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Prevalence of Staphylococci Among Dental Staff and Their Antibiotic Resistance Pattern.

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Abstract

Aims: The study aims to estimate the carriage of Staphylococci spp on nares and hands among dental staff and their antibiotic resistance pattern. **Materials and Methods**: 100 samples were collected from the nose and hands of dental workers (dentists and assistants) in the teaching hospital of the College of Dentistry, University of Mosul from the period 17th Dec. 2020 to 9th Feb.2021. The isolates were diagnosed based on phenotypic traits, microscopy, biochemical test, and the use of the vitek – 2compact device to confirm the species, 66 isolates showed that they were able to ferment mannitol, 32 isolates from the hands, and 34 isolates from the nose while the other isolates showed an inability to ferment mannitol sugar. All Staphylococcus isolates were Gram-positive, catalase 100%, coagulase (18.75%) for hand isolates, and (52.9%) for nosal isolates. Using the vitek 2-compact (40) isolates of fermented mannitol were diagnosed and the results were as follows (20) isolates Staph.aureus 14; (70%) from nose and 6(30%) from hand, 5 isolates of Staph. lugdunensis ;4 (80%) from nose ,1 (20%) from hand .4 isolates Staph. Saprophyticus; 2 (50%) from nose, 2(50%) from hand,2 isolates of Staph. hominis; (100%) from hand,3 isolates of Staph. warneri 2;(66.67%) from nose, 1(33.33%) from hand and one isolate of Staph.sciuri 1,(100) from hand and one isolate of Leuconostoc mesenteroides from hand, four isolates the device couldn't diagnose. The sensitivity of the different isolates to oxacillin was determined with minimum inhibitory concentration (MIC) using vitek2-compact device as oxacillin indicates methicillin in the device. The isolates differed in their resistance to the antibiotic oxacillin where the highest resistance was the percentage to each of Staph.lugdunensis and Staph.sciuri the resistance rate reached 100% followed by Staph.aureus were the resistance rate reached 80% then Staph.warneri 66.67% , Staph.hominis 50% and Staph. Saprophyticus 25% . The sensitivity of different isolates to antibiotics was determined by using Kirby –Bauer method using seven types of antibiotics Staphylococcus isolates showed resistance to both oxacillin and methicillin at percentage 100% except for Staph. Saprophyticus where the resistance was 50%.

الخلاصة

الاهداف: تهدف الدراسة السريرية الى تقييم انتشار المكورات العنقودية بين اطباء الاسنان ونمط مقاومتها للمضادات الحيوية. **المواد وطرائق العمل** : جمعت (100) عينة من الانف والايدي للعاملين في مجال طب الاسنان (اطباء الاسنان والمساعدين) في المستشفى التعليمي لكلية طب الاسنان- جامعة الموصل وللفترة من 17كانون الاول2020 الى 9 شباط 2021 وتم تشخيص العزلات بالاعتماد على الصفات المظهرية ، الفحص المجهري ، الاختبارات الكيموحيوية واستخدام جهاز الفايتك vitek 2 compact لتاكيد النوع ، اظهرت (66) عزلة قابليتها على تخمر المانتول ،32 عزلة من الايدي ،34 من الانف فيما اظهرت بقية العزلات عدم القابلية على تخمر سكر المانتول كانت جميع عزلات المكورات العنقودية موجبة لصبغة كرام والكتاليز 100% اما فحص التخثر فكانت النسبة 18.75 % من العزلات المعزولة من الايدي و 52.9% لعزلات الانف موجبة وباستخدام جهاز الفايتك تم تشخيص (40) عزلة مخمرة للمانتول فكانت النتائج كالاتي عشرون عزلة Staph.aureus14 :(70%) من الانف و 6: (30%) من اليد ، خمس عزلات 4 Staph.lugdunensis: (80%) من الانف ، 1(20%) من اليد ، اربع عزلات Staph. Saprophyticus 2،:(50%) من الانف و 2 (50%) من اليد ، وعزلتين Staph.hominis : (100%) من اليد، ثلاث عزلات 2 : 3 Staph.warneri(66.67%) من الانف و 1(33.33%) من اليد وعزلة واحدة 1: S.sciuri (100%) من اليد وعزلة واحدة Leuconostoc mesenteroides معزولة من اليد . تم تحديد حساسية العزلات المختلفة للاوكساسلين MIC وباستخدام الفايتك حيث ان الاوكساسلين يماثل المثيسلين بالجهاز اختلفت العزلات في مقاومتها للمضاد الحيوي الاوكساسلين حيث كانت اعلى نسبة للمقاومة لكل من Staph.lugdunensis و Staph.sciuriاذ بلغت نسبة المقاومة 100% ، S.aureus اذ بلغت نسبة المقاومة 80% ثم Staph.hominis، 66.67 Staph.warneri % و 50% ، Staph. Saprophyticus 25% تم تحديد حساسية العزلات المختلفة للمضادات الحيوية وباستخدام طريقة Kirby - Bauer وباستخدام سبعة انواع من المضادات الحيوية اظهرت جميع عزلات Staphylococcus مقاومتها للاوكساسلين والمثيسلين وبنسبة 100% ماعدا عزلات Staph. Saprophyticus فكانت نسبة المقاومة 50%..

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INTROCUTION

Staphylococcus spp. is member of family *Micrococcaceae* appear as Gram-positive cocci arranged singly, in pairs, in short chains or cluster [1] . *Staphylococcus aureus* is the most common microorganism which can cause opportunistic infections and hospital-acquired infections [2], it is a major cause of different infections that range from superficial skin(hair follicle abscesses) to deep tissue infection and systemic infections [3], also causes severe disease such as bacterial endocarditis, toxic shock syndrome, scalded skin syndrome and osteomyelitis [4]. Infections caused by these bacteria are more difficult to treat with common standard antibiotics, so they have potential risks for human health. The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals in different parts of the world is diverse ranging between 2- 70%, 20% on average [5]. Methicillin–resistant *S. aureus* is considered a leading global concern in the health sector, and more recently in the community [6]. MRSA has been resistant to beta-lactam antibiotics including Penicillin, methicillin, amoxicillin, ampicillin oxacillin, cephalosporin. etc. [7], also acquired methicillin-resistant gene (*mec*A) [8] carried on DNA fragment known as the Staphylococcal cassette mec (SCCmec), which encodes the pencillin binding protein -2a (PBP-2a) that inhibits the action of β-lactam antibiotics[6], so PBP2 continues in cell wall synthesis even in the presence of high concentration of β- lactam antibiotics; also MRSA produce a β- lactamase enzyme which damage the functional perfection of β- lactam antibiotics by spliting the β-lactam ring of pencillin molecules [9] which enable them to cause particular clinical syndroms [10]. In addition to their antibacterial resistance, MRSA strains are able to produce biofilm: a dynamic and complex multi-layer cellular matrix which is considered to be a major virulence factor [11], once biofilm was formed, the eradication of *Staphylococcal* infection in patient become very difficult, in addition, biofilm promotes the horizontal spread of antibiotic-resistant determinants [12]. The source of MRSA infection in dental clinics could be infected or colonized patients, dentists and environmental surfaces, air and water syringes, dental chair, pushbuttons, light handles [13]. Resistance of *S.aureus* to antimicrobial agents can take place by intrinsic or acquired resistance, the emergence of multi-drug resistance may indicate the presence of efflux pumps which contributes to the antimicrobial resistance of many bacteria [14]. The study aims to estimate the carriage of Staphylococci spp on nares and hands among dental staff and their antibiotic resistance pattern.

MATERIALS AND METHODS

1- Sample collection:

Samples include swabs from noses and hands of the dental workers (dentists and assistants) in the dental teaching

hospital – University of Mosul, samples were collected for the period of 17th Dec. 2020 to 9th Feb. 2021 by using a sterile cotton swab; 200 samples were collected (100f rom nose) and (100 from hands) , transferred to the laboratory inside vial contains Nutrient broth [8] [15]. From (n=100) dental staff (dentists = 96, and assistants = 4**)**;they were (males 61 ; female 39), their ages ranged between(17-50) years ;100 swabs from nose and 100 swabs from hands were taken.

2- Identification of Staphylococci:

The samples were cultured immediately after collection for the purposes of diagnosis on mannitol salt agar, then incubated in aerobic conditions at $37C^0$ for 24 hours until bacterial colonies appear and the isolated colonies were diagnosed routinely depending on phenotypic traits and microscopic examination and biochemical tests [16] and automated identification by VITEKS 2 compact system (BioMerieux, France) [5].

Antibiotic susceptibility pattern

Antibiotic susceptibility testing of bacterial isolates diagnosed as *Staphylococcus aureus* by routine laboratory methods and Vitek compact system includes seven antibiotic disc (Bio analyses , Turkey) ; namely (Penicillin G 10mcg, Methicillin 10mcg, Oxacillin 5mcg , Ciprofloxacin 10mcg , Rifampicin mcg , Vancomycin 30 mcg , Erythromycin 10 mcg)according to Kirby –Bauer method ; Muller –Hinton (MH) agar used, the plates were inoculated by 1 ml of bacterial suspension ,incubated for 18-24 hours at $37C^0$ and was inoculated to a turbidity of 0.5 McFarland which correspond to 10^8 organism/ml ; the diameters of all zones of inhibition were measured by millimeter and the data compared with a standard zone of growth inhibition a according to Clinical and Laboratory Standards Institute (CLSI) [10] [17] ; and by VITEKS 2 compact system (BioMerieux, France) using the card type AST-P592 including: Cefoxitin, Benzylpenicillin, Oxacillin, Methicillin, Gentamicin, Ciprofloxacin, Moxifloxacin, Clindamycin , Erythromycin, clindamycin, linezolid, Teicoplanin, Vancomycin, Tetracyclin, Tigecyclin, Fusidic acid , Rifampicin and Trimethoprim .

RESULTS AND DISCUSSION

All Staphylococcus isolates were Gram positive cocci arranged in cluster, the samples also grow on mannitol salt agar showing mannitol fermenter colonies (66 of the samples), others (113) grow without fermenting mannitol ; all the isolates catalyze H_2O_2 giving + ve result of catalase test, and (71.65%) of the isolates were coagulase-positive;as shown in table (1) [8] identify *S.aureus* isolated from the different sites of the body (wound, blood, tracheas, ear, lungs, nostrils, skin and throats) depending on colony morphology, mannitol salt agar, Gram staining and different biochemical tests (catalase,

coagulase and mannitol fermentation test) . Alkhafaji identified *S.aureus* by growth on mannitol salt agar aerobically, changing the color of the medium from pink to yellow due to fermenting mannitol sugar [16] and microscopic examination of *S.aureus* showing that gram-positive globular cell with pairs or quadric or in the form of bunches of grapes .

Table (1): phenotypic identification of Staphylococcal isolates:

Total specimen number	Growth on MSA	No growth on MSA	Mannitol fermenters	Mannitol Non-fermenters	Gram stain	Catalase test	Coagulase test
Hand (100)	86	14	32	54	+ ve cocci clusters	+ ve 100%	18.75%
Nose (100)	93	7	34	59	+ ve cocci clusters	+ ve 100%	52.9%
200	179	21	66	113			

Using the automated method of identification; all the Staphylococci which ferment mannitol re-identified by the vitek 2 compact device; using the GP: for ID (identify) and 592 kits for AST (Antimicrobial sensitivity test), the results are shown in table (2).

Table (2): Diagnosis of the Staphylococcal isolates by VITEK 2 compact device:

Bacterial Isolates	No. of isolates	Nose	%	Hand	%
Staph.aureus	20	14	70%	6	30%
Staph.lugdunensis	5	4	80%	1	20%
Staph. Saprophyticus	4	2	50%	2	50%
Staph.hominis	2	_	_	2	100%
Staph.warneri	3	2	66.67%	1	33.33%
Staph.sciuri	1	_	_	1	100%
Leuconostoc mesenteroides	1	_	_	1	100%
Total of isolates	36	22		14	

In the present study (40) isolates were chosen to identify by vitek 2 compact system 20 (*s.aureus*) ; 14 (70%) from nose and 6 (30%) from hand as shown in table (3) while *Staph. lugdunensis* 5 ; 4 (80%) from nose and 1 (20%) from hand, *Staph. Saprophyticus* 4 ; 2 (50%) from the nose and 2 (50%) from hand while *Staph.hominis* 2 (100%) from hand only ; *Staph.warneri* 3; 2(66.67%) from nose and 1(33.33%) from hand , *Staph.sciuri* 1 (100%) from hand , *Leuconostoc mesenteroides* 1; 100% from hand while 4 isolates the device couldn't identify them *s.aureus* represent higher percentage from the isolates .In one of local studies [18] 38.1 % of the isolates were *Staphylococcus spp ;* (43.75%) of them were *S.epidermis* and

18.7% *S.hominis* , 18.7% , *S. haemolyticus* with percentage (18.7%) *S.aureus* isolates with percentage (5%) and *S. warrneri* with percentage (6.25%) . *S.aureus* is one of the major pathogens causing serious infections both in the hospital setting and in a community this pathogen is characterized by rapid acquisition of resistance to antibiotics the methicillin-resistant *S.aureus* (MRSA) emerged first in hospital setting and then spread to the community (CA-MRSA) [19]. MRSA are mostly spread via transiently contaminated hands of health care professionals but contaminated surface and objects may play a minor role in MRSA transmission [20].The percentage 20 and 23% numbers reported in other studies on *s.aureus* isolated from environmental surfaces , hands , anterior nares and nasal swab in dental health care facilities [21],another study show that staphylococci were the most prevalent bacteria in nature as there on human skin , mucus membrane, respiratory tract [22]. Another study showed that *S.aureus is* present in the nose and throat with the same percentage [23].

On studying the antibiotic sensitivity of the isolates, they show resistance to oxacillin; as in table (3)

Table 3 - sensitivity of Staphylococcal isolates to oxacillin using minimum inhibitory concentration (MIC) using Vitek-2

Bacteria	Number	Sensitive (S)			Intermediate (I)			Resistance (R)		
		Numbers	Percentage	MIC	Numbers	Percentage	MIC	Numbers	Percentage	MIC
Staph.aureus	20	4	20%	<=0.25	-	-	-	16	80%	>=4
Staph. lugdunensis	5	-	-	-	-	-	-	5	100%	>=0.5
Staph. Saprophyticus	4	3	75%	0.5	-	-	-	1	25%	>=4
Staph.hominis	2	1	50%	0.5	-	-	-	1	50%	>=4
Staph.warneri	3	1	33.33%	<=0.25	-	-	-	2	66.67%	>=4
Staph.sciuri	1	-	-	-	-	-	-	1	100%	<=0.25

The oxacillin was used as an indicator of methicillin resistance according to the instruction of the company [24]. The result agreed with [10] they found that 89.58% of *s.aureus* isolates were oxacillin resistant; other studies showed that70% of *s.aureus* were resistant to methicillin (MRSA) and 62.7% were resistant to oxacillin [3] while [25] showed high resistance of *s.aureus* against methicillin (93.4%) and oxacillin (100%) . The results of the antibiotic susceptibility test using Kirby –Bauer method were explained in table (4) shows that all the Staphylococcal species isolated in this study were resistant to methicillin and oxacillin at 100% except for *S. Saprophyticus* resistance to oxacillin at 50% only. A study confirmed that *S.aureus* was resistant to all beta-lactam antibiotics

[24], it was also resistant to oxacillin. Another study showed that 35 *S.aureus* clinical isolates were highly resistant to beta-lactam antibiotics viz oxacillin (94.28%) and cefoxitin (94.28%)) [8]. Another study confirm that the transconjugants of *s.aureus* were resistant to oxacillin [26].

Table 4- Result of sensitivity testing of samples using Kirby –Bauer method**.**

		Penicillin G P- 10	**Methicillin ME-10**	**Ciprofloxacin CIP-10**	**Rifampicin RA-5**	**Vancomycin VA-30**	**Erthromycin E-10**	**Oxacillin OX-5**
S.aureus **n = 20**	S	12 60%	0 0%	5 25%	7 35%	2 10%	1 5%	0 0%
	I	0 0%	0 0%	3 15%	2 10%	1 5%	6 30%	0 0%
	R	8 40%	20 100%	12 60%	11 55%	17 85%	13 65%	20 100%
Staph.lugdunens**is** **n = 5**	S	2 40%	0 0%	1 20%	0 0%	0 0%	2 40%	0 0%
	I	0 0%	0 0%	2 40%	0 0%	2 40%	0 0%	0 0%
	R	3 60%	5 100%	2 40%	5 100%	3 60%	3 60%	5 100%
Staph. Saprophyticus **n = 4**	S	1 25%	0 0%	0 0%	0 0%	0 0%	2 50%	2 50%
	I	0 0%	0 0%	2 50%	1 25%	2 50%	1 25%	0 0%
	R	3 75%	4 100%	2 50%	3 75%	2 50%	1 25%	2 50%
Staph.hominis **n = 2**	S	1 50%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
	I	0 0%	0 0%	0 0%	0 0%	2 100%	0 0%	0 0%
	R	1 50%	2 100%	2 100%	2 100%	0 0%	2 100%	2 100%
Staph.warneri **n = 3**	S	0 0%	0 0%	0 0%	1 33.33%	0 0%	0 0%	0 0%
	I	1 33.33%	0 0%	1 33.33%	0 0%	0 0%	0 0%	0 0%
	R	2 66.66%	3 100%	2 66.66%	2 66.66%	3 100%	3 100%	3 100%
Staph.sciuri **n = 1**	S	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
	I	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
	R	1 100%	1 100%	1 100%	1 100	1 100%	1 100%	1 100%

CONCLUSION

in conclusion *Staph. Aureus* is the most isolated bacteria in our samples than other bacteria, especially in nose samples. An obvious level of resistance was exhibited against β – lactam antibiotics. the

higher resistance was against oxacillin and methicillin.

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