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ISOLATION, IDENTIFICATION AND ANTIBIOGRAM OF *Staphylocoocus* SPECIES

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Abstract
The purpose of the study is to isolate and identify the staphylococcal swabs collected from hospitalized patients of Kovai Medical College and Hospital. The samples were cultured on nutrient broth and mannitol salt agar plates. These staphylocoocal swabs were tested on different antibiotics by using disk diffusion (Kirby Bauer) method. Antibiotic impenem shows higher activity in *S.haemolyticus* and *S.hominis*. *Staphylococcus aureus* are resistant/intermediate to amoxicillin,cefoxitin,linezolid,vancomycin and ticarcillin. These two species of *S.hominis* and *S.haemolyticus* are resistant to cefpodoxime. Thus these antibiotics can be preferred for Staphylococcal infections.
Keywords:

I. INTRODUCTION

Staphylococci are gram(+) cocci which appear to be arranged in clusters. These organisms are salt tolerant and catalase positive. *Staphylococcus aureus* lives as a part of the normal skin flora in the nose or on the skin in 20-30% of healthy people (staph carriers). *Staphylococcus aureus* causes boils, carbuncles, impetigo, abcesses, postsurgical wounds, pneumonia and osteomyelitis. There are several toxin associated pathologies associated with this organism including: toxic epidermal necrosis (scalded skin syndrome), toxic shock syndrome, food poisoning.

Many of these toxins are Super-Antigens which can cause the host's T-cells to over produce interleukin-2(IL-2) and other cytokines. This over production of immune cytokines is responsible for some of the profound pathology associated with these staphylococcal toxins. This organism also has Protein A, a substance which binds to the Fc part of the antibody molecule. Many strains of *staphylococcus aureus* are resistant to antibiotics. Methicillin resistant staphylococcus aureus (MRSA) is particularly threatening. *Staphylococcus aureus* is mannitol(+), coagulase(+), often beta-hemolytic and sensitive to novobiocin.

Staphylococcus haemolyticus is a member of the coagulase-negative staphylococci. It is a part of the skin flora of humans, and its largest populations are usually found at the axillae, perineum, and inguinal areas. It also colonizes primates and domestic animals. It is a well-known opportunistic pathogen and is the second most frequently isolated CoNS. However, recent studies indicate that coagulase-negative staphylococci have emerged as a major cause of opportunistic infection. *Staphylococcus haemolyticus* itself is also a remarkable opportunistic bacterial pathogen that is well-known for its highly antibiotic-resistant phenotype.

The bacteria can cause meningitis, skin or soft tissue infections, prosthetic join infections, or bacteremia. The ability of the bacteria to simultaneously resist against multiple types of antibiotic has been observed and studied for a long time. Common antibiotics that are subject to resistance in Staphylococcus haemolyticus include methicillin, gentamycin, erythromycin and uniquely among staphylococci, glycopeptides antibiotics.

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Staphylococcus hominis is a coagulase-negative member of the bacterial genus staphylococcus, consisting of gram-positive, spherical cells in cluster. It occurs very commensals on human and animal skin and is known for producing thioalcohol compounds that contribute to body odour. Like many other coagulase-negative staphylococci, *staphylococcus hominis* may occasionally cause infection in patients whose immune systems are compromised, for example by chemotherapy or predisposing illness. It is one of only two species of staphylococcus to display sensitivity to desferrioxamine, the other being *S.epidermidis*. *S.hominis* tends to colonize in areas with numerous apocrine glands, such as axillae and the pubic region. Most, if not all, strains are susceptible to penicillin, erythromycin, and novobiocin, but a divergent strain, *S.hominis* subsp. novobiosepticus(SHN), was found recently.

In 1946 almost all strains of Staphylococcus were penicillin sensitive. Today most hospital strains are resistant to penicillin G, and some are now also resistant to methicillin and/or gentamycin and only treated with vancomycin. During the late 1950s and 1960s, *Staphylococcus aureus* caused considerable morbidity and mortality as a nosocomial, or hospital-acquired, pathogen. Penicillinase-resistant, semisynthetic penicillins have been successful antimicrobial agents in the treatment of staphylococcal infections. Unfortunately methicillin-resistant *S. aureus* (MRSA) strains have emerged as a major nosocomial problem. One way in which staphylococci become resistant is through acquisition of a chromosomal gene (mecA) that encodes an alternate target protein which is not inactivated by methicillin and its relatives, all belonging to the metacillin family of β-lactam drugs. The majority of the strains are also resistant to several of the most commonly used antimicrobial agents, including macrolides, amino glycosides, and other beta-lactam antibiotics, including the latest generation of cephalosporins.

2. MATERIALS AND METHODS

2.1 Isolation and Identification

Staphylococcus species swabs were collected from hospitalized patients obtained from Kovai Medical College and Hospital. Then the collected swabs were cultured onto nutrient broth and then swabbed onto nutrient agar and mannitol salt agar media.

Phenotypic characteristics of the obtained isolates were performed according to the Bergey's manual of systemic bacteriology.

2.2 Antibiogram of isolates

Antibiotic sensitivity tests (ASTs) basically measures the ability of an antibiotic or other antimicrobial agent to inhibit the invitro microbial growth. Mueller Hinton Agar is considered as best for the routine susceptibility testing since it is has batch-to-batch reproducibility, low concentration of inhibitors of sulphonamide, trimethoprim and tetracyclines and produce satisfactory results for most of the non-fastidious pathogens. Fastidious organisms which require specific growth supplements need different media to grow for studying the susceptibility patterns.

The Kirby Bauer test is a qualitative assay whereby disks of filter paper are impregnated with a single concentration of different antibiotics or any chemicals that will diffuse from the disk into the agar. The selected antibiotic disks are placed on the surface of an agar plate which has already been inoculated with test bacteria. During the incubation period, the antibiotics/chemicals diffuse outward from the disks into the agar. This will create a concentration gradient in the agar which depends on the solubility of the chemical and its molecular size. The absence of growth of the organism around the antibiotic disks indicates that, the respected organism is susceptible to that antibiotic and the presence of growth around the antibiotic disk indicates the organism is resistant to that particular antibiotic. This area of no growth around the disk is known as a zone of inhibition, which is uniformly circular with a confluent lawn of growth in the media.

3. RESULT & DISCUSSION

3.1 Cultural characteristic:

3.1.1 Staphylococcus aureus

Staphylococcus aureus grow readily on ordinary media within a temperature range of 10 to 42°C, the optimum being 37°C, and a pH of 7.4 to 7.6. They are aerobes and facultative anaerobes. On nutrient agar medium, after incubation for 24 hours, the colonies are large (2-4mm diameter), circular, convex, smooth, opaque and easily emulsifiable. Pigment production occurs optimally at 22°C and only in aerobic cultures. Pigment production is enhanced when 1% glycerol monoacetate or milk is incorporated in the medium. Individual colonies on agar are round, convex, and 1-4 mm in diameter with a sharp border. The golden appearance of colonies of some strains is the etymological root of the bacteria's name; aureus meaning "golden" in Latin. Methicillin-resistant strains of *Staphylococcus aureus* (i.e. MRSA) often have only weak or no beta-hemolysis and special cultivation media with oxacillin, mannitol and NaCl for their isolation are used. MRSA is able to grow on this media and produce colonies of certain color, depending on used pH indicator (pink).

3.1.2 Staphylococcus haemolyticus

Staphylococcus haemolyticus is non-motile, non-sporulating, facultatively anaerobic. Cells are typically occurs coccus shaped and range from 0.8 to 1.3µm in diameter. Optimal growth occurs between 30-40°C in the presence of oxygen and 10% NaCl. However, some strains can grow at temperatures that range between 18-45°C. Growth at 15°C or 15% NaCl is poor or absent. On blood agar, it shows beta-hemolysis.

3.1.3 Staphylococcus hominis

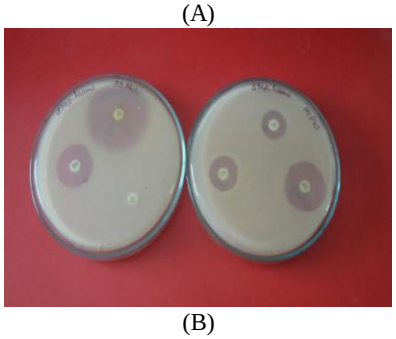
When grown in agar cultures, colonies are usually circular, 4.0 to 4.5 mm in diameter. Agar colonies usually have wide edges and an elevated center. They are commonly smooth with dull surfaces, and are yellow-orange pigmented in the center of the opaque colonies. They grow both in aerobic and anaerobic conditions, but tend to grow significantly less in the latter. Optimal NaCl concentrations of the agar culture for the growth of *S. hominis* seem to be around 7.5%, and a salt concentration of 15% yielded poor growth to no growth at all. The optimal growth temperature range was around 28 to 40 °C, but good growth is still observed at 45 °C, while no growth is observed at 15 °C. *S. hominis* can be differentiated from staphylococci by its colony morphology and pigmentation patterns, predominant tetrad cell arrangement, poor growth in thioglycolate, low tolerance of NaCl, and carbohydrate reaction pattern. Each species is also significantly different in cell wall composition, lactic acid configuration, temperature extremes of growth, coagulase activity, hemolysis acetylmethylcarbinol production, nitrate reduction, and phosphatase, DNase, and bacteriolytic activities. Similarities in these properties between S. hominis and several other species suggest a close relationship between *S. hominis* and *S. epidermidis*, *S. haemolyticus*, and *S. warneri*.

3.2 Antibiogram of isolates

From the observed results all the three species of *staphylococcus* are resistant to methicillin (Fig 1). Compared to other two species, *S.haemolyticus* show high activity of antibiotic sensitivity. Antibiotic impenem shows higher activity in *S.haemolyticus* and *S.hominis*. *Staphylococcus aureus* are resistant/intermediate to amoxicillin, cefoxitin, linezolid, vancomycin and ticarcillin. These two species of *S.hominis* and *S.haemolyticus* are resistant to cefpodoxime. Thus these antibiotics can be preferred for Staphylococcal infections.

Staphylococcus aureus is a medically important organism associated with a variety of diseases. MRSA represents a major challenge to hospitals in all countries due to the emergence and spread of isolates with decreased susceptibilities to several antibiotic classes, in addition to methicillin and the other members of the β-lactum family. The result of the study shows that the significant *Staphylococcus aureus* are resistant to the antibiotics (amoxicillin, cefoxitin, linezolid, vancomycin, ticarcillin), *S.haemolyticus* and *S.hominis* are resistant to cefpodoxime antibiotic.

(A)



(B)

(C)

Figure 1. (A,B & C) Antibiogram of isolates

TESTS	Sample 1	Sample 2	Sample 3
Indole	Negative	Negative	Negative
Methyl red	Positive	-	Negative
Voges Proskauer	Positive	Positive	Negative
Citrate	Positive	-	Negative
Coagulase	Positive	Negative	Negative
Urease	Positive	Negative	Positive
Oxidase	Negative	Negative	Negative
Catalase	Positive	Positive	Positive

Table 1. Biochemical characteristic (Microscopy: On gram staining, purple cocci in clusters were observed).

Antibiotics used	Disc content	Diameter of zone of inhibition in mm					
		S.aureus		*S.haemolyticus*		*S.hominis*	
Amikacin	30 mcg	26mm	S	24mm	S	17mm	S
Amoxycillin	30 mcg	-	I	22mm	S	17mm	S
Chloramphenicol	30 mcg	24mm	S	28mm	S	22mm	S
Cefotaxime	30 mcg	28mm	S	22mm	I	19mm	I
Cefpodoxime	10 mcg	7mm	R	-	R	-	R
Cefoxitin	30 mcg	-	R	22mm	S	22mm	S
Ceftriaxone	30 mcg	27mm	S	18mm	I	18mm	I
Amoxyclav	10 mcg	11mm	I	29mm	S	17mm	I
Doxycycline Hcl	30 mcg	16mm	S	25mm	S	24mm	S
Furazolidone	50 mcg	18mm	S	18mm	S	19mm	S
Imipenem	10 mcg	26mm	S	40mm	S	40mm	S
Linezolid	30 mcg	-	R	32mm	S	30mm	S
Nalidixic acid	30 mcg	18mm	I	15mm	I	18mm	I
Norfloxacin	10 mcg	34mm	S	25mm	S	25mm	S
Nitrofurantoin	300 mcg	15mm	I	22mm	I	17mm	S
Neomycin	30 mcg	21mm	S	16mm	S	16mm	I
Ofloxacin	5 mcg	29mm	S	29mm	S	28mm	S
Oxytetracycline	30 mcg	15mm	I	13mm	I	12mm	I
Polymycin-B	300 units	12mm	S	12mm	S	9mm	R
Rifampicin	5 mcg	10mm	R	17mm	I	14mm	R
Methicillin	5 mcg	-	R	-	R	-	R
Vancomycin	30 mcg	-	I	14mm	I	20mm	I
Ticarcillin	75 mcg	-	I	27mm	S	19mm	S
Novobiocin	30 mcg	11mm	R	23mm	S	17mm	R

S- Sensitive, I- Intermediate, R- Resistant

Table 2. Antibiotics

5. REFERANCE

[1] Abreu, A. C., Serra, S., Borges, A., Saavedra, M. J., Salgado, A. and Simões, M., 2013b, Synergism between selected alkaloids and antibiotics against Staphylococcus aureus, ChemMedCom (Submitted).

[2] Adwan, G. and Mhanna, M., 2008, Synergistic effects of plant extracts and antibiotics on Staphylococcus aureus strains isolated from clinical specimens, Middle-East Journal of Scientific Research, Vol. 3, no. 3, p: 134-139.

[3] Anon. Methicillin-resistant Staphylococcus aureus. Commun Dis Rep Weekly 1998;8:372.

[4] Childs C, Edwards-Jones V, Heathcote DM, Dawson M, Davenport P.Patterns of Staphylococcus aureus colonisation, toxin production,immunity and illness in burned children. Burns 1994;20:514–21.

[5] Donaldson J, Warner S, Cates R, Young D. Assessment of Antimicrobial Activity of Fourteen Essential Oils When Using Dilution and Diffusion Methods. Pharmaceutical Biology 2005;43(8):687-695.

[6] Hammer K, Carson C, Riley T. Antimicrobial activity of essential oils and other plant extracts. J Apply Microbiol 1999;86(6):985-990.

[7] Kloos WE, Bannerman TL. Update on clinical significance of coagulase-negative staphylococci. Clin Microbiol Rev 1994;7:117-40.

[8] Lawless J. The illustrated encyclopedia of essential oils: The complete guide to the use of oils in aromatherapy and herbalism. New York: Barnes & Noble; 1995.

[9] National Center for Infectious Diseases [Internet]. Atlanta: Centers for Disease Control and Prevention (US); [updated 2011 Apr 15; cited 2012 Jun 21]. Methicillin resistant

[10] Prax M, Lee CY, Bertram R. An update on the molecular genetics toolbox for staphylococci. Microbiology 2013;159:421-35.

[11] R. Oprean; M. Tamas; R. Sandulescu; L. Roman "Essential oil analysis. I. Evaluation of essential oil composition using both GC and MS " fingerprints. J. Pharm. Biomed.

[12] Staphylococcus aureus (MRSA) infections; [about 2 screens]. Available from: http://www.cdc.gov/mrsa/

[13] Ugur A, Varol O, Ceylan O. Antibacterial Activity of Sideritis curvidens and Sideritis lanata from Turkey. Pharmaceutical Biology 2005;43(1):47-52.

[14] Voss A, Milatovic D, Wallrauch-Schwarz C, Rosdahl VT, Braveny I. Methicillin-Resistant Staphylococcus aureus in Europe. Eur J Clin-Microbiol Infect Dis 1994;13:50–5.

[15] Walker J, Borrow R, Edwards-Jones V, Oppenheim BA, Fox AJ. Epidemiological characterisation of methicillin-resistant Staphylococcus aureus isolated in the North West of England by protein A (spa) and coagulase (coa) gene polymorphisms. Epidemiol Infect 1998;121: 507–514.