.

All the 118 *S. aureus* were processed for the determination of antibiotic resistance and sensitivity using KirbyBauer disc diffusion method against Aminoglycoside antibiotics (i.e. Kanamycin, Amikacin, Gentamycin, Neomycin and Tobramycin). The percentages of resistance and sensitivity against each antibiotic was then calculated (Table 1). The highest percentage of resistance was seen against Neomycin (60.1) followed by Kanamycin (57.6%), Amikacin (48.3%), Tobramycin (34.7%) and Gentamycin (17.7%) **(Fig.2).** Categorical percentage of resistance and sensitivity was then calculated for both clinical and non-clinical *S. aureus* isolates (Table 1). Comparatively the frequency of Aminoglycoside resistance in *S. aureus*was higher in clinical isolates than the non-clinical isolates **(Fig. 3).** To probe the differences of resistance between clinical and non-clinical isolates we determined the percentage difference of resistance for each member of Aminoglycoside antibiotic used in this study **(Table 1).** About 75% and 90% difference were seen against Neomycin and Kanamycin, respectively,

while more than 100% differences were seen against Amikacin, Gentamycin and Tobramycin. The highest percentage of difference (153.9%) was seen for Gentamycin **(Fig.3).** For further evaluation of the differences statistical analyses were performed. We calculated Odd Ratio (OR) at 95% CI and applied Fisher's Exact test (employing 2x2 contingency table) to determine the *p*-values in each category. The values are given in **(Table 1).** Intriguingly in each case significant ($p < 0.05$) differences were observed, indicating the impact of source on the frequency of antibiotic resistance in *S.aureus.*

Table 1Aminoglycoside resistance/sensitivity profile of *S.aureus* isolated from both clinical and non-clinical sources. Absolute and relative values along with percentage of difference, OR, CI and *p*-values are expressed.

Antibiotic	Source	Profile	No.	%	% of difference	OR	CI [95%]	*p*-value
kanamycin	Clinical	Resistant	46	88.4	90.5505	15.33	5.68 - 41.38	0.0001
		Sensitive	6	11.6				
	Non-Clinical	Resistant	22	33.3				
		Sensitive	44	66.7				
Amikacin	Clinical	Resistant	43	82.6	118.304	17.75	7 - 44.97	0.0001
		Sensitive	9	17.4				
	Non-Clinical	Resistant	14	21.2				
		Sensitive	52	78.8				
Gentamycin	Clinical	Resistant	18	34.6	153.964	11.12	3.06 - 40.45	0.0001
		Sensitive	34	65.4				
	Non-Clinical	Resistant	3	4.5				
		Sensitive	63	95.5				
Neomycin	Clinical	Resistant	45	86.5	75.0397	9.89	3.88 - 25.24	0.0001
		Sensitive	7	13.5				
	Non-Clinical	Resistant	26	39.3				
		Sensitive	40	60.7				
Tobramycin	Clinical	Resistant	35	67.3	152.818	20.59	7.43 - 57.04	0.0001
		Sensitive	17	32.7				
	Non-Clinical	Resistant	6	9				
		Sensitive	60	91				

Key: OR = Odds Ratio, CI = Confidence Interval.

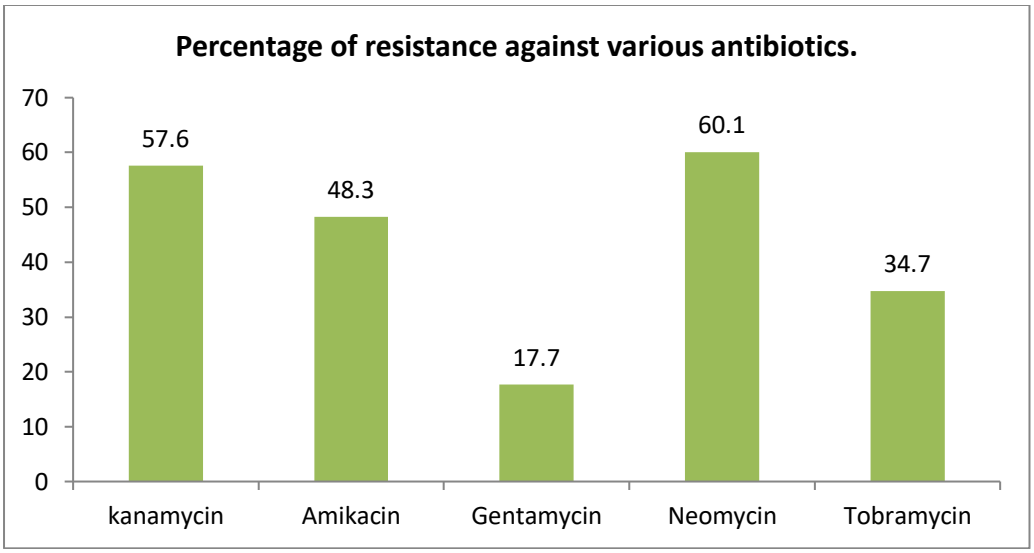

Fig. 2 Bar Diagram Displaying the Cumulative Percentages of Resistance in the *S. aureus*isolates against each Member of Aminoglycoside Group of Antibiotic used in this Study.

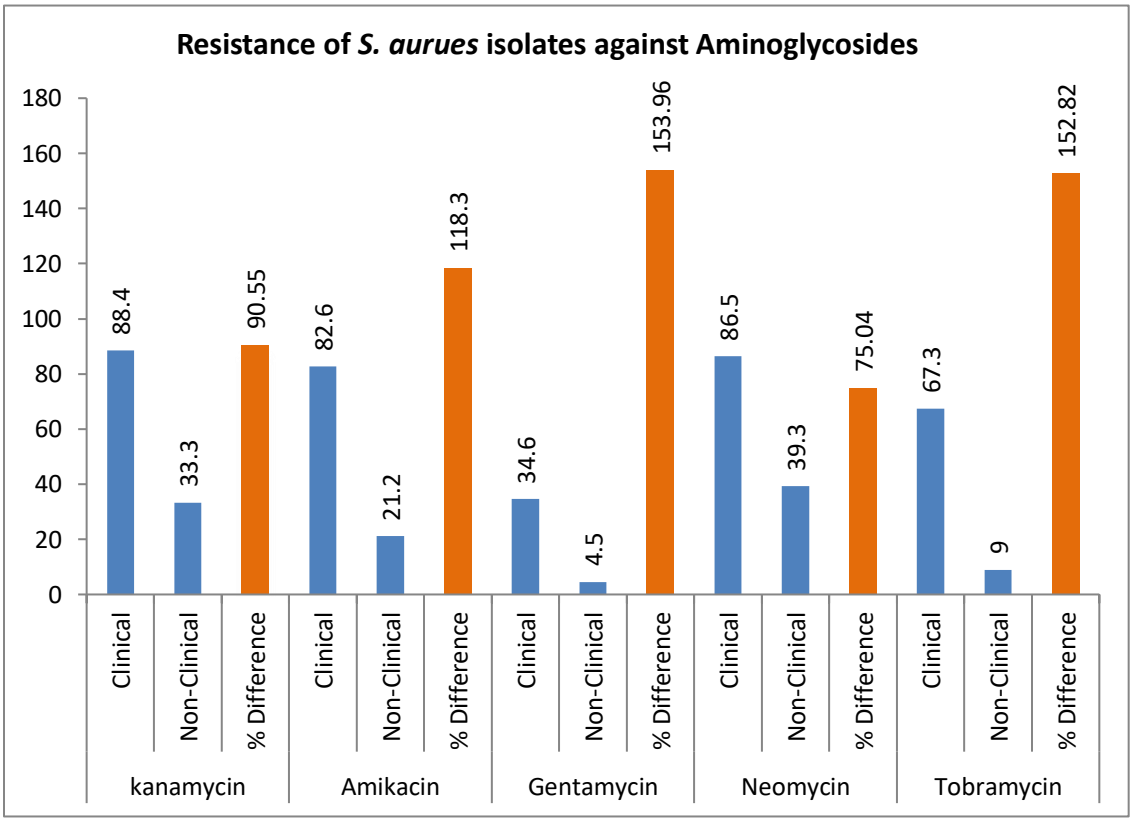

Fig. 3. Bar Diagram Displaying the Categorical Percentages of Resistance Against various Antibiotics in Clinical and non-clinical *S. aureus*isolates along with percentage of difference for each member of Aminoglycoside Group of Antibiotics.

4. DISCUSSION

In this current study we aimed to evaluate and compare the Aminoglycoside resistance in *S.aureus* isolated both from clinical non-clinical sources. The clinical sources included; pus, blood and nose, while non-clinical *S. aureus* were recovered from the skin surfaces of healthy volunteers. (Franz-Josef Schmitz *et al.* 1990), reported about 23% of the *S. aureus* to be resistant against Neomycin, whereas the Neomycin resistance in MRSA was reported to be 96% by Azar

Dokht Khosravi *et. al*. 2017.In this study we report about 60.1% of the *S. aureus* strains isolated either from clinical or non-clinical origin to be resistant to Neomycin. The *S. aureus* resistance to Kanamycin was determined to be 57.6% where as in studies conducted in Netherland and Malaysia reported 31% and 00% Kanamycin resistant *S. aureus* strains, respectively (Sakr *et al.,* 2018) (Mahdiyoun, *et al.,* 2016) .In the current study we however divided the *S. aureus* strains based on their origin. The clinical *S. aureus* strains were found to show more (i.e. 88.4%) resistance against Kanamycin than non-clinical *S. aureus* strains (i.e. 33.3%). Two independent studies conducted in Iran report about 99%(18)and 83.3% (Mahdiyoun, *et al.,* 2016) Kanamycin resistance in MRSA of clinical origin. In Pakistan, *S. aureus* resistance against

Amikacin has been reported through a few studies. In 2010, a study conducted in Karachi reported 10.4% of the *S. aureus* strains to be resistant against Amikacin (Shamila-Syuhada, *et al.,* 2016) whereas 17.5% and 41% of the *S. aureus* strains were reported Amikacin resistant in Peshawar and Mirpurkhas, respectively (20,21).In the current study about 48.3% of the *S.aureus* strains were determined to be Amikacin resistant. The prevalence of Amikacin resistance in clinical and non-clinical *S. aureus* was evaluated to be 82.6% and 21.2%, respectively. Almost similar kind of Amikacin resistance (i.e. 82% and 77.6%) has been reported in MRSA of clinical origin in two different studies conducted in Iran (Shamila-Syuhada, *et al.,* 2016). In the current study the lowest percentage of resistance was seen against Gentamycin (17.7%) followed by Tobramycin (34.7%). When probed for the comparison, more resistance against both antibiotics was witnessed in clinical *S. aureus* isolates than non-clinical isolates. More than 150% differences of resistance were calculated between clinical and non-clinical isolates for both the antibiotics (Gentamycin and Tobramycin).Study conducted in Karachi reported almost similar percentage (34.8%) of resistance against Tobramycin whereas 92% of resistance in clinical MRSA was reported from Lahore in 2016 (Eriksen *et al.,* 1995). In 2016, a study conducted in Peshawar reported 24.6% of the *S. aureus* strains to be resistant against gentamycin (Ullah, *et al.,* 2016) while comparatively higher Gentamycin resistance (87%) was reported in MRSA of clinical origin in the same year from Lahore (Woodford 2005). (Taj, *et al.,* 2010) In the current study we however determined the significance of differences for Kanamycin and Tobramycin resistance between clinical and non-clinical isolates. Odd Ratio (OR) at 95% CI were calculated and *p*-values were calculated using Fisher's Exact Test to determine the level of significance (Table 1). The values suggest statistically significant differences of Kanamycin and Tobramycin resistance between clinical and non-clinical isolates in the Hyderabad. The data for other aminoglycosides used in this study (i.e. Amikacin, Neomycin and Gentamycin) was also processed in a similar way. Interestingly significant differences of aminoglycoside resistance between clinical and non-clinical *S.aureus*isolates were determined for Hyderabad region.

5. CONCLUSION

Being the common and significant therapeutic agents for the treatment of Staphylococcal infections, the aminoglycosides effectively provide synergistic action in severe infections when given in combination with Beta-lactam antibiotics. The development of comparatively higher resistance against aminoglycosides mainly Amikacin, Gentamycin and Tobramycin in clinical *S. aureus* isolates is of great concern. Given the resistance determinants for these are encoded by transferable genetic elements, specific line of action may be proposed by health care authorities to curb further development of resistance.

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Conflict of interest

The author declares that there is no conflict of interest.

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