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RESEARCH ARTICLE

Isolation and Diagnosis of *Staphylococcus spp.* using VITEK-2 System from Nasal and Ear of Healthy Carriers

Zainab Mohammed Jassim[1], Aaya Hamid Al-Hakeem[2], Sddiq Ghani Al-Muhanna[2] *

[1.] *Department of Genetic Engineering, Collage of Biotechnology, AL-Qassim Green university/Iraq.*

[2.] *Department of Medical Laboratory Techniques, Faculty of Medical and Health Techniques, University of Al Kafeel, Najaf, Iraq.*

***Corresponding Author: Sddiq Ghani Al-Muhanna**

Abstract

One hundred of nasal and ear swabs were collected from healthy students of Babylon University who not suffering from any upper respiratory tract infection and have not received any antibiotics from the period of two. All they were cultured on suitable media .The positivity of culturing on Mannitol salt agar (selective media for Staphylococci) was 20% distributed as 14% for nasal swabs and 6% for ear swabs. When Biochemical and Serological tests were used for identifying Staphylococcus species, the results showed that 45% of these isolates were Coagulase positive while 55% were Coagulase negative .*Staphylococcus* species were identified by using VITEK-2: *Staphylococcus aureus* 45%, *S. epidermidis* 20%, *S.capitis sub.capitis*20% and *S.cohni. Sub. cohni* 10% .Antibiotic sensitivity test for *Staphylococcus* isolates showed sensitivity for ciprofloxacin to all isolates while appeared resistant to Nalidixic acid, Kanamycin and Gentamycine, most of *Staphylococcus aureus* isolates were resisted to Methicillin antibiotic.

Keywords: *Coagulase-negative staphylococci, Colonization, Nasal carriage.*

Introduction

Healthy people are naturally contaminated. Feces contain about 10^{13} bacteria per gram, and the number of microorganisms on skin varies between 100 and 10000 per cm^2. Many species of microorganisms live on mucous membranes where they form a normal flora [1].None of these tissues, however, is infected. Microorganisms that penetrate the skin or the mucous membrane barrier reach subcutaneous tissue, muscles, bones, and body cavities (e.g. peritoneal cavity, pleural cavity, bladder), which are normally sterile (i.e. contain no detectable organisms).

If a general or local reaction to this contamination develops, with clinical symptoms, there is an infection [2].Healthy individuals have a normal general resistance to infection. Patients with underlying disease, newborn babies, and the elderly have less resistance and will probably develop an infection after contamination. Health-care workers are thus less likely to become infected than patient. Local resistance of the tissue to infection also plays an important role: the skin and the mucous membranes of nose act as barriers in contact with the environment. Infection may follow when these barriers are breached. Local resistance may also be overcome by the long-term presence of an irritant, such as a cannula or catheter; the likely hood of infection increases daily in a patient with an indwelling catheter [3].

Staphylococcus aureus is among the leading Gram positive bacteria (GPB) causing diseases in humans and animals. It is also the leading bacteria in the normal flora of humans especially, in the skin and nasal vestibule. Besides, this bacterial colonization is very common in certain areas in the body namely-axilla, umbilicus, perineal region and mammary folds [4].The carrier rate of *Staphylococcus aureus* in the nasal canal among the healthy people range from 20-

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30%.From the healthy carriers among the health care personnel, there are more chances of spreading from their hands, nose or throat by way of touching, sneezing, talking, coughing etc [2].Nasal carriage of *Staphylococcus aureus* plays a key role in the epidemiology and pathogenesis of staphylococcal infection. Eradication of *S. aureus* from the nose has proved to be effective in reducing the incidence of staphylococcal infection.

This indicates that the anterior nasal region is a primary ecological reservoir of *S. aureus,* although the throat and the perineum are also important reservoirs. However, nasal recolonization may occur within weeks to months in those who have successfully been decolonized. In light of the emergence of antibiotic resistance and the lack of long-term elimination strategies against *S. aureus* nasal carriage, new approaches are needed for the prevention of staphylococcal disease. To develop new strategies, it is important to acquire additional knowledge about the underlying mechanisms of *S. aureus* nasal carriage [5].Unrecognized carriers may spread MRSA and render infection control programs futile).

Three main carriage patterns have been described when individuals are repeatedly sampled in the anterior nares for *S. aureus* over longer periods of time: the so-called no carriers [4].Historically, individuals have been assigned to 1 of 3 groups with regard to carriage of *S. aureus:* persistent carriers (20% of individuals), intermittent carriers (30%), and non-carriers (50%). The prevalence of *S. aureus* nasal carriage varies, however, and is higher in young children, men, white persons, hospitalized patients, and a number of patient groups, including patients with diabetes mellitus, those undergoing hemodialysis or chronic ambulatory peritoneal dialysis, those with S. *aureus* skin infection, and HIV-infected patients It has been documented that certain individuals may carry their res [6].

Ident strain for extensive periods, sometimes even for years. How nasal carriage is established and maintained is still largely unknown. In addition to host factors, several bacterial carriage determinants have been studied. Cell wall teichoic acid, lipoteichoic acid and fibronectin-binding proteins have been shown to represent major ligands in the adherence of *S. aureus* to epithelial cells. Recently, such a role for the protein clumping factor (Clf) B was ascertained by use of human and animal artificial colonization. Meanwhile, current knowledge still does not explain why some individuals are persistent carriers and others are intermittent carriers or no carriers [7].

Nasal carriage was first described as persistent or intermittent. However, recent results suggested that intermittent carriers should be grouped together with non-carriers, because only persistent carriers were at high risk of autoinfection, whereas intermittent and non-carriers were at a similarly low risk. Risk factors for colonization include young age, male sex, underlying co morbidities, sharing of a carrier's household, smoking, having been hospitalized, and recently, contact with animals. Antibiotic exposure affects the susceptibility of the colonizing strains [8].

Highly automated identification systems are now days widely distributed in many medium-to-high-through put clinical microbiology laboratories. These systems improve the quality of patient care and enable more-cost-effective management of the same by enabling clinical microbiologists to identify medically relevant bacteria more rapidly and accurately.

An important measure of the value of a highly standardized commercial identification system must be the capability of the manufacturer to maintain or even improve the performance of an identification system over time. The new VITEK 2 gram positive (GP) identification card (bioMerieux, Marcy l_Etoile, France) for identification of GP cocci was created in recent years as research and development related to the VITEK 2 instrument continued [9]. The rationale for designing the new VITEK 2 GP identification card was to broaden the VITEK 2 database while maintaining the quality of the identification results in the routine clinical laboratory.

The GP identification card contains 43 tests (27 tests that had been included in the previous card and 16 new tests), GPC),, the GP identification card tests are based on colorimetric detection, tests are subjected to measurements every 15 min, and the total incubation time is up to 2 h [10].

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The aim of the present study was to evaluate the newly developed VITEK 2 GP identification card in a routine clinical laboratory by a weighted laboratory profile to identify Persistent nasal carriers which have an increased risk of *Staphylococcus aureus* infection, whereas intermittent carriers and non-carriers share the same low risk. Also, study was performed to provide additional insight into staphylococcal carriage types and their antibiotics sensitivity.

Figure1:1: VITEK -2 Systems

Material & Methods

Study Subject

Healthy students not suffering from any upper respiratory tract infection (50 sample from ear and 50 sample from nose) who have not received any antibiotics are selected for the present study.

Swabs Collection

Cotton swabs sterilized by hot air oven at 160C° for 1 hour were used for nasal swabbing of the anterior nares and ear of (100) healthy students. The swabs were rubbed very well by rotating 5 times over the inner wall of the nasal and ear then immediately processed for culture and isolation [11].

Cultivation

The swabs collected were cultured on Mannitol Salt agar (selective medium for *Staphylococcus*) within one hour after collection by streaking as per the conventional technique. The culture plates were incubated at 37C for 24-48 hours in the incubator [11].

Identification of Staphylococcus

The suspected Staphylococcus colonies-yellow colonies showing Mannitol fermentation and non-yellow colonies (Mannitol negative) were selected and subject to Gram staining and sub-cultured into nutrient agar slopes. The isolates showing gram-positive cocci in clusters were subjected to coagulase test by slide and test tube technique using undiluted and 1:6 diluted human plasma respectively. All the coagulase positive staphylococci are stored in +4C and Later they are sub cultured to carry out varying phenotypic characterization like Mannitol fermentation and antibiotic susceptibility testing etc [12].

New GP Identification Card and VITEK 2 Instrument

A bacterial suspension was adjusted to a McFarland standard of 0.5 in 2.5 ml of a 0.45% sodium chloride solution with a VITEK 2 instrument (DensiChek; bioMerieux). The time between preparation of the inoculum and the card filling was always less than 30 min. The GP identification card includes test for the following reactions: phosphatidylinositol phospholipase C, arginine dihydrolase (two tests), galactosidase, glucosidase, alanine-phenylalanine-proline arylamidase, L-aspartic acid arylamidase, galactosidase, mannosidase, alkaline phosphatase, L-leucine arylamidase, proline arylamidase, glucuronidase (two tests), galactosidase, Lpyroglutamic acid arylamidase, alanine arylamidase, tyrosine arylamidase, and urease. The GP identification card also tests acid production from the following substrates: amygdalin, xylose,-cyclodextrin, sorbitol, galactose, ribose, lactate, lactose, *N*-acetyl-glucosamine, maltose, mannitol, mannose, methyl-D-glucopyranoside,

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pullulan, raffinose, salicin, sucrose, and trehalose.

Finally, growth in 6.5% NaCl as well as tests for resistance to polymyxin B, bacitracin, novobiocin, O129, and optochin are also included in the GP identification card. The card was put on the cassette designed for use with the VITEK 2 system, placed in the instrument, automatically filled in a vacuum chamber sealed, incubated at 35.5°C, and automatically subjected to colorimetric measurement every 15 min for a maximum incubation period of 8 hr. Data were analyzed using VITEK 2 database version 4.01, which allows organism identification in a kinetic mode beginning 180 min after the start of incubation based on the color change of the reaction [13].

Results & Discussion

Cultural Characteristics

A total of 100 swabs were collected from nose and ear of healthy students , 20 bacterial isolates were grown on Mannitol agar plates subjected to hemolytic test on blood agar, gram staining, and Catalase test in an attempt to screen the-hemolytic gram positive, and Catalase. These strains are subjected to various morphological and biochemical tests. It was found only 9 (45 %) isolates were identified as *Staphylococcus aureus* which is coagulase positive while 11 (55%) were identified as another types of *Staphylococcus* (CONS) (Table 1,2) according to their growth morphology and biochemical reactions patterns such as coagulase, tests.

In respect to cultural characteristics and colonial morphology it was found that on blood agar the colonies are circular, golden yellow, white or milky on nutrient agar. On microscopic observation it was revealed that the cells were arranged in pair or short chain and clusters, the isolates were coagulase positive for *Staphylococcus aureus* and negative for others (CONS). The studies available from various parts of the world have indicated that *Staph.aureus* is causing serious and fatal infections among the hospitalized patients especially in 5 intensive care facilities. It is well known that throughout the world 20% to 30% of healthy people 5. Although the amount of knowledge about host factors and bacterial determinants of *S. aureus* colonization is increasing, it remains unclear why certain individuals are persistent carriers, whereas others are intermittent carriers or non-carriers.

This knowledge is important, because persistent carriers are a higher risk for development of *S. aureus* infections although intermittent carriers carry *S. aureus* in the nose at times, their risk of infection is similar to that among no carriers [14]. Recently (CONS) considered as opportunistic pathogen and has uncertain relationship of causing disease because it has the ability to produce the bio- film which is one of virulence factor [7].Previous investigations had found a variable frequency of *Staph. aureus* nasal carriage ranging from 4% up to 64%.

Several studies had shown that individuals may have colonization exclusively in the nose that would be missed on screening limited to the anterior nares. As in a previous study by Nilsson and Ripa, our results also suggest that the presence of *S. aureus* is frequent in the nares (Table 2). This is important because social and economic circumstances are different for peoples, and the nose and ear is an important habitat of Staphylococcus spp., including MRSA, and the bacteria could persist as colonizers for many years. In conclusion, any screening for *Staph. aureus*, in particular screening for MRSA within a community, should include cultures from both the anterior nares and the ear [14].

Table 1: Positive bacterial culture of nose and ear swabs for Staphylococcus healthy carriers

Type of sample	Culture Positivity		
	No. Of positive culture	Total	(%)
Nose	14	(50)	(14%)
Ear	6	(50)	(6%)
Total	20	(100%)	(20%)

Table.2: Frequency of Coagulase positive Staphylococcus (COPS) & Coagulase Negative Staphylococcus (CONS) bacteria from ear and nasal carriers

Samples	COPS			CONS			Total	
	Total	(%)	NO.	Total	(%)	NO.		
Nose	(30%)	6	20	8	(20)	(40%)	14	(70%)

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Ear	**3 20 (15%)**	**3 (20) (15%)**	**6 (30%)**
Total	**9 20 (45%)**	**11 (20) (55%)**	**20 (100%)**

Identification by using VITEK- 2 System

This study examined the Vitek-2 GPI card's ability to identify 9 (45%) isolates of of Coagulase positive Staphylococcus (CoPS) and 11 (55%) isolates of Coagulase Negative Staphylococcus (CoNS) species and subspecies, 20 clinical isolates were used, these were sequential isolates from a healthy student. The distribution of species is what might be expected in a clinical setting, and the performance of the GPI card, as demonstrated in this study, should reflect the expected performance of the system in most clinical settings.

Previous studies have examined the GPI card produced by bioMerieux Vitek-2. However, these studies had several limitation because they used a relatively small number of isolates of CoNS, newly described *Staphylococcus lugdunensis* and S. *capitis subsp. Ureolyticus* were not included in this studies, and bioMerieux Vitek-2 has now updated the GPI card [5]. Even though *S. capitis subsp. capitis* has been recently described and not much research has been done on its clinical significance, some preliminary studies have shown its ability to produce a polysaccharide adhesion and its ability to cause abscess formation in a mouse model. The need to correctly identify and give accurate susceptibility patterns for the **CoNS** is becoming more evident with the recognition of their clinical significance. Conventional methods can provide accurate identifications; however, there remains a need for rapid systems [13].*S. capitis subsp. ureolyticus*, which was considered correctly identified if called *S. capitis subsp capitis*, was correctly identified 25% of the time (Table 3) and when misidentified was called either *S. epidrmidis* (20%), *S. cohni subsp. cohni* (10%), Therefore, the future may show that this subspecies is a clinically significant CoNS, and the need to be able to distinguish it from other CoNS [15].

Incorporation into the card of additional substrates such as fructose, maltose, or mannose might be helpful in improving the accuracy of staphylococcal identification Although attempts have been made to simplify the identification of coagulase negative staphylococci, it appears that there is some degree of physiological heterogeneity among these species and that a large battery of biochemical tests is needed to identify certain strains accurately [9].

Table 3 Diagnosis of *Staphylococcus* spp. by using VITEK- 2 System:

Type of sample	Staphylococcus spp.	Number & Percentage of isolates	
		No.	(%)
Nose	***Staphylococcus aureus*** ***Staphylococcus epidrmidis*** ***Staphylococcus cohni subp.conhi*** ***Staphylococcus capitis subp.capitis***	**6** **1**	**30** **5**
Ear	***Staphylococcus aureus*** ***Staphylococcus epidrmidis***	**3** **2** **3**	**15** **10** **15**
Total		**5** 20	**25** 100

Antibiotic Susceptibility Testing

All nasal and ear isolates of *Staphylococcus spp.* were subjected to *in vitro* anti-microbial testing method on Muller-Hinton agar, using 2-hour-old nutrient broth culture and HIMEDIA make antibiotic discs as per the method described by [16]. The zone of inhibition around the discs were measured and interpreted as sensitive, moderately sensitive and resistant using the interpretation chart supplied by the antibiotic disc manufacturers (HIMEDIA).

The results of this study demonstrated that the majority of Staphylococcus strains that were isolated from the nose and ear showed a high level of sensitivity to Ciprofloxacin (100%), (60%), (100%) and (50%) for Staphylococcus cohni sub. conh, Staphylococcus capitis sub. capitis, Staphylococcus aureu and Staphylococcus

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epidrmidis respective this antibiotic is from the third generation of antibiotics and are rare in use this prevent the organisms to develop resistance to the [17]. While showed resistance Nalidixic acid (50, 80, 66.6%), Kanamycin (100, 80, 66.6 and 50%), Gentamicin (50, 80, 77.7 and 75%) respectively. Development of moderate susceptibility to Gentamycin despite the fact that they are not third generation antibiotics, so this indicates that they are not being abused or commonly prescribed , and there was no resistance found to Chloramphenicol and Ciprofloxacin.

These antibiotics the most effective against *Staphylococcus* isolated from middle ear [18]. All isolates of *S.aureus* were(100%) resistant to Ampicillin and Amoxicillin *S. aureus* resistance to ampicillin was seen in clinical practice as early as the 1950s, by acquiring a plasmid that encodes the production of beta-lactamase enzymes causing resistance to beta-lactam antibiotics. The exceedingly increases and emergence f resistance pathogens in the developing countries can be attributed to the indiscriminate use of antibiotics, complex socio-economic, behavioral antecedents and the dissemination of drug-resistant pathogens in human medicine Antibiotic resistance of pathogens typically causative of acute continues to increase as the emergence of multi-drug resistant strains especially *Staphylococcus aureus* complicate the management disease and increase the risk for treatment failure [6]. The available study indicates the MRSA carrier rate ranges from 5-50% in many countries.

The maximum carrier state was found among the UK hospitalized patients (5-50%) followed by USA (10-60%), northern Europe (1-5%), southern Europe (10-30%) and Asian countries (5-40%) and this agree with our study. Recently, the epidemiology of *S. aureus*, in particular methicillin- resistant *S. aureus* (MRSA), has changed with the emergence of community-acquired MRSA) (Reports from different parts of the world indicate that MRSA has emerged as a new pathogen, *S. aureus* is a transient or persistent part of the resident flora in the anterior nares of the population [19].

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