Isolation and Identification of Some Bacterial Isolates from Mercury and Photo Filling of Teeth and Study the Antibacterial Effect of *Pimpinella anisum*

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Abstract

The purpose of this study was the isolation and identification of some bacterial isolates like *staphylococcus aureus*, *staphylococcus epidermidis*, *streptococci* and *entrococci* from mercury and photo filling indifferent ages in male and femal. These bacteria were predominant in male compared with female in the older ages from the normal pH (6.8 – 7.0). We also investigate the antibacterial activity for the plant *Pimpinella anisum* and some antibiotics by disc diffusion methods and minimum inhibitory cocentration,the results showed these bacterial isolates were sensitive to the aqueous extract compared with methanol, acetone and petroleum ether and were more sensitiveto vancomycin compared with other antibiotics.

Key Words: Mercury & photo filling bacterial teeth, Antibacterial effect, *PimPinella anisum staphylococcus epidermidis*, *enterococci*, *streptococci.*

Introduction

We all have little pockets between our teeth which tend to accumulate food, even when we brush regularly, the presence of these moist food nutrients combined with neutral PH body warmth to provide excellent growth conditions for bacteria (1). Dentinal amalgam has been used for 150 years is relatively low-cost, durable and easy to use. The majority of human mercury (Hg) exposure has been reported to be from dietary source and from dental amalgams (50% mercury) which emit mercury vapor (2, 3). Concluded that children receiving amalgam did not on average have differences in neuro behavioral assessments or nerve condition. Velocity compared with children treated with composite materials (4).

Antibiotic and mercury resistance (Hg^f) genes are often associated some conjugative elements of bacteria. Therefore, it might be possible that mercury released from amalgam may select for increased numbers of antibiotic resistance commensally bacteria which could pass these trails to pathogenic bacteria and increase the risk of bacterial disease resistance to common therapies (4, 5). Such infections are more difficult and costly to treat and increased morbidity and mortality (6).

At study on 6 monkeys suggested that amalgam did increase the number of (Hg^f) *Enterobacteriaceae, Streptococcus* and *Enterococcus* species (7). Another study found that 71% of children without amalgam restorations carried Hg^f ($\geq$ 32mM) oral Gram Positive bacteria and 56% carried antibiotic resistance bacteria indicating that both Hg^f and antibiotic resistance bacteria are commonly found in children (8). In our study we used

anise (*Pimpinella anisum L*.) extracts that a flowing plant in the family *Apiaceae*, native to the India and south west Asia, it is herbaceous annual plant growing to 1m tall. The leaves at the base of the plant are simple, 2-5cm. long and shallowly lobed. While leaves higher of the stems are feathery pinnate, divided into a numerous leaflets, the flowers are white, 3mm diameter a produce in dense umbels (9). The fruit is an oblong dry schizo carp (3-5mm) long. As a medical plant, *pimpinella anisum* has been used as a stimulating effect of digesting and antiparasitic, antifungal (10) and antipyretic (11).

Additionally, the plant especially its fruits essential oil has been used for treatment of some disease including seizures and epilepsy (12). Furthermore it has been to have anticonval sant-effect and has been used for treatment of dental problem (9,10) and possesses muscle relax ant effect (15) recently its oil has been reported to be used as antibiotic substitute in broilerration (13). There are a few reports (14,15) on systematic studies pertaining to antibacterial evaluation of *pimpinella anisum*, hence considering its therapeutic potential it was essential to prove it for its exact rational use a medicine by scientific means. Therefore, the present investigation was undertaken to evaluate antibacterial activity of some bacteria isolated from tooth filling with *pimpinella anisum* dried fruits.

Materials and Methods

Isolation and Identification:

Thirty seven samples were collected from dental private by sterilized swabs impregnated in normal saline from different persons (20 male and 15 female) aged from 16 to 48 years in Basrah in order to investigate the bacteria associated with tooth filling thirteen samples were collected from normal persons the isolated bacteria were identified using appropate selective media and biochemical tests as described in (16).

Plant material and extraction:

The fruit of *pimpinella anisum* were purchased from the local market of Basrah. The fruits were sun dried and ground to powder by using the grinder mixer, fifty gram dried fruit were soaked separately for 48 hrs, In 200ml distilled water, 50% (V/V) methanol, acetone and petroleum ether extraction respectively. The soaked material was agitated at regular time intervals. After 48 hrs. the soaked material was filtered using muslin cloth then the filtrate were again filtered using Wattman filter paper No.1 on separate filtration setups. The final filtrates were collected in a wide mouthed evaporating bowls and dried under room temperature. The dried extracts were weighed to calculate the extractability percentage (9).

Sensitivity test:

To study the antibacterial activity of for five types of antibiotics (Vancomycin, Bacitracin, Ciprofloxacin, Nalidixicacid, Tobramycin) Muller –Hinton medium used for bacterial growth 10^{5} cell/ml assays on sensitivity test of *pimpinella anisum* were performed as previously described (17). In brief, these bacteria were determined by using disk diffusion method. Circular paper disk measuring 7.0 mm was cut from Watt man No.1 filter paper. Each group of plates was inoculated with each of the test organism which was fully spread on the Muller-Hinton agar medium. Finally the discs impregnated with

extracts (D.W. acetone-petroleum ether, ethanol). Culture bacteria with halos equal to greater than 7 mm were considered susceptible to tested extract (9, 10). The zone of inhibition was determined by measuring diameter of clearance across the disc with a ruler.

The Results and Discussion

From (50) samples we obtained 8 (16%) isolates *staphylococcus aureus*, 6 (12%) *staphylococcus epidermidis* and 4 (8%) isolates enterococci 2(4%) isolates Streptococci. (Table1).

These bacteria characterized by using biochemical tests as described (16). We showed that. The percentage frequency of bacterial isolates were higher in male (24%) than in female (16%) table 2 and increased with increase the age of the persons that may be due to the decreased of immunity and resistance to infection by bacteria and all these isolates obtained from amalgam tooth filling (26%) compared with photo tooth filling (10%) and control 4% (Table 1) High percentage frequency also were obtained from normal pH 5.8 – 7.0 (30%) compared with acidic pH (10%) and alkaloid pH (Table 3). These results provide that normal pH oral cavity make suitable condition to growth of bacteria compared with those of alkaloid and acidic pH. As we know mercury is toxic to bacteria and as a result bacteria have quired genes that confer Hg^f (19, 20).

These Hg^f genes may predate man since they were identified in Gram negative *pseudomonas* and *Acinetobacter* and Gram positive *Staphylococcus* (21). Other studies suggest that the presence of Hg^f genes in bacterial amalgam is ancient and evolved initialty due to environmental mercury rather than irresponse to human activities (18, 22). Hg^f genes are thought to differ from most bacterial resistant genes, which have clearly appeared irresponce to human that use these agents over the last 50 years (22). Previously the mer.genes from Individual Hg^f Gram negative bacteria and all the Gram positive mergenes were able to transfer these genes to Gram negative *E coli* recipients (23).

Results of disc diffusion test (Table 4) which illustrated by photograph showed that these bacteria were sensetive to vancomycin and resistant to other antibiotics(figure 1-3) that agree with (21) who shows that these bacteria able to grow on 3 different antibiotic and Hg^f supplemented media± over seven years on the other hand. The results of *pimpinella anisum* extracts revealed high effect on the bacterial isolates (Table 5) compared with low inhibition zones of the antibiotic disc uses in this study (figure 4-6) also the aqueous extract was found to be effective against all the Pathogenic bacteria under study by disc diffusion assay, these study suggest that the potential use of *pimpinella anisum* as abroad spectrum of antibacterial agents and the medical importance of this plant through antibacterial activity that surely enhance their application among other uses as alternative to antibiotics for effective treatment of bacterial infections. (9) also this plant could be used to treat some stubborn *Staph*ylococcus & *Streptococcus* and *Enterococcus* infections Further considering the cost, availability and extractability percentage of the aqueous extractability percentage of the aqueous extract if can be considered & used as a cheap alternative to substitute antibiotics especially in animal and poultry feeds (24).

Table (1): Percentage frequency of bacterial isolates from mercury and photo tooth filling compared with control.

Bacterial isolates	Number of isolates	Mercury filling N=23	Photo filling N=14	Control N= 13
staphylococcus aureus	8 16%	n = 5 21.7%	n=3 21.4%	n = 0
staphylococcus epidermidis	6 12%	n = 4 17.3%	n = 0	n = 0 4%
Enteroccci	4 8%	n = 3 13%	n = 1 7.1%	n = 0
Streptococci	2 4%	n = 1 4.3%	n = 1 7.1%	n =2 15.3%
Total of Bacterial positive	20 40%	n = 13 26%	n = 5 10%	n = 2 4%
Total of Bacterial negative	30	n = 10	n = 9	n = 11

n = Number of isolates N = Total Number of isolates.

Table (2): Percentage frequency of bacterial isolates in male and female in age groups

Age (year)	Number of isolates	Percentage occurance	Percentage Occurance In female	Percentage occurance In male
16 – 26	18	n = 9 50 %	n = 2 11.1%	n = 7 38.8 %
27 – 37	19	n = 4 21 %	n = 2 10.5 %	n = 2 10.5 %
38 – 48	13	n = 7 53.8 %	n = 4 30.7 %	n = 3 23 %
Total sample of bacterial isolates	50	n = 20 40 %	n = 8 16 %	n = 12 24 %

n = Number of isolates

Table (3): Percentage frequency of bacterial isolates depend on pH of mouth

Age (year)	Number of isolates	Percentage frequency in normal pH (6.8 – 7.0)	Percentage frequency in acidic pH (1 – 6)	Percentage frequency in alkaloid pH (8 – 14)
16 – 26	18	n = 7 38.8 %	n = 1 5.5 %	n = 0
27 – 37	19	n = 1 5.2 %	n = 3 15.7 %	n = 0
38 – 48	13	n = 7 53.8 %	n = 1 7.6 %	n = 0
Total	50	n = 15 30 %	n = 5 10 %	n = 0

n = Number of isolates.

Table (4): The minimum inhibitory concentrations (MICs) of antibiotics

Bacterial isolates	Vancomycin	Bacitracin	Ciprofloxacin	Nalidixicacid	Tobramycin
	Mean ± Standard deviation				
Staph. aureus	3.5 ± 1	5.62 ± 1.187	4.25 ± 3.57	2.25 ± 2.54	4.00 ± 2.26
Staph. epidermidis	4.5 ± 1.91	3.33 ± 1.751	4.33 ± 3.50	3.66 ± 2.58	2.50 ± 2.073
Entero coccus	8.25 ± 1.25	2.75 ± 2.061	2.25 ± 1.707	2.50 ± 1.63	1.75 ± 2.06
Streptococcus	4.25 ± 1.41	0.00 ± 0.00	2.50 ± 3.53	1.63 ± 0.00	2.00 ± 0.00

MICs = Minimum inhibitory concentrations measured in µg/ml . ($p < 0.05$)

Table (5): The minimum inhibitory concentrations (MICs) of pimpinella anisum extract

Bacterial isolates	Distilled Water	Methanol	Acetone	Petrollum ether
	Mean ± Standard deviation			
Staph. aureus	0.21 ± 1.03	0.63 ± 0.103	0.40 ± 0.109	0.67 ± 3.165
Staph. epidermidis	0.1 ± 1.03	0.57 ± 0.10	0.60 ± 0.109	0.50 ± 0.103
Entero coccus	0.27 ± 8.165	0.20 ± 0.103	0.57 ± 0.103	0.57 ± 0.103
Streptococcus	0.20 ± 0.00	0.23 ± 0.103	0.43 ± 0.103	0.20 ± 0.109

MICs = Minimum inhibitory concentrations measured in µg/ml . ($p < 0.05$)

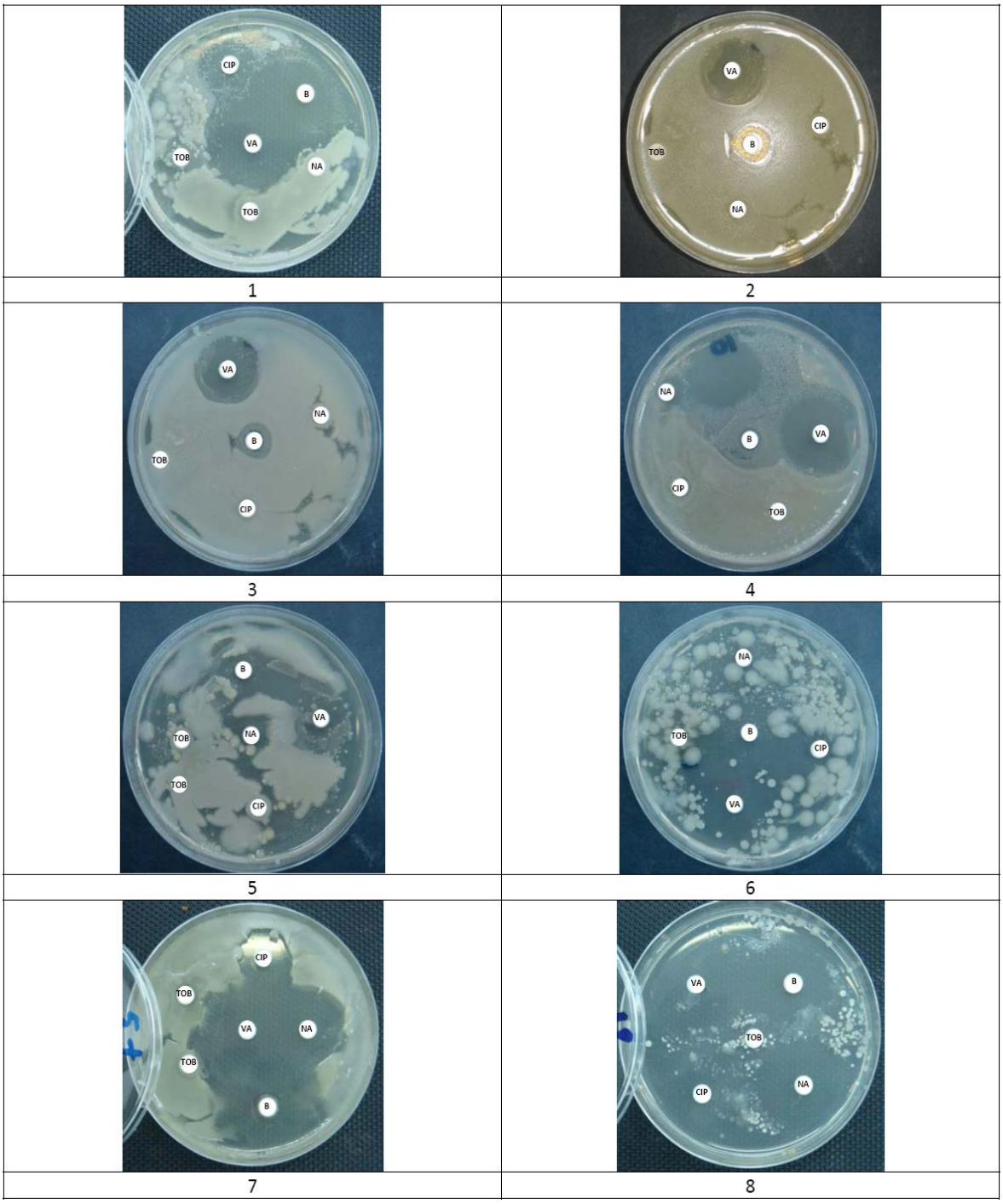

Figure (1): Antibiotics sensitivity to eight isolates of Staphylococcusaureus (1 5) from mercury filling; 68) from photo filling.

B=Bacitracin; VA=Vancomycin;NA=Nalidixicacid;CIP=Ciprofloxacin;TOB=Tobramycin

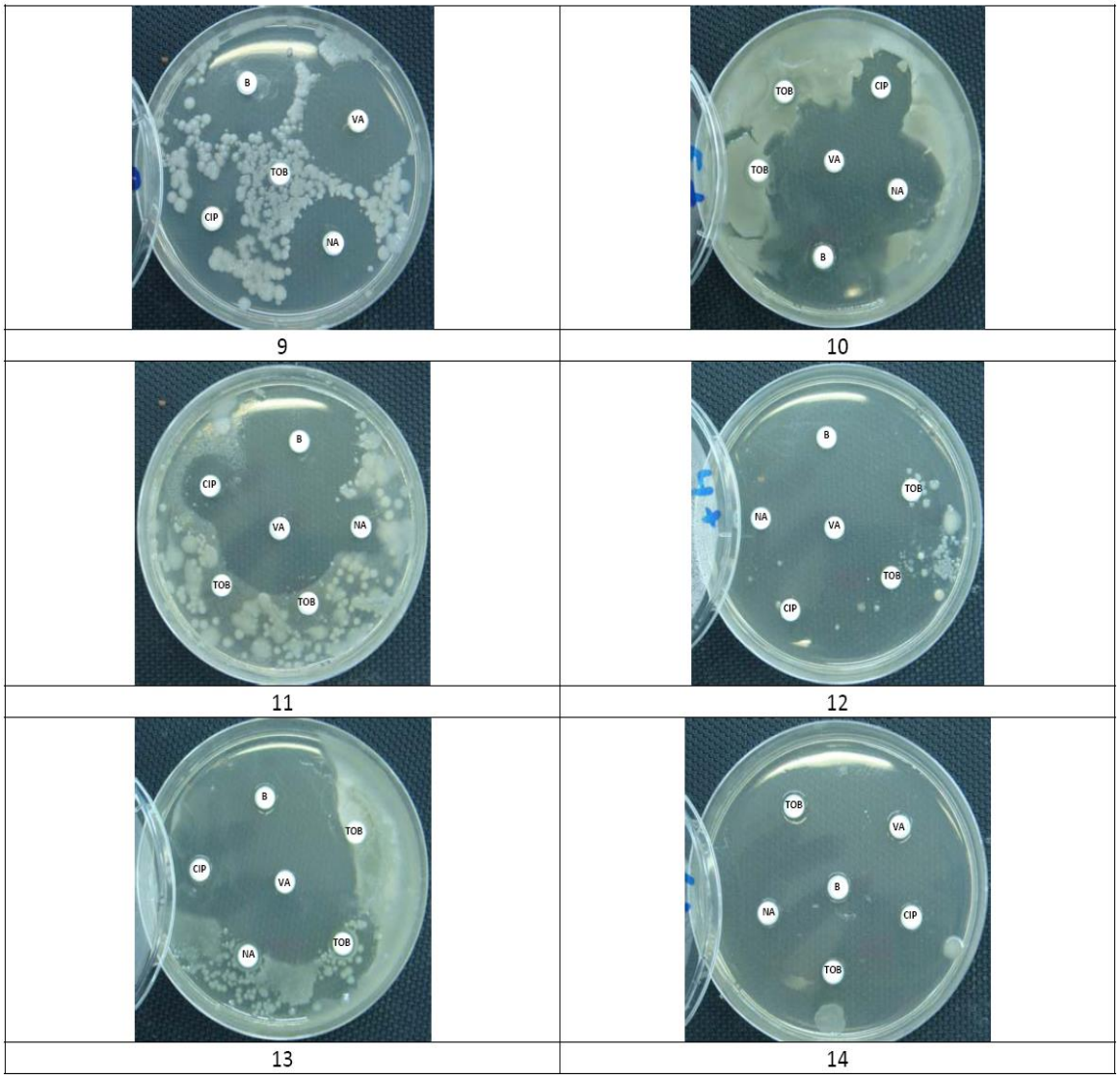

Figure (2): Antibiotics sensitivity to Six isolate of *Staphylococcusepidermidis* (9 12) from mercury filling; 13 14) from control.

B=Bacitracin; VA=Vancomycin;NA=Nalidixicacid;CIP=Ciprofloxacin;TOB=Tobramycin

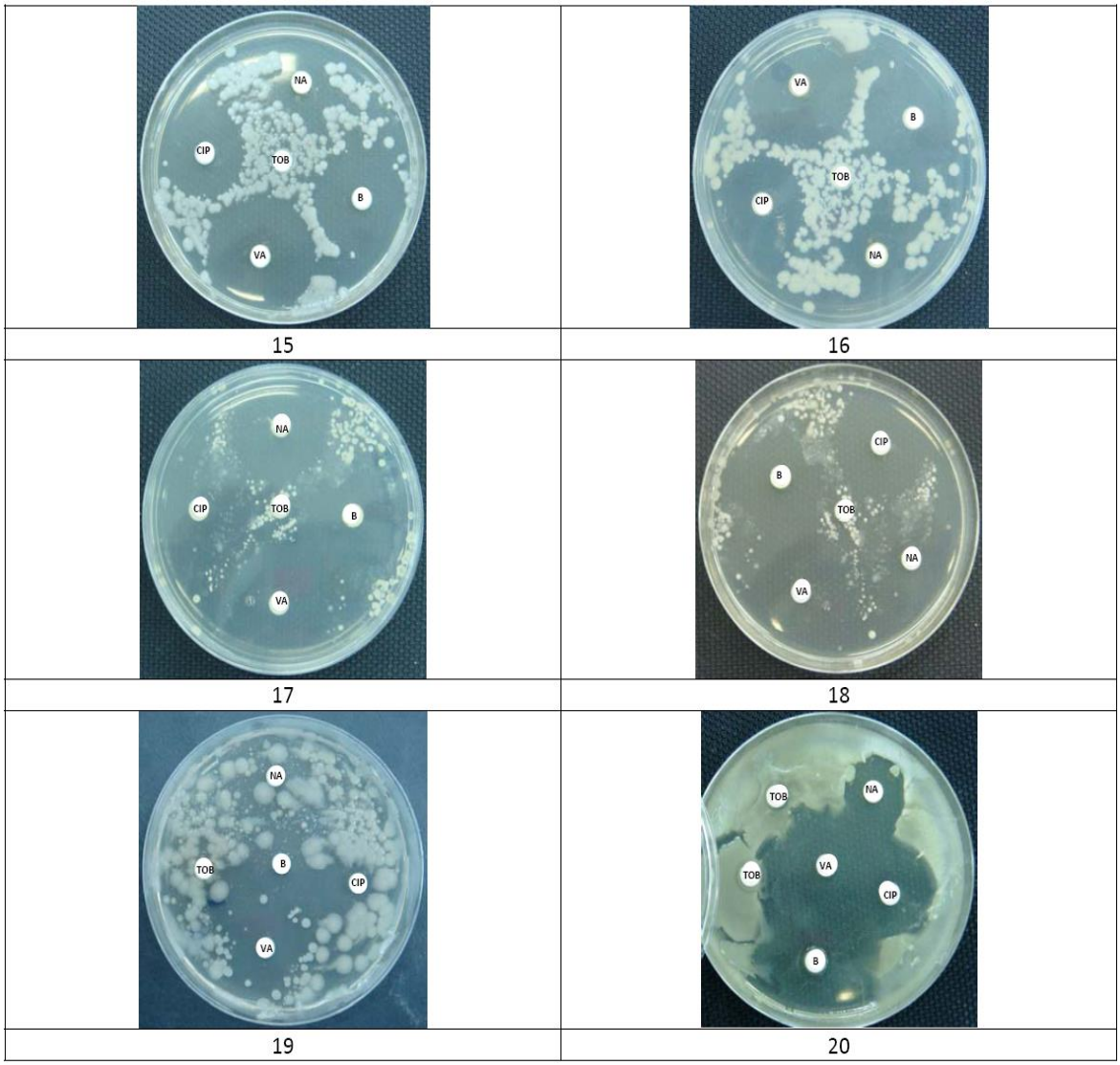

Figure (3): Antibiotics sensitivity to four isolates of *Enterococci* (15 18) and two isolates of *Streptococci* (19-20).

B=Bacitracin; VA=Vancomycin;NA=Nalidixicacid;CIP=Ciprofloxacin;TOB=Tobramycin

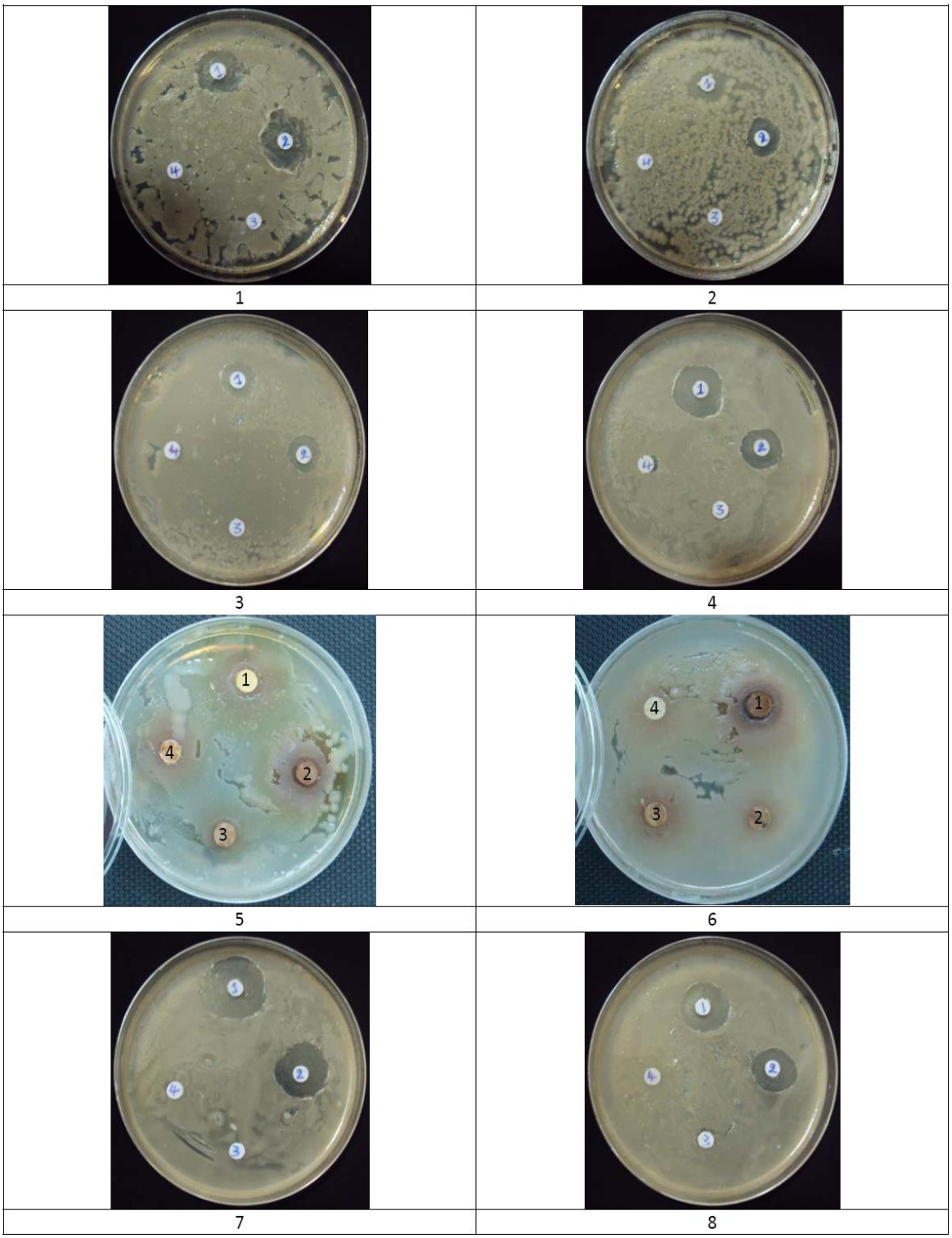

Figure (4): Inhibition zone sinduced by *Pimpinellaanisum* on *Staphylococcusaureus*.

1=Dislledwater; 2=Methanol; 3=Acetone; 4=Petroleumether

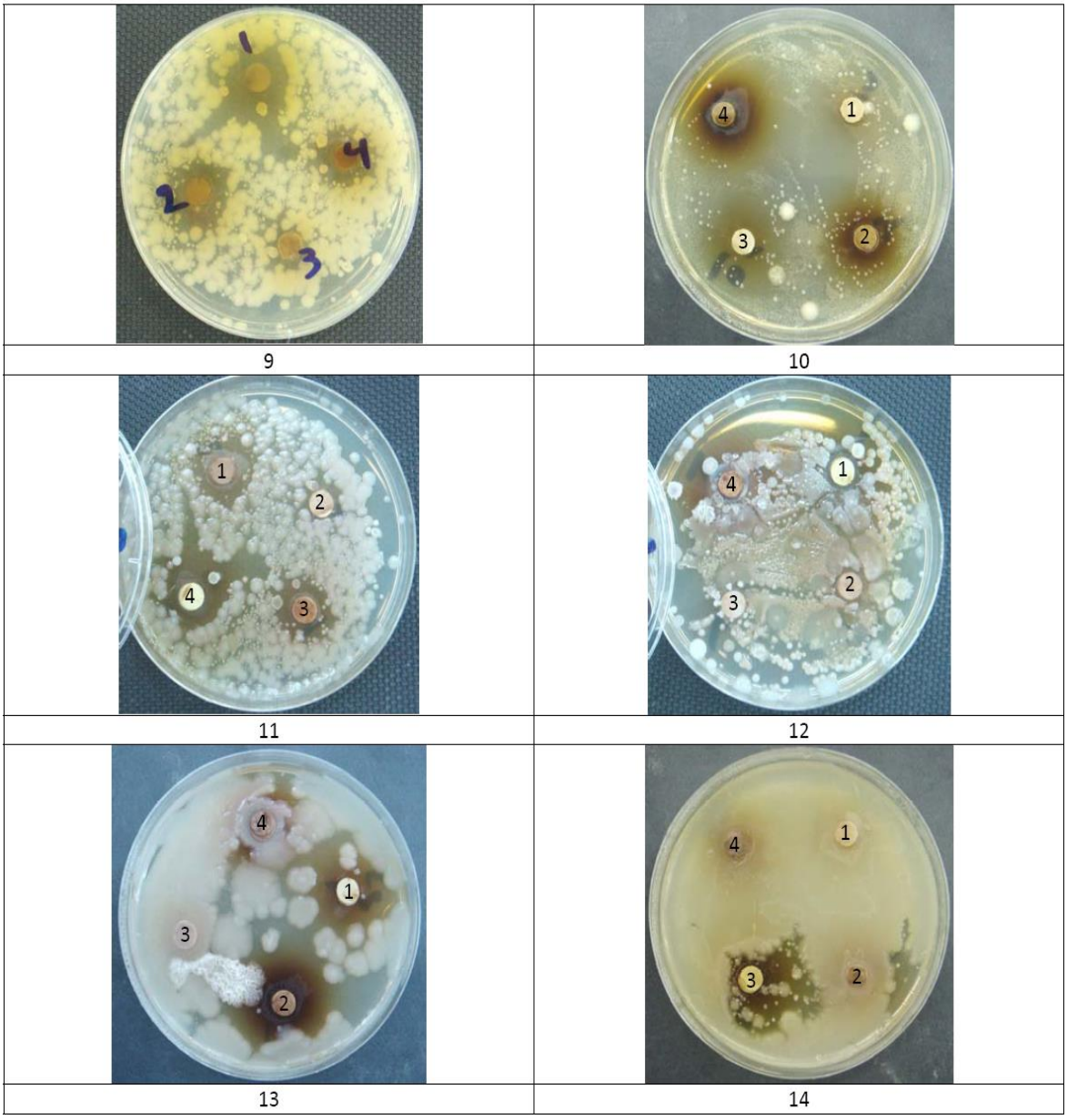

Figure (5): Inhibion zone sinduced by *Pimpinellaanisum* on six isolates of *Staphylococcusepidermidis*.

1=Dislledwater; 2=Methanol; 3=Acetone; 4=Petroleumether.

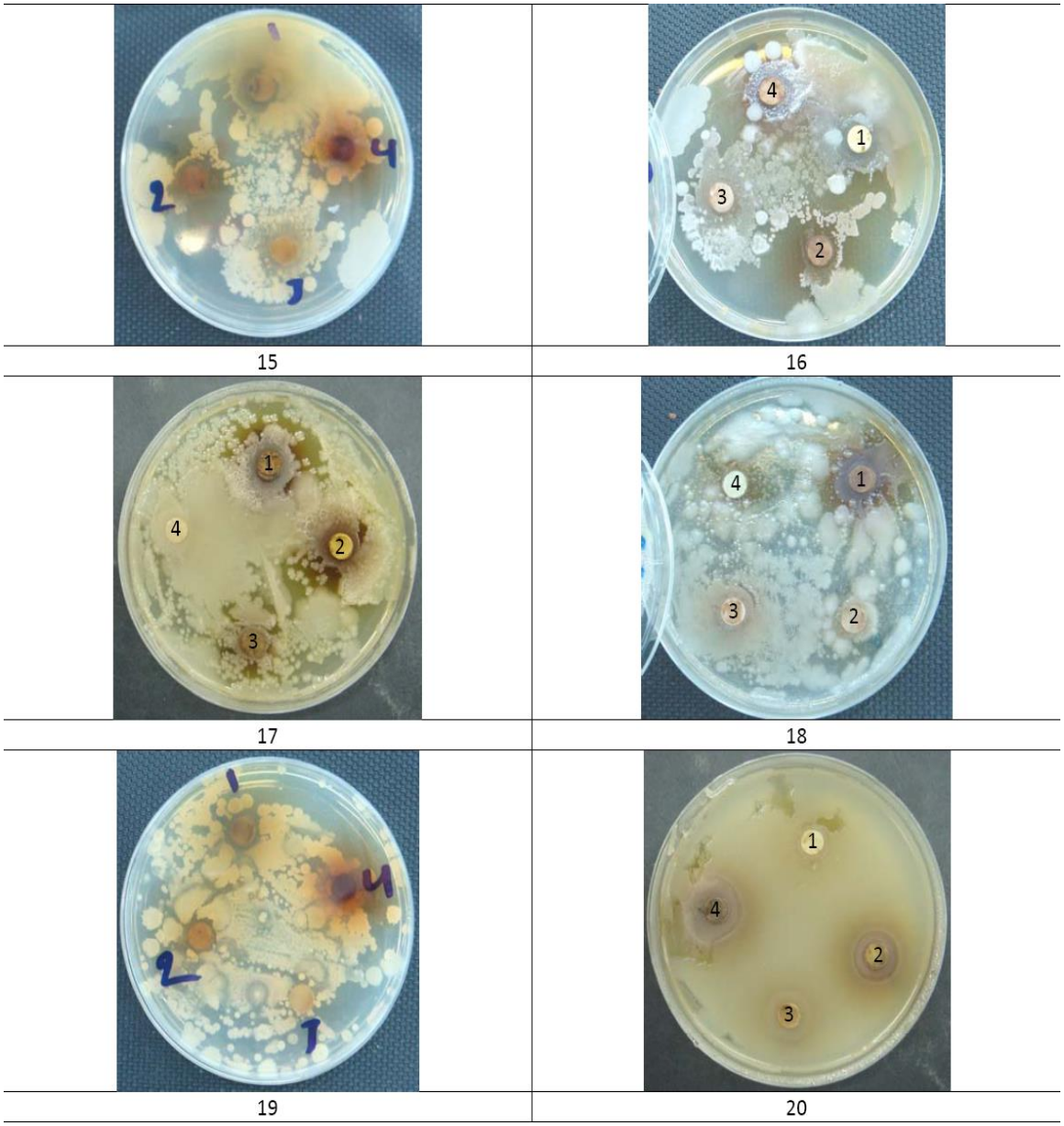

Figure (6): Inhibition zone sinduced by Pimpinellaanisumon Four isolates of *Enterococci* (15–18) and two isolates of *Streptococci* (19–20).

1=Dislledwater; 2=Methanol; 3=Acetone; 4=Petroleumether

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عزل وتشخيص بعض الأنواع البكتيرية من حشوات الأسنان الزئبقية والضوئية ودراسة الفعالية الضدجرثومية لمستخلص نبات الينسون *PimPinella anisum*

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الخلاصة

الغرض من الدراسة، هو التحري عن وجود بعض الأنواع الجرثومية مثل *staphylococcus aureus* المرضية و*staphylococcus epidermidis* و *streptococci* و *enterrococci* من حشوات الأسنان الزئبقية والضوئية في الأعمار المختلفة للذكور والإناث. أظهرت النتائج إن هذه الجراثيم هي أكثر شيوعًا في الذكور مقارنة مع الإناث ضمن الأعمار المختلفة والتي الدالة الحامضية لها متعادلة (6.8 – 7.0). كما تم دراسة الفعالية الضدجرثومية لنبات الينسون لبعض المضادات الحياتية قيد الدراسة بطريقة الانتشار ضمن الأقراص وطريقة التركيزالمثبط الادنى، أظهرت النتائج إن هذه العزلات حساسة للمستخلص المائي للينسون بالمقارنة مع الميثانول، الاسيتون والبتروليم أيثر، وكانت هذه العزلات أكثر حساسية للمضاد الحيوي vancomycin مقارنة مع بقية المضادات الحياتية.