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MAR Index Study and MDR Character Analysis of a few Golden Staph Isolates

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Abstract

The study was carried out with an objective to isolate Staphylococcus aureus from a few clinical pus samples. The isolates were subjected to antibiotic sensitivity tests using 17 different antibiotics belonging to three different modes of action namely β-lactams, aminoglycosides and quinolones. Based on the resistance pattern of the isolates, the Multiple Antibiotic Resistance (MAR) Index was calculated. The results of the study revealed that 50% of the isolates exhibited Multi Drug Resistance (MDR) character and all the isolates had a very high MAR Index, suggesting the origin of the isolates to be of high antibiotic usage.

Keywords*: Antibiotic Sensitivity test, β-lactams, aminoglycosides, Quinolones, MAR Index, Multi Drug Resistance.*

Introduction:

Staphylococcus aureus aka Golden Staph are often found in the human nasal cavity (and on other mucous membranes) as well as on the skin. There are five species of Staphylococcus commonly associated with clinical infections: Staphylococcus aureus, S. epidermidis, S. haemolyticus, S. hominis and S. saprophyticus. *Staphylococcus aureus* is the most pathogenic species and is implicated in a variety of infections. Approximately 30% of adults and most children are healthy nasal carriers of S. aureus. In the majority of S. aureus infections the source of the organism is either the healthy nasal carrier or contact with an abscess from an infected individual. The portal of entry is usually the skin. S. aureus cause pus-filled inflammatory lesions, infections of accidental wounds and postoperative wounds.

Multi Drug Resistance is a common problem that hurdles chemotherapy. To overcome this problem, it is obligatory to identify the Multi Drug Resistance (MDR) character of an isolate. An attempt was done in this study to isolate a few *Staphylococcus aureus* from pus samples, to determine their MAR Index and to determine their MDR character by subjecting the isolates to antibiotic study

Materials and Methods:

Isolation of *Staphylococcus aureus*:

Pus samples were collected from patients suffering from purulent wound infections who were admitted to private hospitals. A total of ten different pus samples were collected by needle aspiration from different patients. The samples were quadrant streak inoculated on ten Mannitol Salt Agar (MSA) plates within 1 hour of sampling and after the period of incubation of 48 hours, the plates were observed for the presence of golden yellow colonies. Of the ten MSA plates, four plates contained golden yellow coloured colonies. The other plates that did not developed golden yellow colonies, which might had been due to infections by members of other genera or have had no

infection at all, were discarded. The colonies from the four plates were subcultured on Nutrient agar and were designated as Isolates I, II, III and IV respectively.

Identification and Biotyping of the Isolates:

All the four strains were Gram stained and subjected to Coagulase test. A volume of 100µls of the four isolates were mixed with0.5ml of human plasma in individual test tubes and incubated at 37^{o}C for 6 hours. Biotyping of the isolates were done based on their ability to hydrolyse Tween80 and their sensitivity to Gentamicin [1]. Tween 80 (1% v/v) was incorporated in nutrient agar and the test isolates were spread over an area of 1cm in diameter and incubated at 37^{o}C for 48 hours. Gentamicin sensitivity was tested by disc diffusion test [2], using commercially available Gentamicin (10) discs using Muller Hinton Agar.

Antibacterial study:

Antibacterial study was done using 17 different antibiotics namely Penicillin (10), Methicillin (10), Ampicillin (25), Vancomycin (30), Cefoxitin (5), Ampicillin/Sulbactam (10) - that belong to β lactam group, Amickacin (30), Gentamicin (10), Sreptomycin (10), Kanamycin (30) – that belong to aminoglycoside group, Ciprofloxacin (5), Ofloxacin (5), Lemofloxacin (5) – that belong to Quinolone group, Rifampicin (30), Rifabutin (30), Clindamycin (10) and Linezolid (30). The susceptibility patterns of the isolates to the different antibiotics were noted as Sensitive (S) or Resistant (R) as per CLSI standards. (Table-I)

Identification of MDR (Multi Drug Resistance):

Multi Drug Resistance is defined as resistance to all the tested antibiotics in atleast two of the following three classes: β-lactams, aminoglycosides and quinolones[3]. The Multi Drug Resistance (MDR) character of the isolates were identified by observing the resistance pattern of the isolates to the antibiotics.

MAR (Multiple Antibiotic Resistance) Index Study:

The MAR Index of an isolate is defined as a/b, where a represents the number of antibiotics to which the isolate was resistant and b represents the number of antibiotics to which the isolate was subjected [4]. The MAR indexes of the isolates were calculated and noted (Table – II).

Result and Discussion:

All the four isolates were Gram Positive Coccus, Coagulase positive and were positive for Tween 80 hydrolysis and showed sensitivity to Gentamycin. (Table I). The ability to clot plasma by Staphylococcus is mediated by the Staphylocoagulase enzyme and it is one of the most widely used and generally accepted criterion for the identification of *Staphylococcus aureus*[5].

The antibiotic susceptibility study revealed that both Isolate – I and Isolate – II were resistant to all the β-lactam group of antibiotics tested, aminoglycoside group of antibiotics and the quinolone group antibiotics, fulfilling the criteria to be designated as Multi Drug Resistant. Isolate – III was susceptible to Ofloxacin and Isolate – IV was susceptible to Amikacin, which make them ineligible to be categorised as Multi Drug Resistant.

The MAR Index analysis (Table II) reveals that all the four isolates had a very high MAR index value (>0.2). Bacteria having MAR Index > 0.2 originate from an environment where several antibiotics are used.[6] This suggested that though isolate – III and Isolate – IV were not Multi Drug Resistant, all the isolates would have spread from a niche of high antibiotic use.

Conclusion:

Multiple Drug Resistance has become a common feature of many microorganisms especially the human pathogens. Of the different isolates tested, 50% of the isolates exhibited the MDR character, suggesting the existence of greater

frequency of MDR strains in the community. This demands the introduction of antibiotics with novel modes of action

Table I. Susceptibility of the Isolates to antibiotics

S. No	Antibiotics (µgm/disc)	Isolate - I	Isolate – II	Isolate – III	Isolate - IV
1.	Cn 30	R	R	R	R
2.	R 30	R	R	R	R
3.	Ak 30	R	R	S	S
4.	AS 10	R	R	R	R
5.	Cd 10	S	S	S	S
6.	G 10	S	S	S	S
7.	Va 30	R	R	R	R
8.	Cf 5	R	R	R	R
9.	A 25	R	R	R	R
10.	S 10	R	R	R	R
11.	M 10	R	R	R	R
12.	Of 5	R	R	S	R
13.	Lz 30	S	S	S	S
14.	P 10	R	R	R	R
15.	K 30	R	R	R	R
16.	Lf 5	R	R	S	R
17.	Rb 30	R	S	S	S

LEGENDS: Cn-Cefoxitin , R -Rifampicin , Ak-Amikacin
AS - Ampicillin / Sulbactam, Cd - Clindamycin
G - Gentamicin, Va - Vancomycin, Cf-Ciprofloxacin
A - Ampicillin , S - Streptomycin
M - Methicillin, Of - Ofloxacin
Lz - Linezolid, P - Penicillin
K - Kanamycin, Lf - Lomefloxacin , Rb-Rifabutin

Table II: MAR index analysis of the Isolates:

No. of antibiotics to which the isolates were subjected = 17 (b)

Isolate	No. of antibiotics to which the isolate was resistant (a)	MAR Index (a/b)
I	13	0.76
II	12	0.70
III	11	0.64
IV	13	0.76

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