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PROFILE OF ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN CLINICAL ISOLATES STAPHYLOCOCCI AND TO OBSERVE THE SENSITIVITY PATTERN OF ISOLATED ORGANISMS.

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Conflicts of Interest: Nil
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ABSTRACT

Background: Staphylococcus (from the Greek staphyle grape and kokkos granual) is a Gram-positive bacteria genus that produces clusters that look like grapes. There are at least 40 species of Staphylococcus, nine of which have two subspecies. The majority is non-toxic and live on the skin and mucous membranes of humans and other animals.
Aims & objectives: To study the Profile of antimicrobial susceptibility pattern in clinical isolates staphylococci and to observe the sensitivity pattern of isolated organisms.
Material & methods: A total of 85 clinical Staphylococci isolates were obtained from various samples. The Coagulase Test was carried out on a slide. Kirby Bauer Method was used to assess the antimicrobial resistance of the collected strains.
Results: The isolates' antimicrobial susceptibility pattern revealed that they were generally multi-drug immune. Coagulase-positive Staphylococci were more resistant than those that were Coagulase-negative. Despite this, the sensitivity to Vancomycin and Linezolid was excellent.
Conclusion: The organism must be isolated from clinical specimens and its antimicrobial susceptibility pattern studied. It is therefore essential to assess the various factors and methods by which it acquires antimicrobial resistance.

Keywords: antimicrobial susceptibility, staphylococci, sensitivity pattern

INTRODUCTION

Staphylococci have a spherical morphology with a diameter of one micro metre. They're clustered in clusters that look like grapes. Staphylococci are nonmotile and nonsporing bacteria that stain with aniline dyes, but they can transition to L-forms when exposed to penicillin and certain chemicals[1]. Von Reckling Hausen discovered staphylococci in human pyogenic lesions in 1871, and they were divided into two classes. Staphylococcus aureus, also known as Staphylococci pyogenes, contains coagulase-positive strains, while Staphylococcus epidermis, also known as Staphylococcus albus, contains coagulase-negative strains[2]. Staphylococcus saprophyticus, Staphylococcus capitus, and Staphylococcus hominis are the other coagulase negative bacteria. Staphylococcus aureus is a well-known nosocomial pathogen, and the widespread and indiscriminate use of antibiotics has resulted in the emergence of increasingly drug-resistant strains[3]. Antibiotic overuse has resulted in the rise of bacterial resistance. Antibiotic resistance, a natural occurrence, has turned into a nightmare for doctors. Staphylococci can grow in ordinary media at temperatures ranging from 10 to 42 degrees Celsius[4]. The ideal temperature for Staphylococci growth is 37°C, with a PH of 7.4–7.6. When colonies are incubated for 24 hours on nutrient agar, they appear on the media as large circular, convex, smooth, glossy, opaque, emulsifiable colonies[5]. Blood agar colonies are similar to nutrient agar colonies. The majority of staphylococci strains are haemolytic, and when incubated under 20-25 percent CO2, they

develop beta type haemolysis on MacConkey's medium[6]. The colonies are tiny, pink, and lactose fermenting. Staphylococcus ferments sugars but does not produce gas; however, they are catalase positive, indole negative, and positive on the Methylene red test and the Voges prosekaur test[7]. When grown on media containing dense opacity egg yolk, the majority of Staphylococci strains are lipolytic. Phosphatase activity is demonstrated by culturing on nutrient agar containing phenolphthalein diphosphate, which produces bright pink colonies when exposed to ammonia due to the presence of free phenolphthalein[7].

Aims & objectives: To study the Profile of antimicrobial susceptibility pattern in clinical isolates staphylococci and to observe the sensitivity pattern of isolated organisms.

Material & Methods:

During the duration of 1 February 2015 to 28 February 2015, a total of 85 clinical isolates of Staphylococci were collected from various samples at Vishakha Clinical Microbiology Laboratory (VCML), Nagpur for this research. For this analysis, the ATCC culture S. aureus 25923 was used as the norm. Normal identification procedures such as colony morphology, Gram stain reaction, catalase test, and urease test were used to identify the strains. Before conducting Antimicrobial Susceptibility Testing, all of the strains were screened for Coagulase activity. The slide coagulase test was used to validate the results of the tube coagulase test. The Kirby Bauer Method (disc diffusion method) was used to measure the antimicrobial susceptibility of the collected strains using discs of Amikacin, Amoxyclav, Ampicillin, Cefuroxime, Cephalexin, Ciprofloxacin, Clindamycin, Erythromycin, Gentamycin, Pristinomycin, Rifampacin, and Vancomycin. All of the strains were tested for Methicillin resistance using the standard disc diffuson method described above. According to NCCLS guidelines, all strains were tested for Inducible Clindamycin resistance using the standard D-Zone Test. At the conclusion of the analysis, the findings were interpreted.

Results:

A total of 85 Staphylococcal isolates obtained from various clinical specimens were included in the study. Of the total isolates, 80 (94.12%) were Coagulase Positive Staphylococci while only 5 (5.88%) were coagulase negative. Their distribution from different specimens is shown in Table 1.

Table 1: Shows frequency of isolates of Staphylococci from different specimens.

SAMPLE	No. of Samples	Coag +ve Staph.	%	Coag Neg Staph	%
BLOOD	12	11	91.67	1	8.33
PUS	18	18	100.00	1	0.00
FLUID	9	8	88.89	0	11.11
SPUTUM	18	17	94.44	1	5.56
STOOL	2	2	100.00	0	0.00
URINE	26	24	92.31	2	7.69
TOTAL	85	80	94.12	5	5.88

Antimicrobial susceptibility pattern of the isolates showed them to be generally multi-drug resistant. The coagulase positive Staphylococci were more resistant than Coagulase negative Staphylococci. Nevertheless the sensitivity was excellent to Vancomycin or Linezolid. The Antimicrobial sensitivity profile is shown in Table 2.

Table 2: Shows the Antimicrobial Sensitivity of Staphylococci to various antibiotics.

ANTIBIOTICS	COAG. +VE STAPH (n=80)	%	COAG. NEG STAPH (n=5)	%
VANCOMYCIN	80	100%	5	100%
LINEZOLID	80	100%	5	100%
PIPERACILLIN	68	85%	5	100%
TETRACYCLLIN	68	85%	5	100%
GENTAMYCIN	66	82.5%	5	100%
AMIKACIN	66	82.5%	4	80%
AMOXYCLAV	59	73.75%	5	100%
CEFTRIAXONE	53	66.25%	5	100%
CEFUROXIME	48	60%	4	80%
ERYTHROMYCIN	45	56.25%	5	100%
CEPHALEXIN	45	56.25%	5	100%
CEFTAZIDIME	43	53.7%	5	100%
CLINDAMYCIN	35	43.75%	5	100%
AMPICILLIN	7	8.75%	2	40%

Discussion:

Penicillin is the medication of choice for S. aureus infection, but penicillin resistance is highly common in most countries, so first-line therapy is usually a penicillinase-resistant penicillin (for example, oxacillin or flucloxacillin). While gentamycin-based combination therapy can be used to treat severe infections including endocarditis, its use is controversial due to the high risk of kidney damage[8]. The length of treatment is determined by the location and severity of the infection. Antibiotic resistance in S. aureus was first noticed about 60 years ago, shortly after the introduction of penicillin. In the years since, Staphylococci's remarkable ability to develop antibiotic resistance has resulted in the development of methicillin-resistant S. aureus (MRSA) and S. epidermidis strains[9]. Methicillin resistance was first detected in nosocomial isolates of S. aureus in 1961, one year after the drug was introduced. Resistance to methicillin is caused by the formation of PBP2a (or PBP2), an altered penicillin binding protein with a lower affinity for most beta-lactam antibiotics[10].

The mecA gene is the only resistance factor in type I SCCmec, while type II and III elements contain, in addition to mecA, several determinants for resistance to non-beta-lactam antibiotics[11]. As a result, multidrug resistance in nosocomial MRSA isolates is caused by type II and III SCCmec components. Type IV SCCmec elements, including type I elements, have no resistance genes other than mecA and are smaller than type II and III elements[12]. This may be an evolutionary advantage, allowing these mobile genetic elements to spread more easily through bacterial populations. In comparison to the other elements, Type V SCCmec elements are small and have a different collection of recombinase genes[13]. The two recombinase genes ccrA and ccrB are found in type I to IV SSCmec elements, while type V elements have a single copy of a gene, ccrC that is homologous to a cassette chromosome recombinase gene[14]. These elements also have two open reading frames, hsdS and hsdM, which encode a restriction-modification system. The genes' phylogenetic analysis revealed a distant association with their homologues in other S. aureus genomes, implying a foreign origin. S. aureus has developed resistance to a wide range of antibiotics[15]. Due to a penicillinase (a form of -lactamase), only 2% of all S. aureus isolates in the UK are susceptible to penicillin, with a similar picture in the rest of the world[16]. To

treat penicillin-resistant S. aureus, the -lactamase-resistant penicillins (methicillin, oxacillin, cloxacillin, and flucloxacillin) were developed and are still used as first-line treatment. Methicillin was the first antibiotic in this class to be used (in 1959), but the first case of methicillin-resistant S. aureus (MRSA) was identified in England just two years later[17].

Despite this, MRSA was relatively rare in hospital settings until the 1990s, when the prevalence of MRSA in hospitals where it is now prevalent skyrocketed. Since Staphylococci is one of the more common species associated with various infections and has a proclivity for developing drug resistance, we decided to investigate the profile of drug resistance among Staphylococci isolated from various specimens[18]. Coagulase positivity was found in 80 (94.12 percent) of the 85 Staphylococci isolates studied, while Coagulase negativity was found in 5 (5.88 percent). The isolation of CONS from blood and urine in this study clearly has a pathogenic role, while the other isolates may also have the potential to cause disease[19]. While not all CONS isolates are pathogenic, some must play a role in the etiopathogenesis of infections, given the CONS's well-documented pathogenic capacity. They're also notorious for being multidrug resistant. Multidrug resistance was found in the present study's antimicrobial susceptibility profile, particularly in Coagulase positive Staphylococci[20]. The frequency of resistance varies depending on where you are. Coagulase positive and Coagulase negative multidrug-resistant Staphylococci have been identified from various locations. Vancomycin and Linezolid sensitivity was standardised across all strains in the sample, but tolerance to other antimicrobial agents was variable[21]. Many of the isolates were immune to the most commonly and empirically used antibiotics, including ampicillin, clindamycin, erythromycin, cephalexin, and ceftazidime. The synthesis of the Beta-Lactamase enzyme is one of the most common mechanisms of drug resistance in Staphylococci. Beta-Lactamase activity correlates well with the low ampicillin sensitivity found in the current study. Some scientists have made similar observations.

Conclusion:

Staphylococci are found in almost any type of specimen, but with varying degrees of frequency. Coagulase positive Staphylococci make up 94.12% of the total isolates, while Coagulase negative Staphylococci make up 5.8%. The isolation of Staphylococcus aureus is excellent in a correctly collected sample, and S. epidermidis is found in only a few specimens. Multidrug resistant strains are used in both Coagulase positive and Coagulase negative strains. There isn't much of a difference between these strains' susceptibility profiles. Ampicillin, Clindamycin, Ceftazidime, and Cephalexin resistance is higher, while Vancomycin, Linezolid, Piperacillin, Tetracycline, Amikacin, and Gentamycin sensitivity is high.

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