جامعة القلمون الخاصة
كلية الصيدلة
قسم الكيمياء الحيوية والأحياء الدقيقة

التحري عن التلوث الجرثومي وتقييم فعالية المطهرات في مشفى في منطقة القلمون

Investigation of Microbial Contamination & Evaluation of Disinfectants Efficacy in Hospital In Alkalamoon Region

د. سندس ياسين
عميد كلية الصيدلة

- **Cleaning:** Physical removal of foreign material.
- **Decontamination:** The removal of disease-producing microorganisms to leave an item safe for further handling

- **Sterilization:** Complete elimination or destruction of all forms of microbial life accomplished in healthcare
- facilities by either physical or chemical processes.

- **Disinfection:** Cleaning some or all pathogenic organisms from an article of which may cause infection.

- **Antisepsis:** Chemical destruction of vegetative pathogens on living tissue.

Kills most vegetative bacteria, some viruses, some fungi. Microorganisms surviving (*Mycobacterium tuberculosis*, bacterial spore, some viruses, prions)

Quaternary Ammonium Compounds

Phenolic compounds

Kills all microorganisms unless extreme challenge or resistance exhibited. Microorganisms surviving (challenge of resistance bacterial spore, prions)

Ethylene oxide

Formaldehyde

Gluteraldehyde

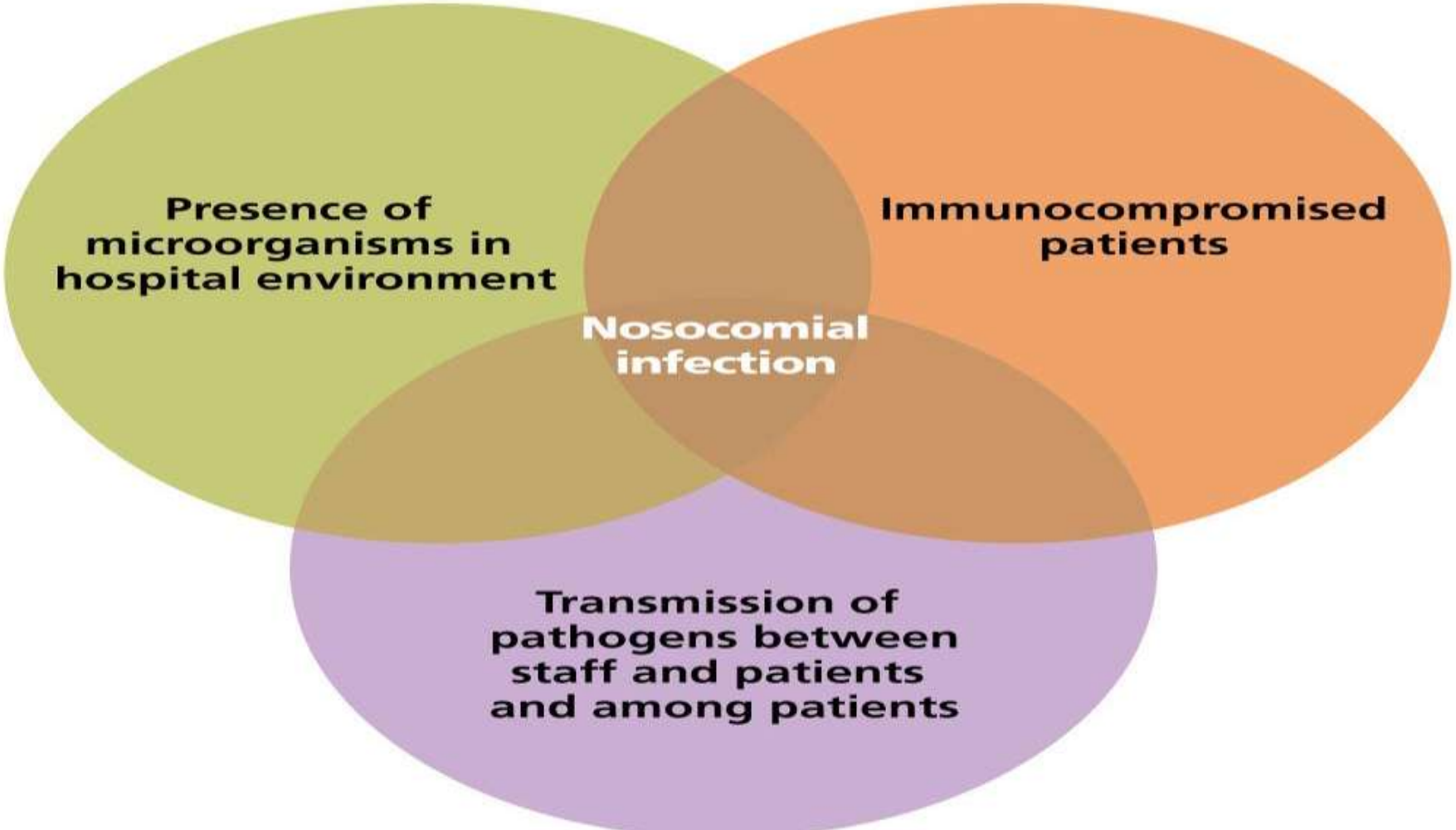
most viruses including hepatitis B virus (HBV), most fungi. Microorganisms surviving (bacterial spores, prions)

Alcohols

Hypochlorite

Iodine And Iodophors

Nosocomial Infection



2 in 10 hospitalized patients will acquire an infection

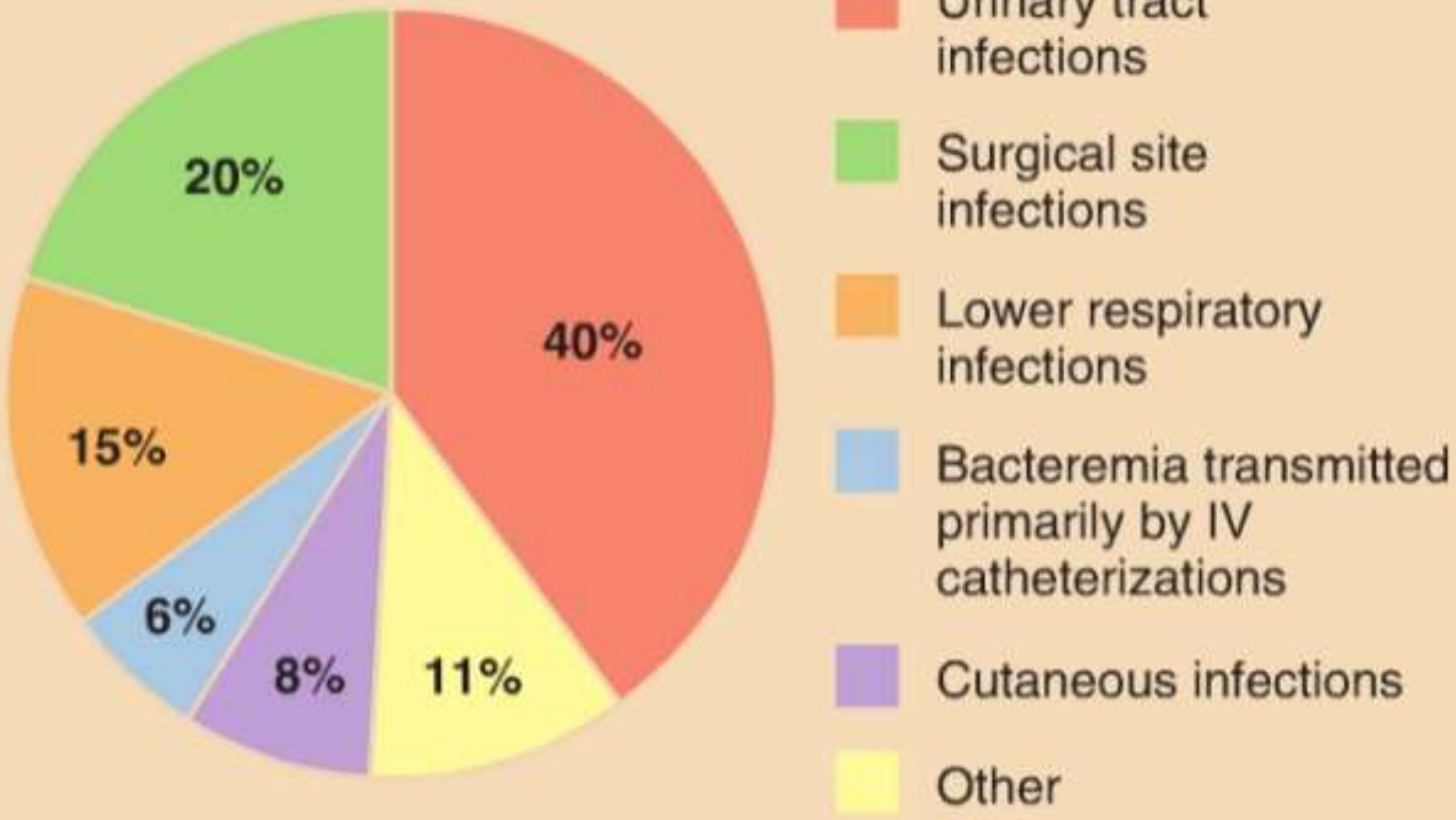
in 2013 the cost was $9.8 billion per year

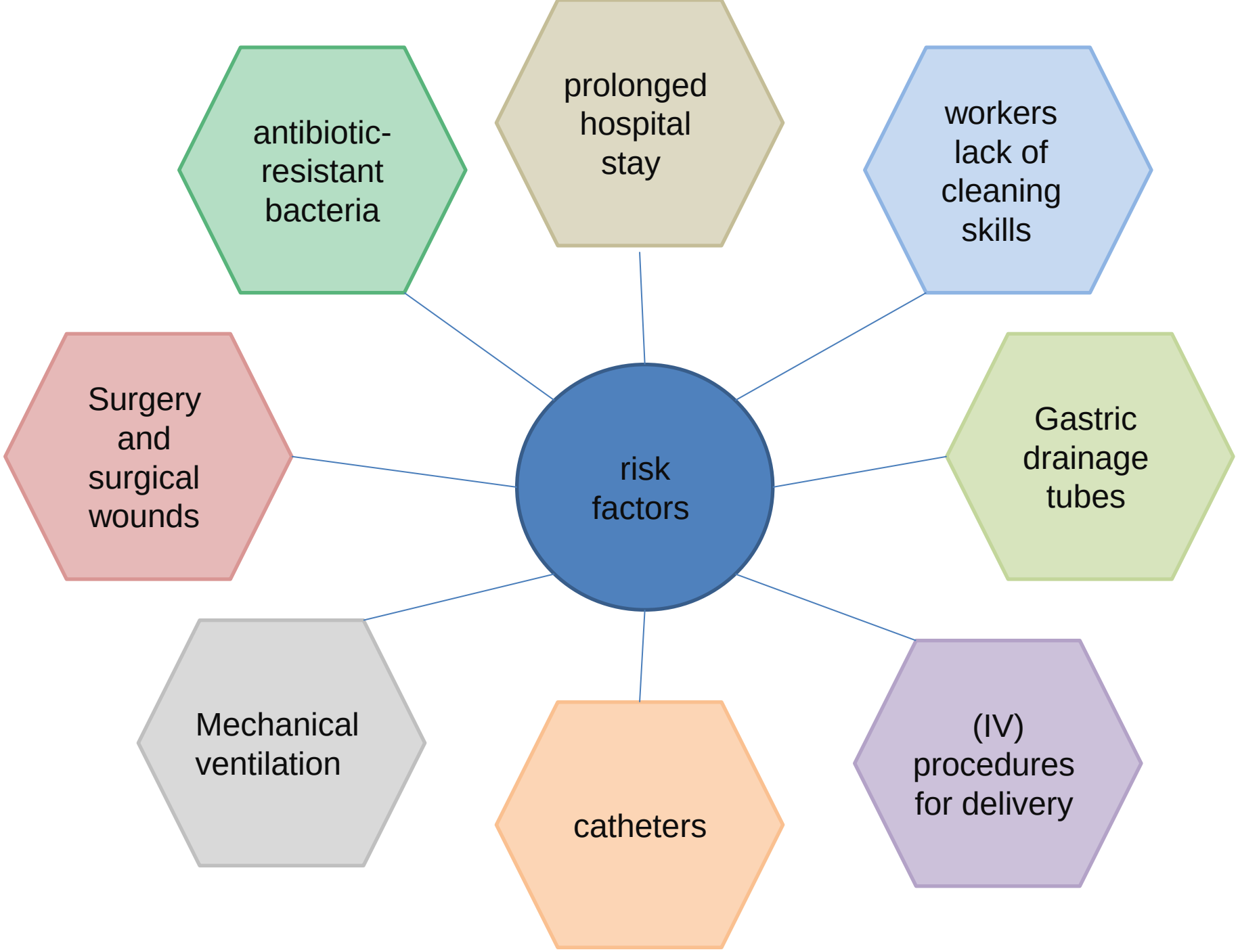
$45,814 Per patient

major cause of mortality world wide

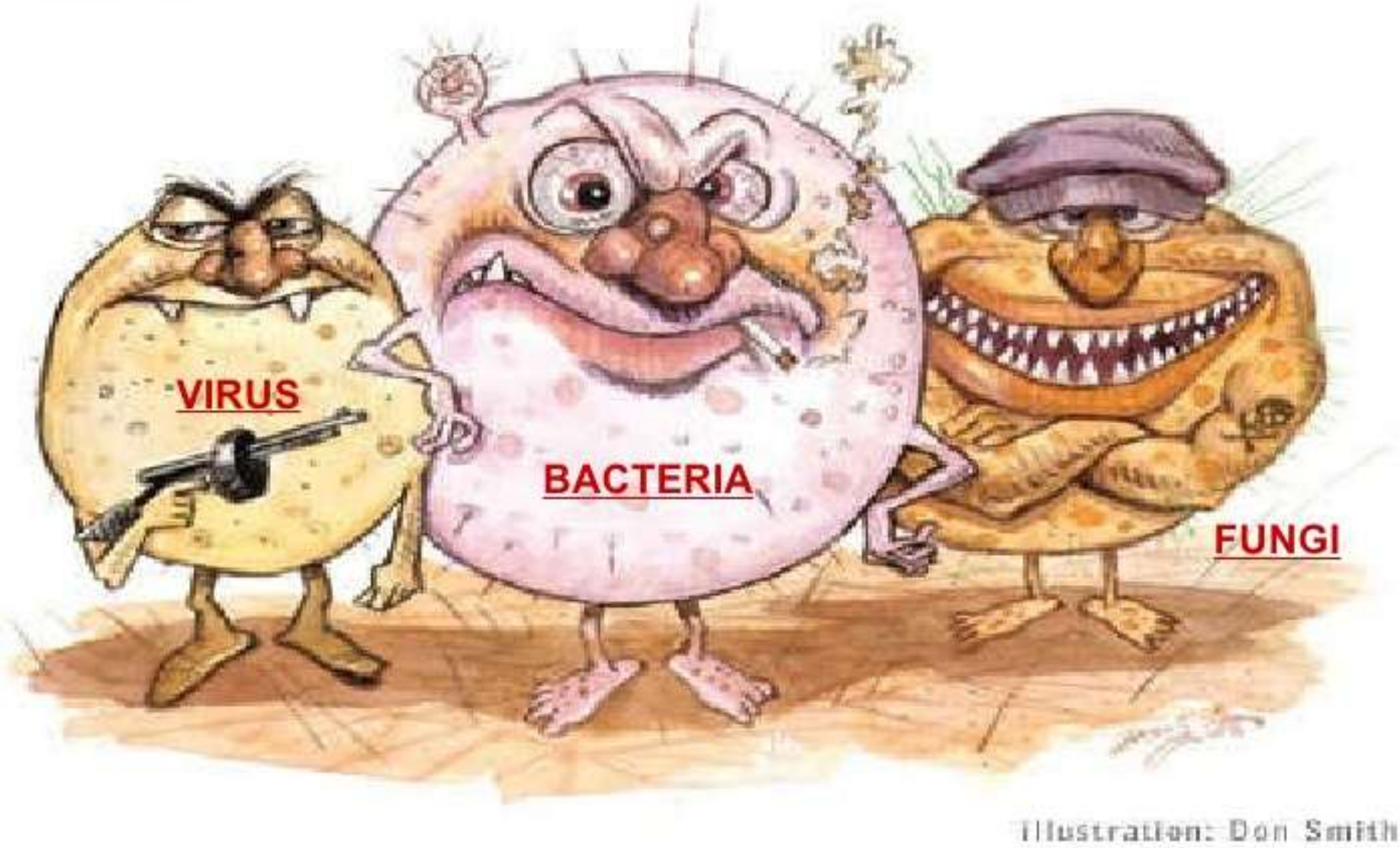
Nosocomial infections: most common sites

Source: Data from CDC, National Nosocomial Infection Surveillance.

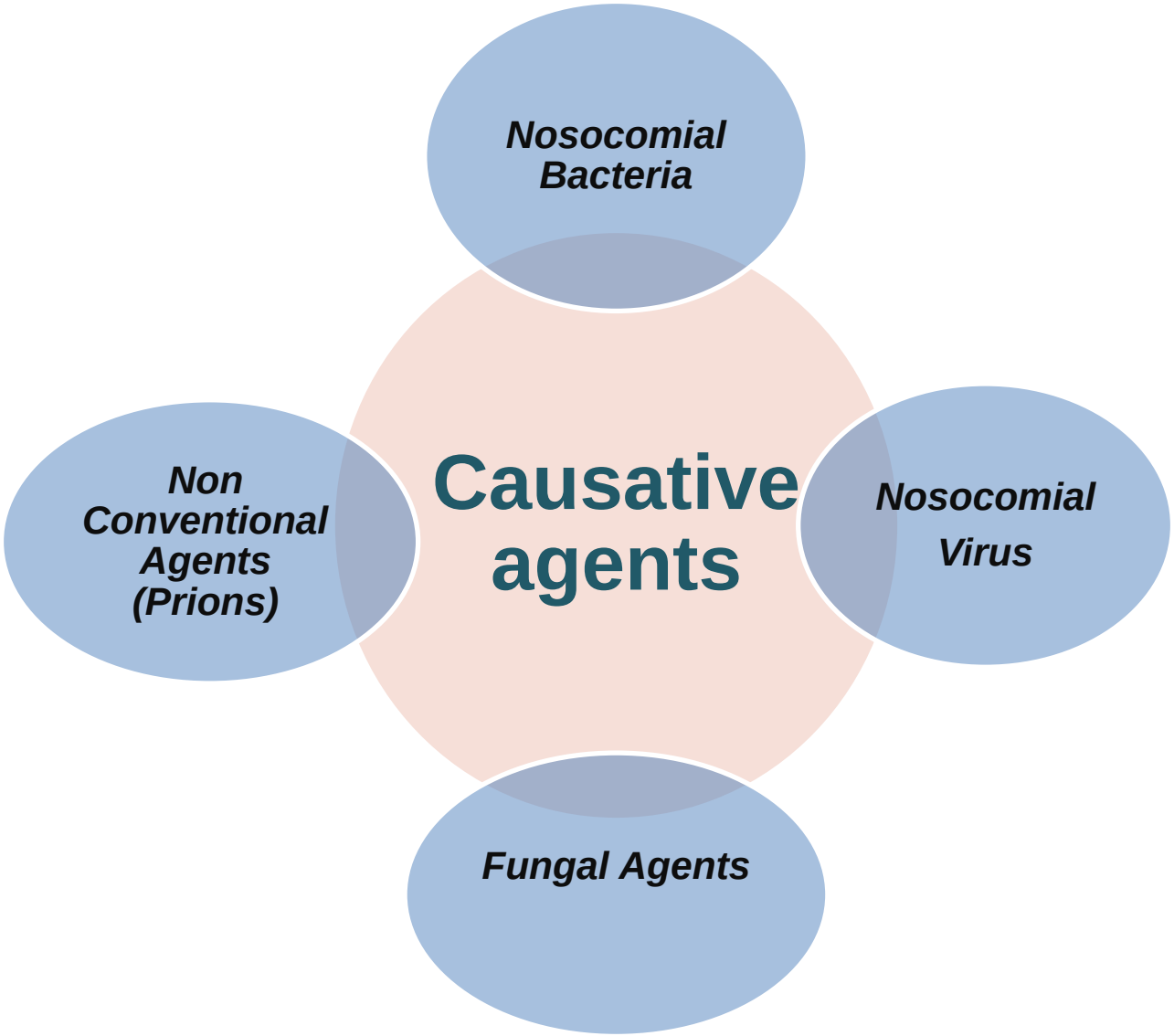



antibiotic-resistant bacteria
prolonged hospital stay
workers lack of cleaning skills
Surgery and surgical wounds
risk factors
Gastric drainage tubes
Mechanical ventilation
catheters
(IV) procedures for delivery

AGENTS OF NOSOCOMIAL INFECTIONS



Nosocomial infection agents



Aims of study

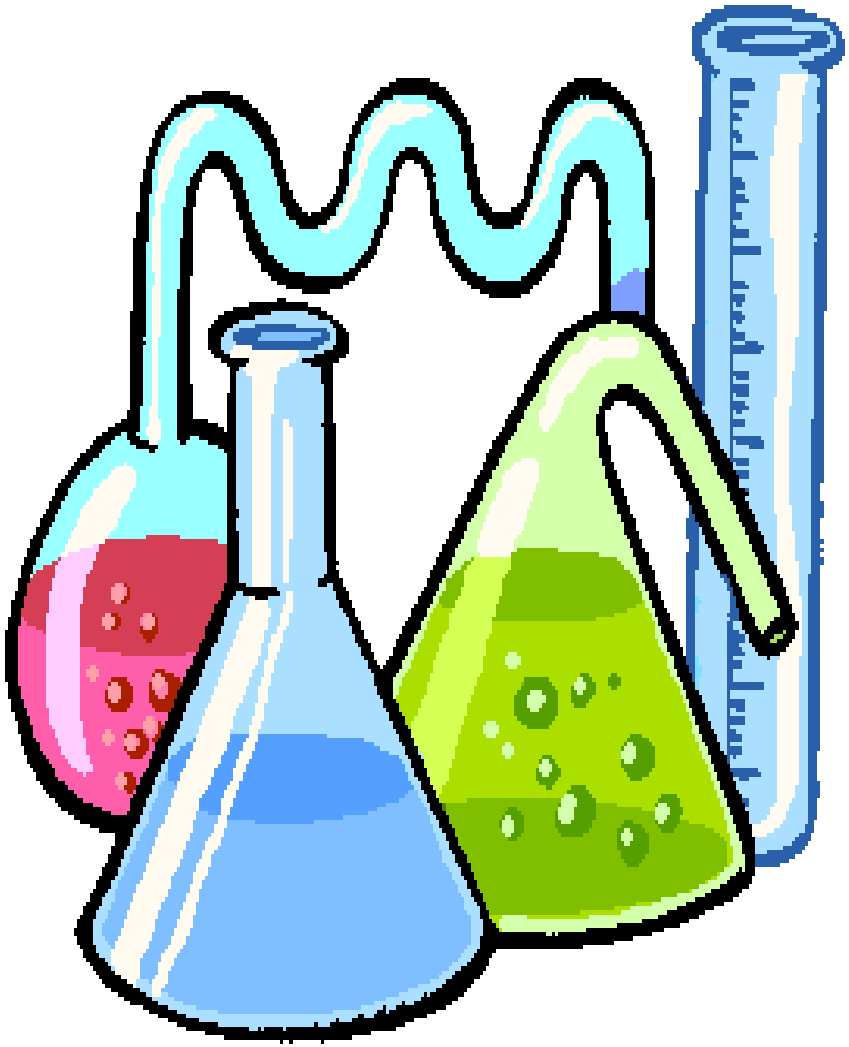
Isolation and identification of clinical common microorganisms in a hospital in Kalamoon region

Determination the sensitivity of the isolates *in vitro* against different types of disinfectants

Evaluate the microbiological Efficacy of used disinfectants

Determination (MIC) in vitro

Comparing our results with global studies



MATERIALS & METHODS

❖ Time And Place Of Specimens Collection:

- (120)samples
- December – february (2016-2017)
- Samples were collected from :

A hospital in Al-Kalamoon region.

Operating room

Intensive care

Incubators

Dental clinics

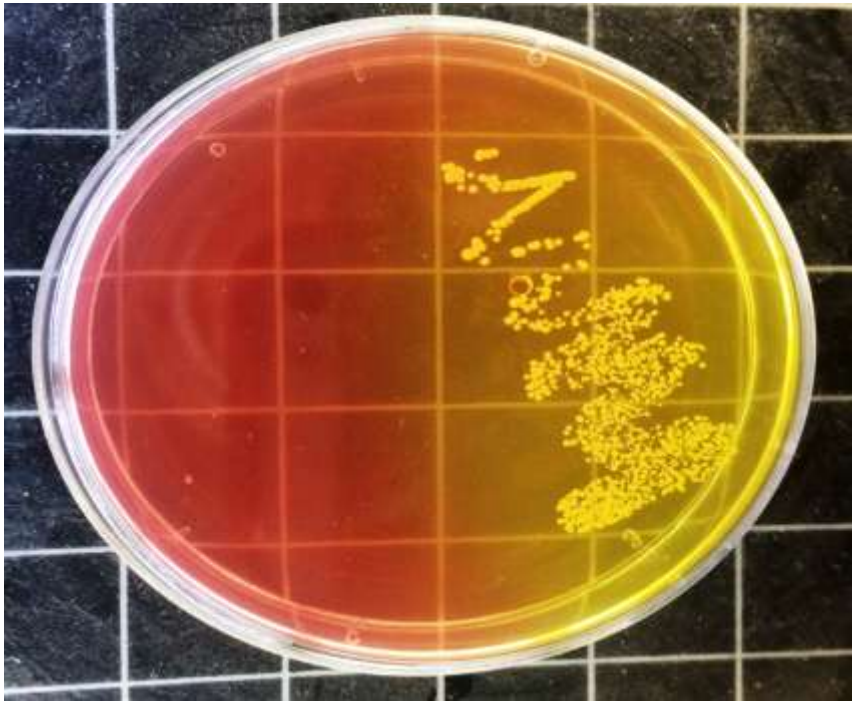
Culture media

Nutrient Agar

Blood Agar

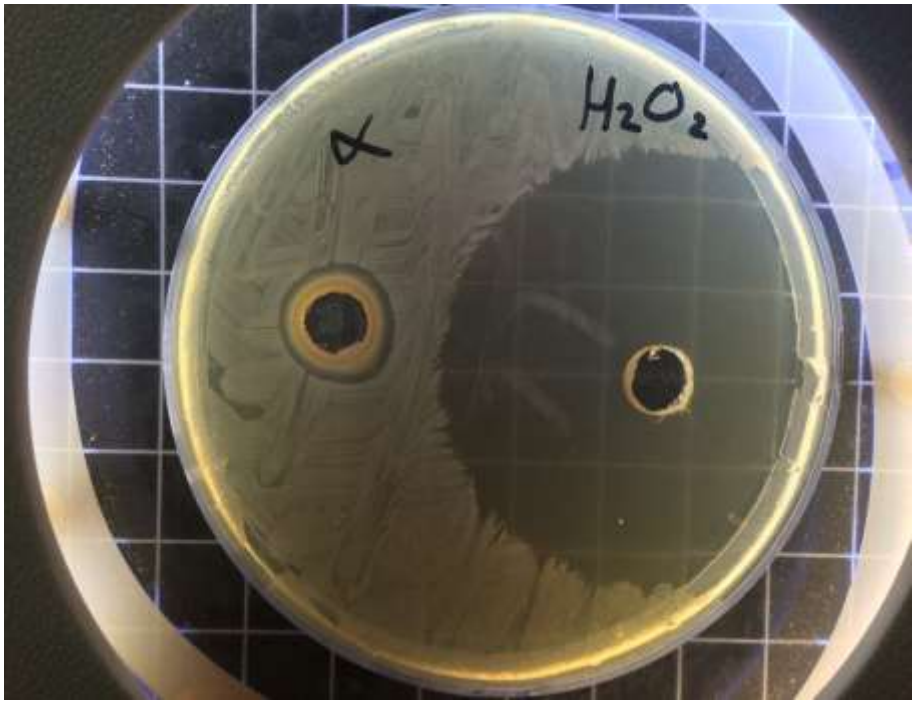
Culture media

EMB agar



MSA agar

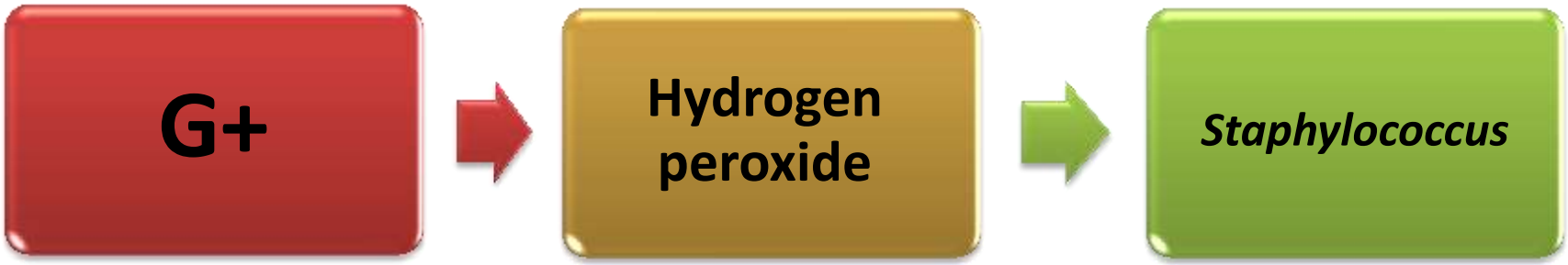
Culture media

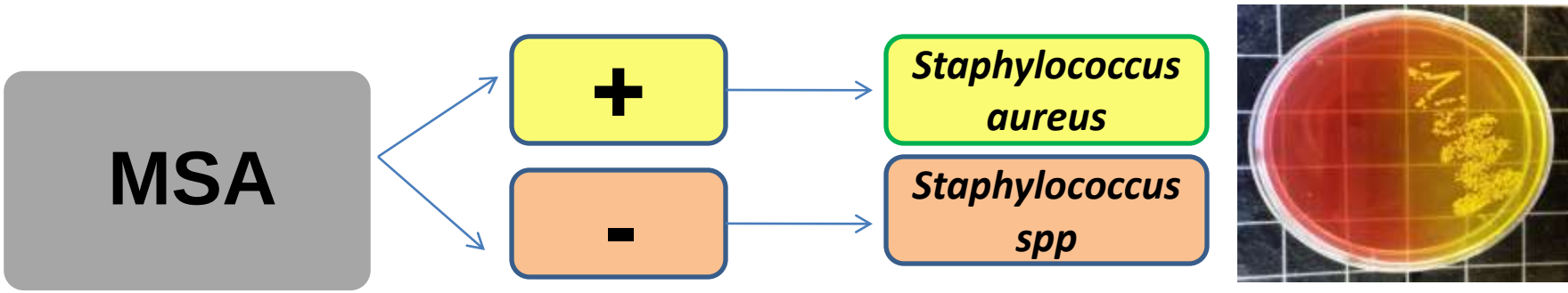


SDA agar

Mueller-Hinton agar

Identification of Gram positive

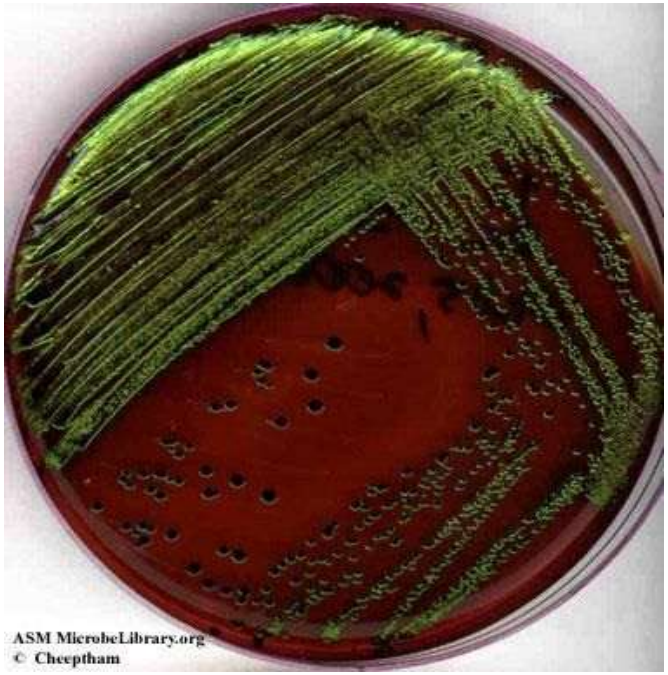




Identification of Gram Negative

❖ **Colony morphogy:**

E.coli



(metallic Green sheen)

Proteus

(Swarming)

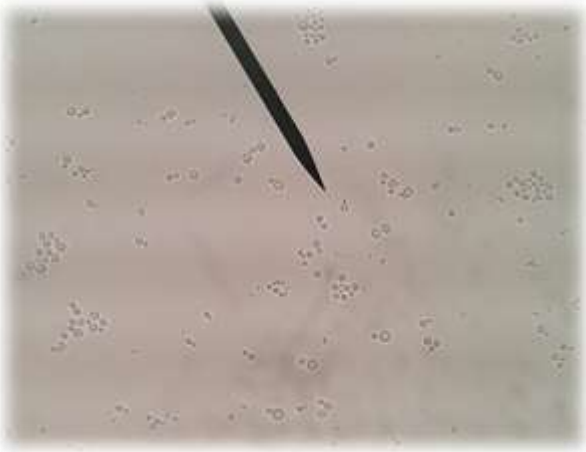
Identification of candida albicans

Candida cultures

+

0.5 mL of human serum

Incubate at 37º C
for 2 hours



biochemical tests

Urease test

- **Positive:** ***Proteus***

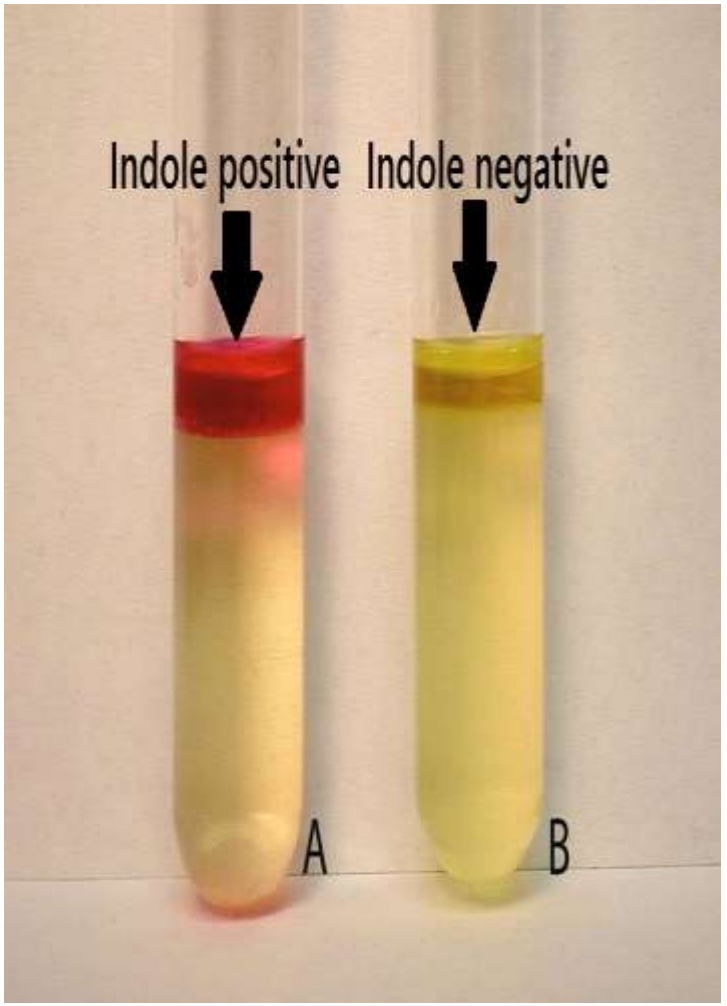
(Bright pink).

- **Negative:** ***Klebsiella***

(orange yellow color).

I M V C tests
Indole test

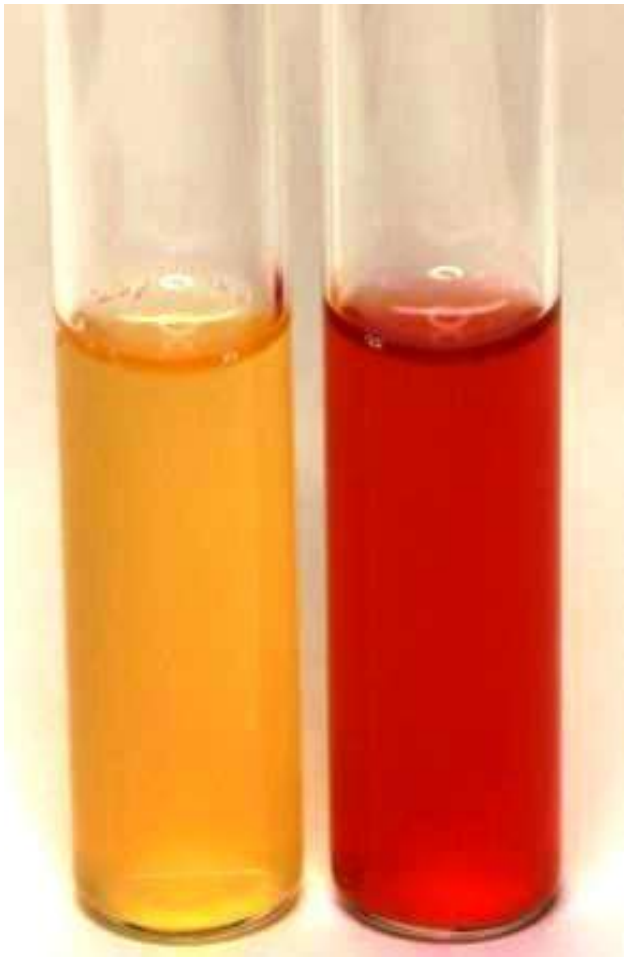
- **Positive:** ***Proteus***
- (red or red-violet color in the surface alcohol layer of the broth.)



- **Negative:** ***Klebsiella***
- (No color change , remain yellow).

Methyl red test

Some bacteria produce large amount of acid from glucose fermentation that they overcome the buffering action of the system pH=4.4 or less



MR test positive: ***Escherichia coli***

appearance of (red color after the addition of methyl red reagent.)

MR test Negative : ***klebsiella***

(the culture medium remains yellow.)

Voges-Proskauer test

Positive : ***Klebsiella***

(cherry red color)

Negative : ***Escherichia coli***

(yellow-brown color)

Citrate test

- Citrate agar is used to test an organism's ability to utilize citrate as a source of energy.

Positive: *Klebsiella*

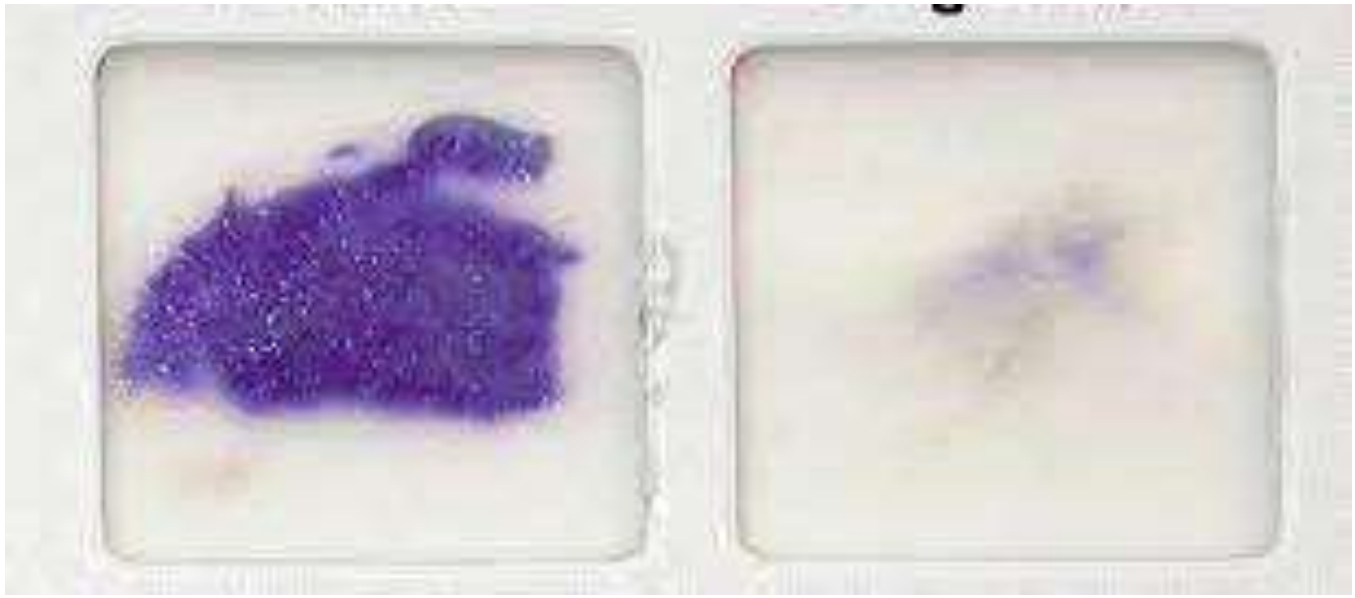
(change from the original **green** color to **blue**)

Negative: *Escherichia coli*

(No color change)

Oxidase test

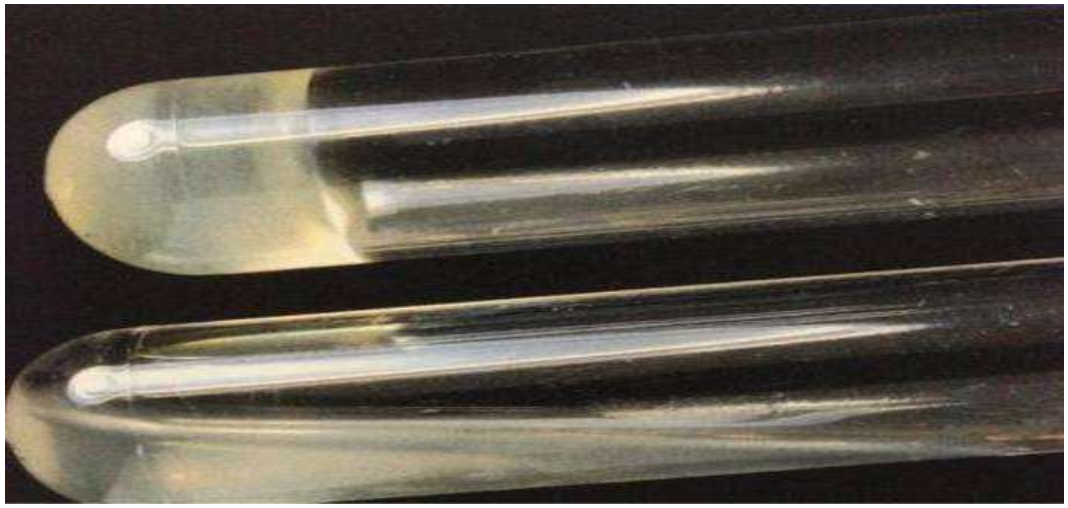
- It used for the differentiation of ***Pseudomonas*.**



Coagulase test

- Coagulase test is used to differentiate *Staphylococcus aureus* (positive) from Coagulase Negative *Staphylococcus*

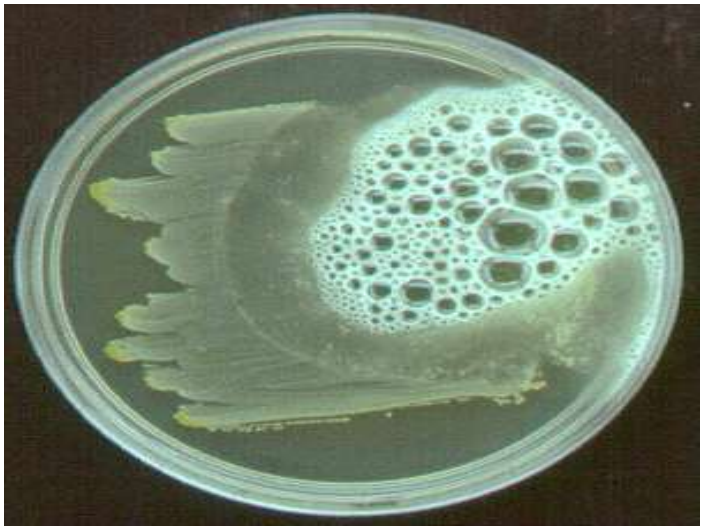
- **Positive(clot) : *Staphylococcus aureus***
- **Negative(no clot): *Staphylococcus spp.***



Catalase test

- $H_2O_2 \xrightarrow{\text{catalase}} H_2O + O_2$

- **Positive*:*** ***Staphylococcus*** (bubble formation)
- **Negative :** No bubble formation (no catalase enzyme to hydrolyze the hydrogen peroxide)



disinfectants used in the hospital in al-kalamoon region

Delta Guard

operating room

(2% in at use concentration)

intensive care

(2% in at use concentration)

dental clinics

(1% in at use concentration)

Alpha Guard GF

Department of incubators

Plus H2O2

Composition of these disinfectants

Delta Guard

- **Glutardialdehyde (8 gr/100gr)**
- **Didecyldimethylammonium chloride (3 gr/100gr)**
- **Benzalkonium chloride (5 gr/100gr)**

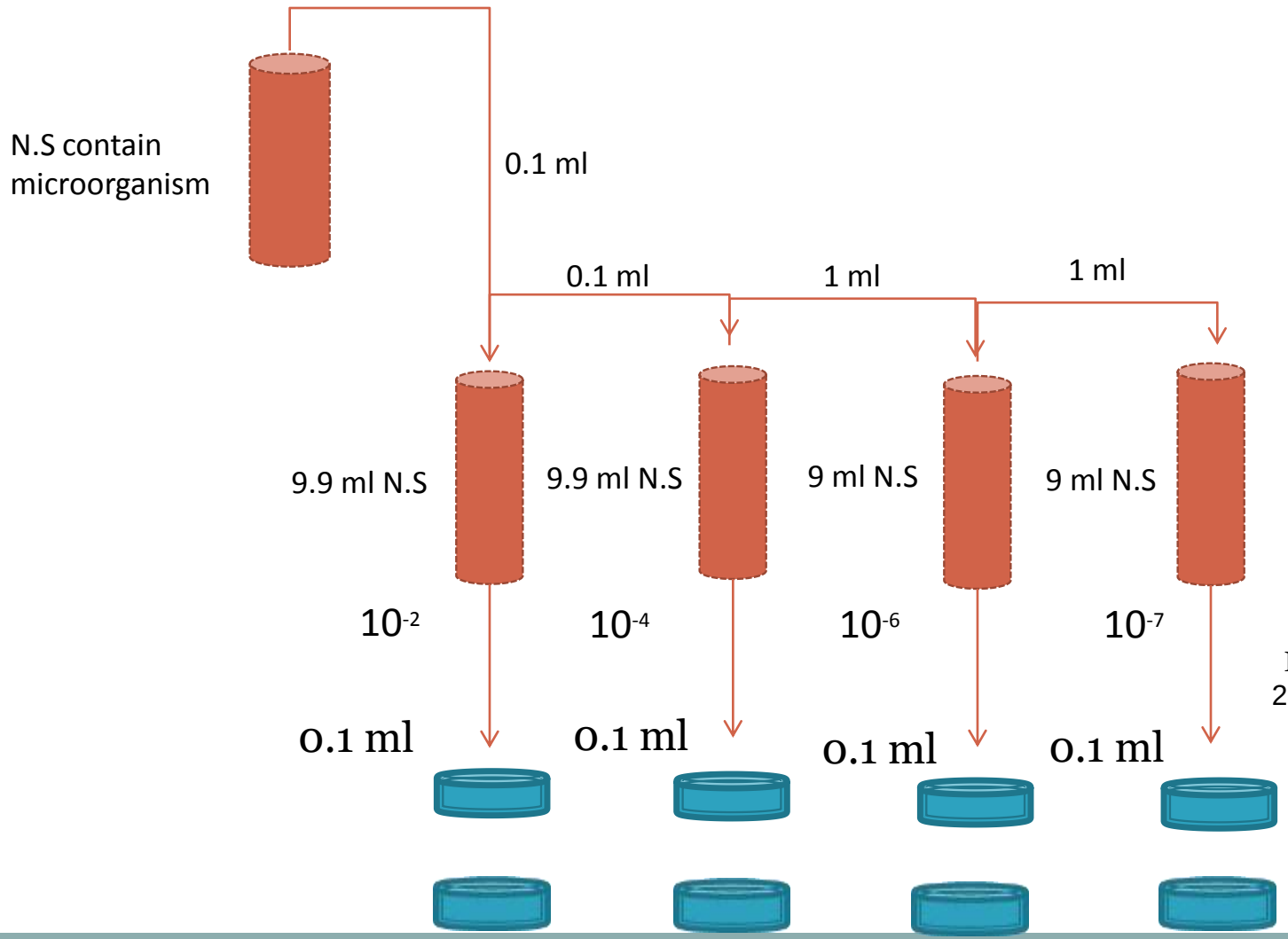
Alpha Guard GF

- **Didecyldimethylammonium chloride (0.84 gr/100gr)**
- **PolyhexamethylenBiguanid - hydrochloride (0.2 gr/100gr)**

Detect the final PH of disinfectants at in use concentration by PH meter

- PH was calibrated by fresh and unused buffer , then PH electrode was rinsed by distilled water.
- PH electrode was dipped into the testing disinfectants.
- The PH was determined when the PH reading was stable .

Inoculums preparations



Incubated petri dishes for 24 hours at 37c and for 48 hours at 25c for bacteria and yeast growth respectively

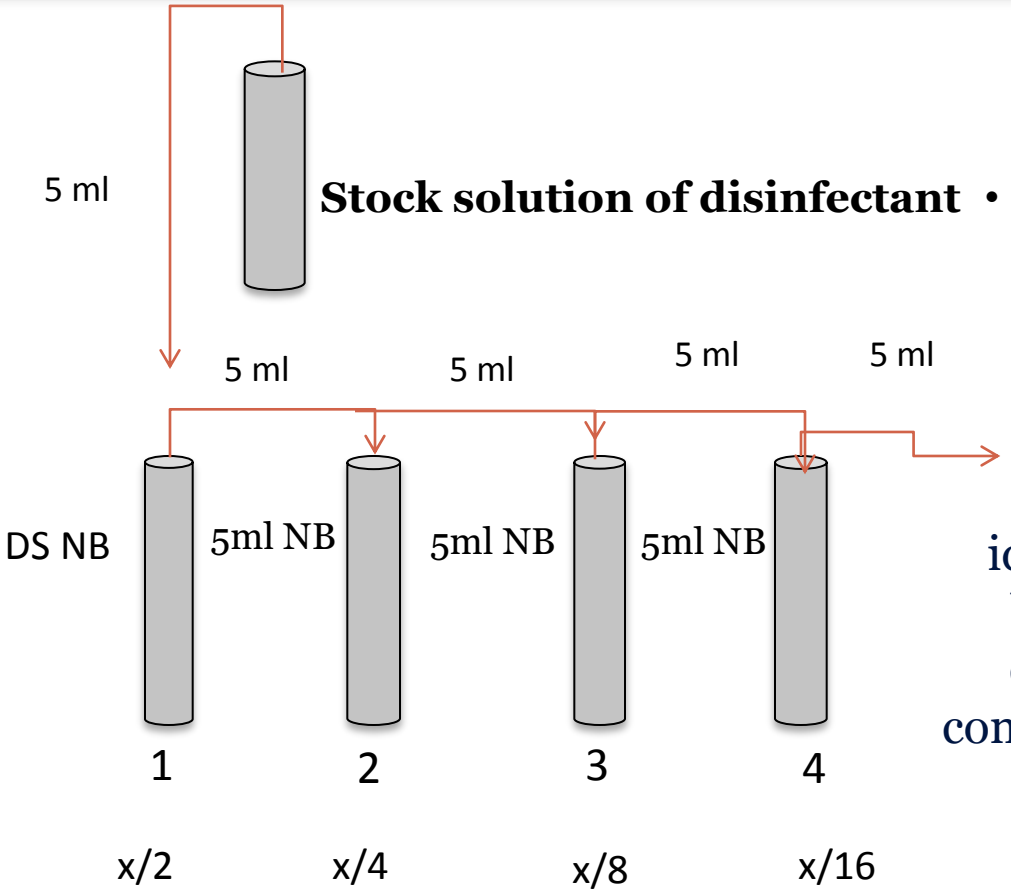
(30-300)

Well diffusion method

Different bacterial suspension was spread on the surface of Mueller-Hinton agar plates , then disinfectants was applied to wells in the plates.

All plates were incubated on 37° c for 24 hours .

Determine the minimum inhibitory concentration (MIC)

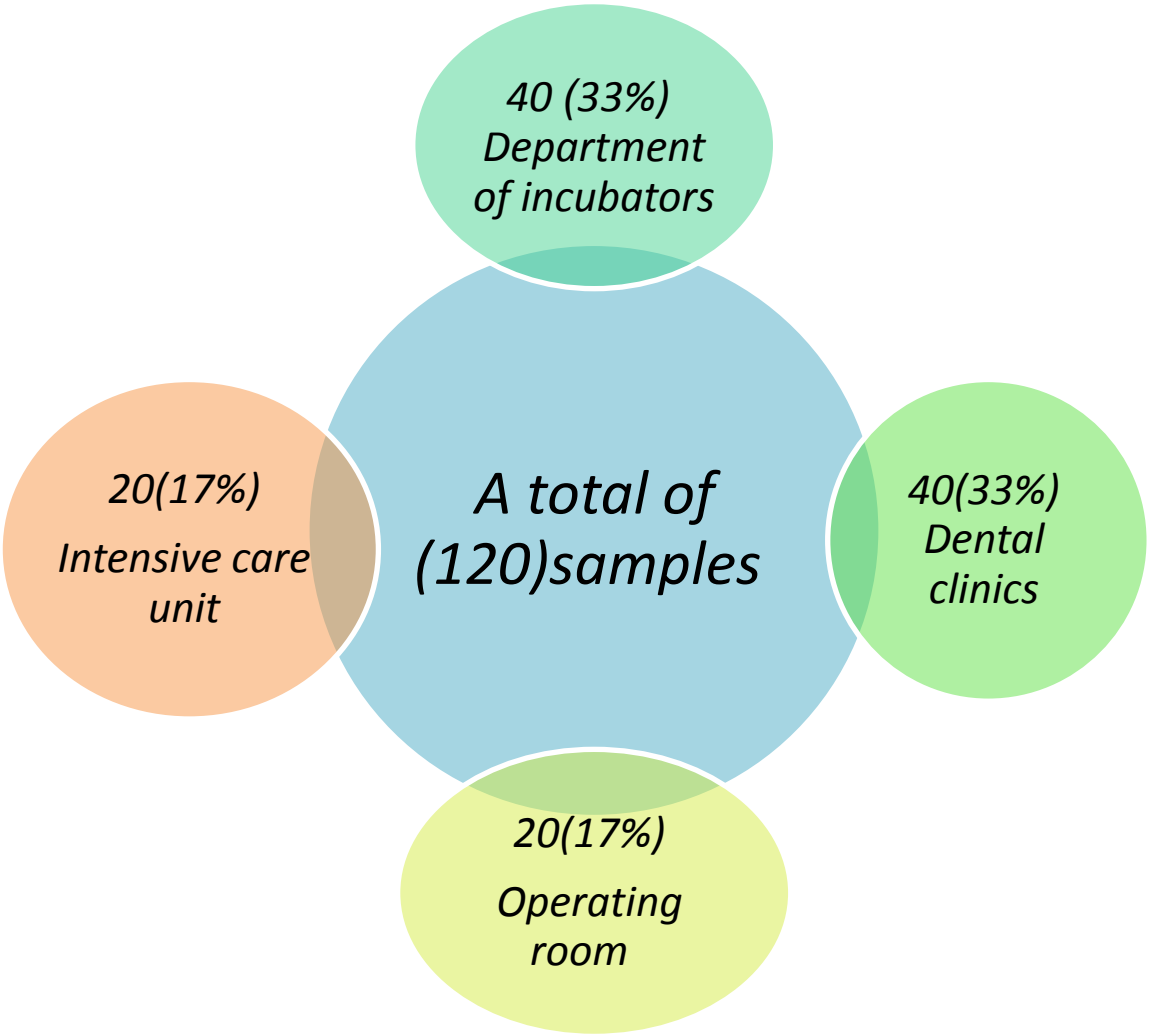


identical doses (0.1 ml) of bacteria and fungi were cultured in (5 ml) broth containing progressively lower concentrations of the disinfectants.

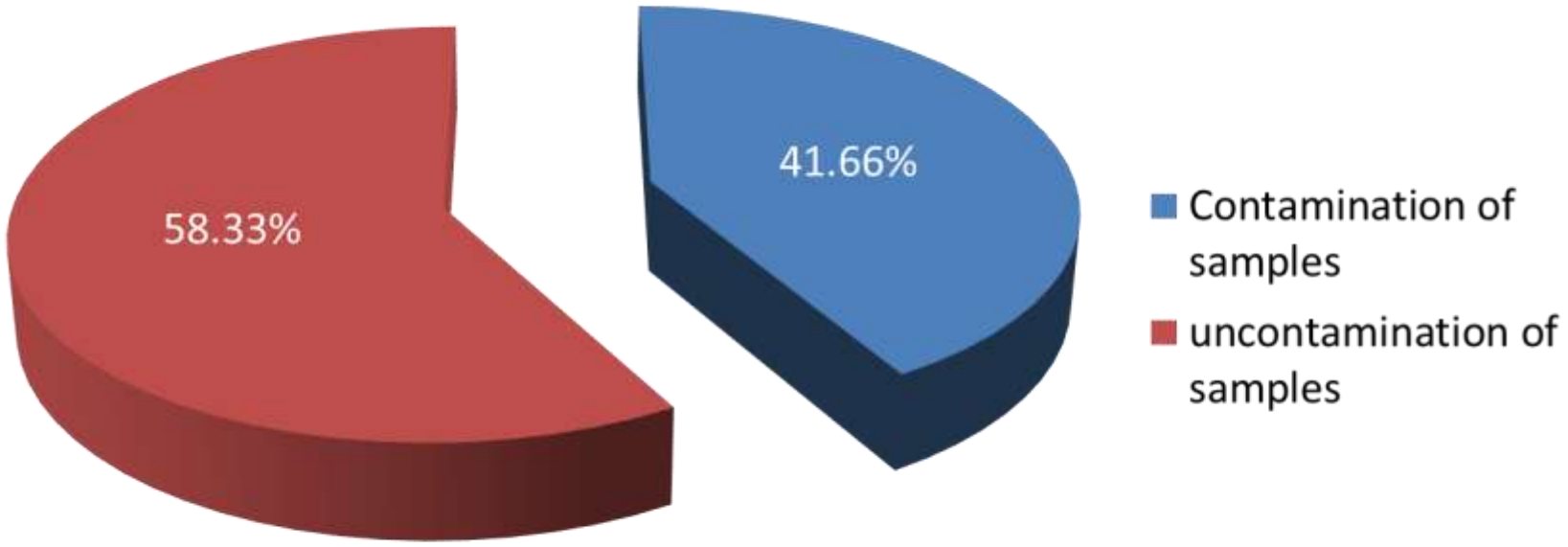
(the tubes incubated at 37c for 24 hours)


Results

Studying samples :

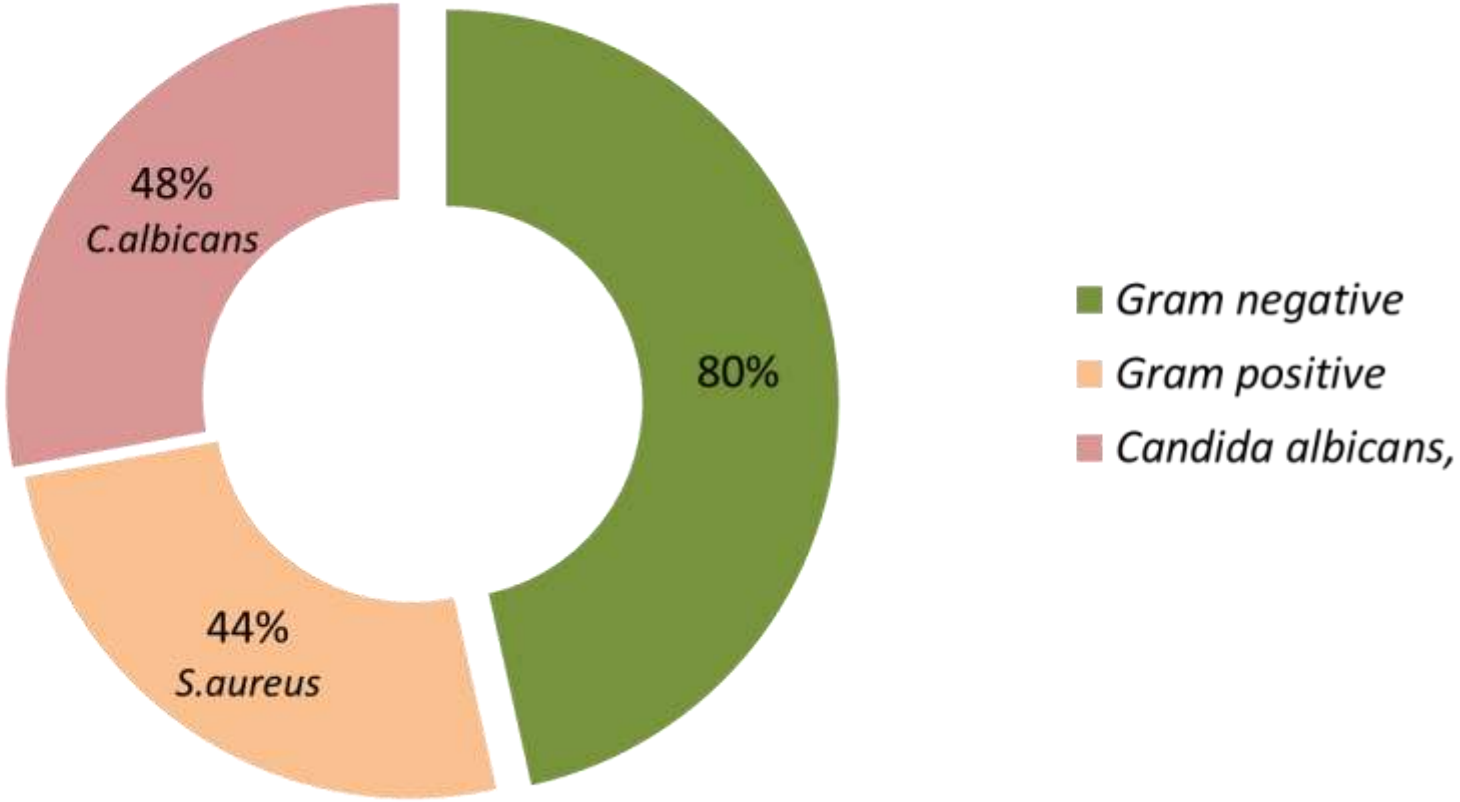


From 120 samples 50 (41.66%) were contaminated and 70 (58.33%) were uncontaminated samples



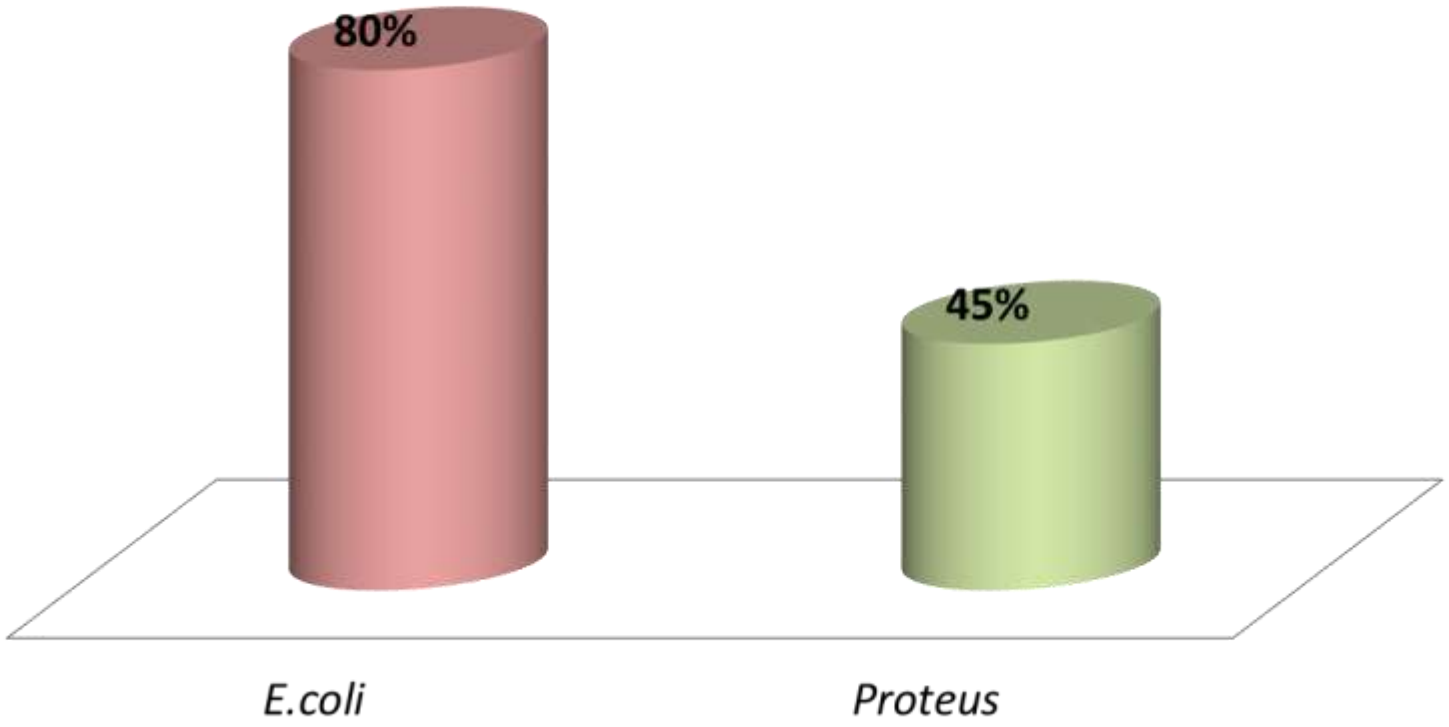
The percentage of contaminated and uncontaminated samples.

From 50 (41.66%) contaminated samples, 40 (80%)samples were contaminated by *Gram negative* , 22 (44%) samples by *Gram positive* and 24 (48%) samples by *Candida albicans*,



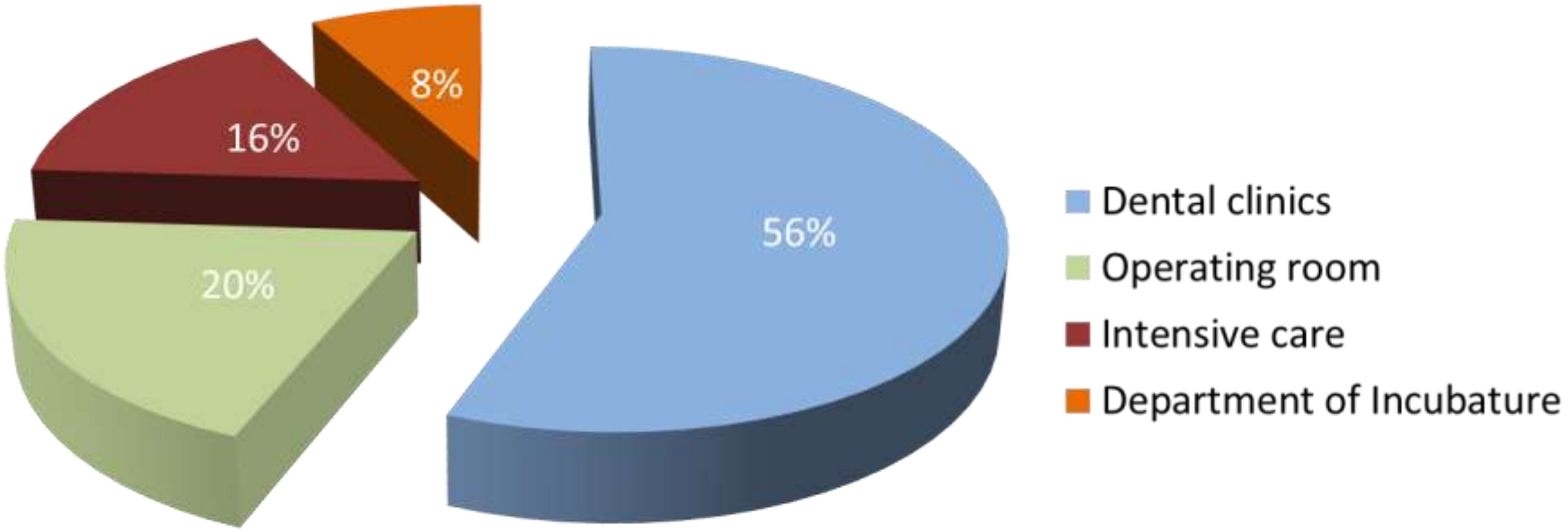
isolation rate of Gram negative and Gram positive from all departments in hospital.

From 40 (80%)contaminated samples,32(80%) samples were contaminated by *E.coli*, and 18 (45%) contaminated by *Proteus*.



Gram negative isolated from all departments in hospital

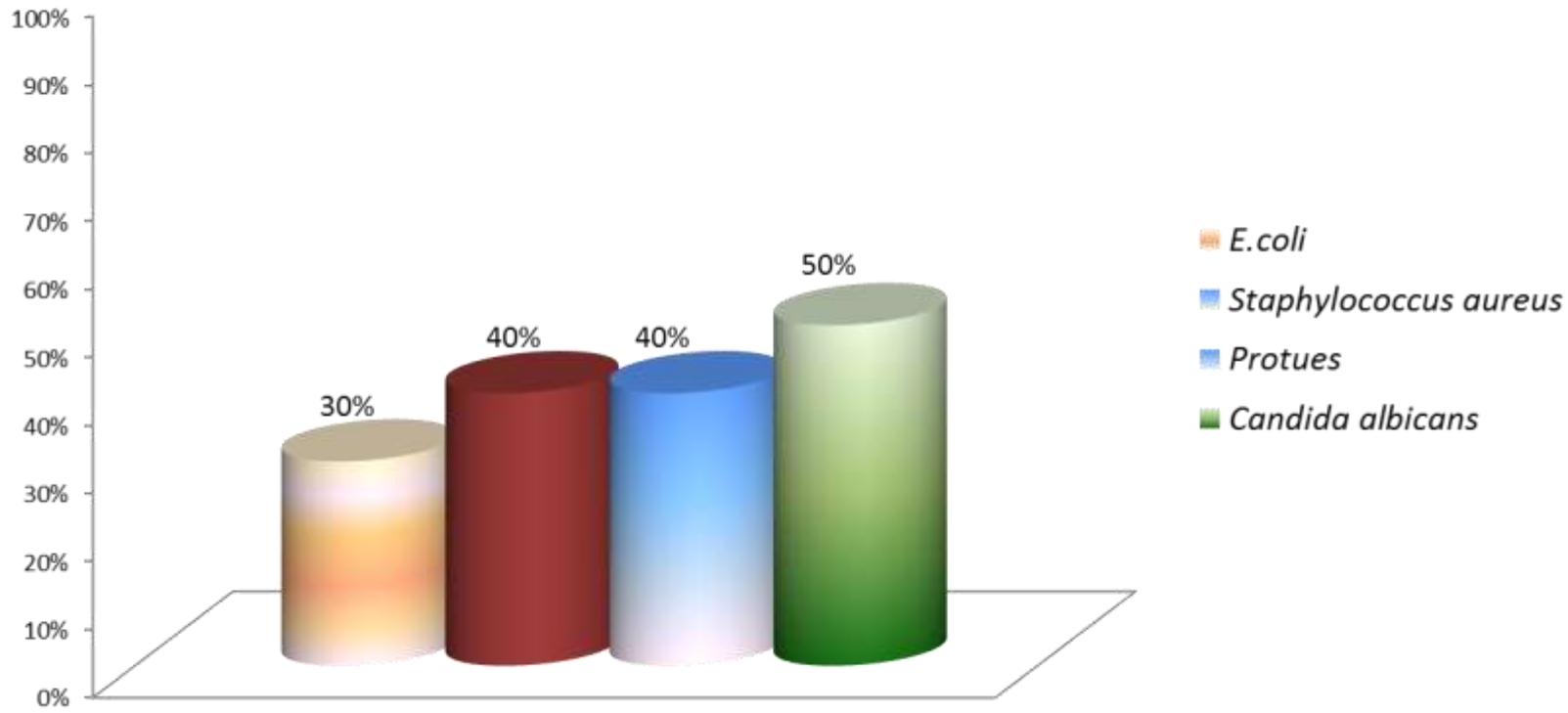
From 50(41.66%) contaminated samples, 24(56%) were from dental clinics ,10(20%) from operating room , 8(16%) from intensive care, 4(8%) from department of incubature.



The percentage of contaminants in each department

❖*In operating room :*

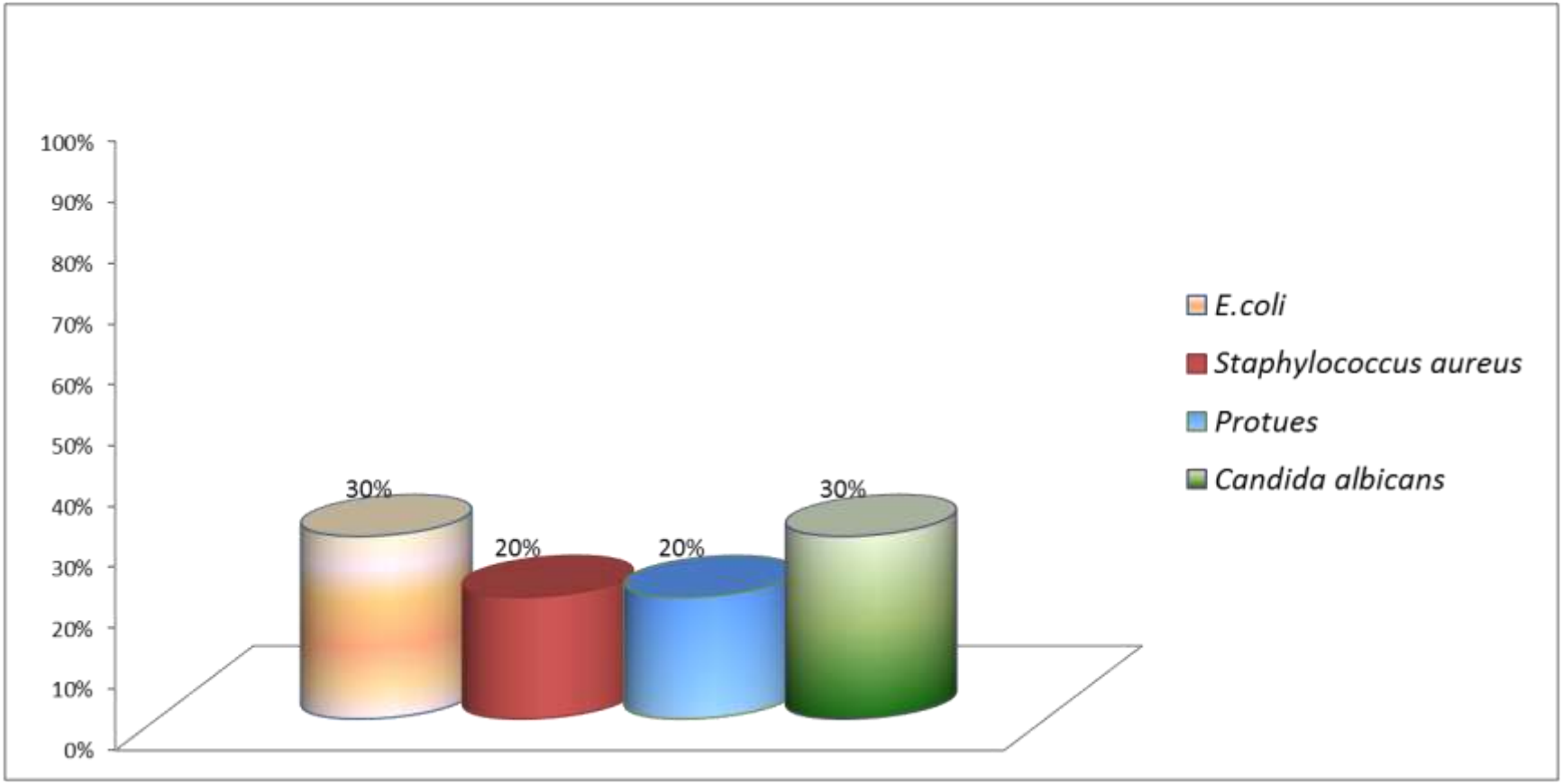
Among 20 (17%) samples ,6 (30%) were contaminated by Escherichia coli ,8 (40%) Staphylococcus aureus,8 (40%) Proteus and 10(50%) Candida albicans.



The percentage of contaminated samples In operating room

❖ In intensive care:

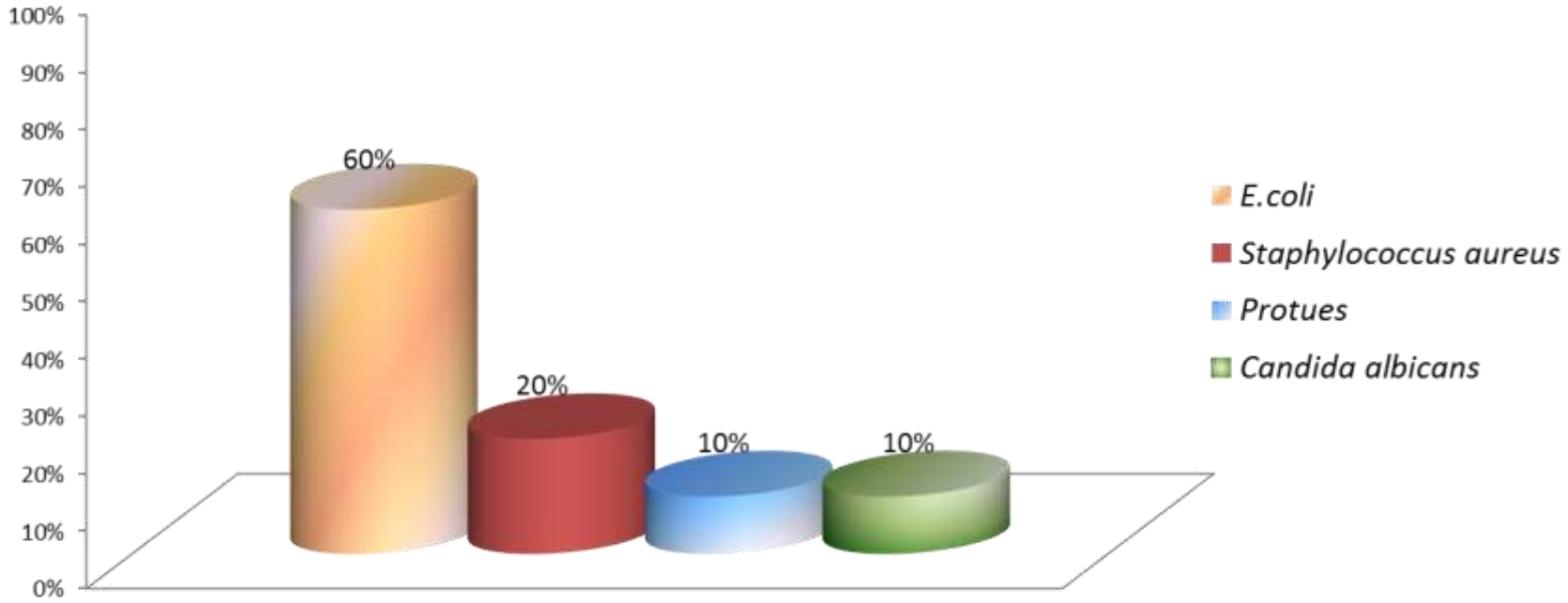
Among 20 (17%) samples ,6(30%) were contaminated by Escherichia coli ,4(20%) Staphylococcus aureus, 4(20%) Proteus and 6 (30%) Candida albicans.



The percentage of contaminated samples In intensive care

❖In dental clinics:

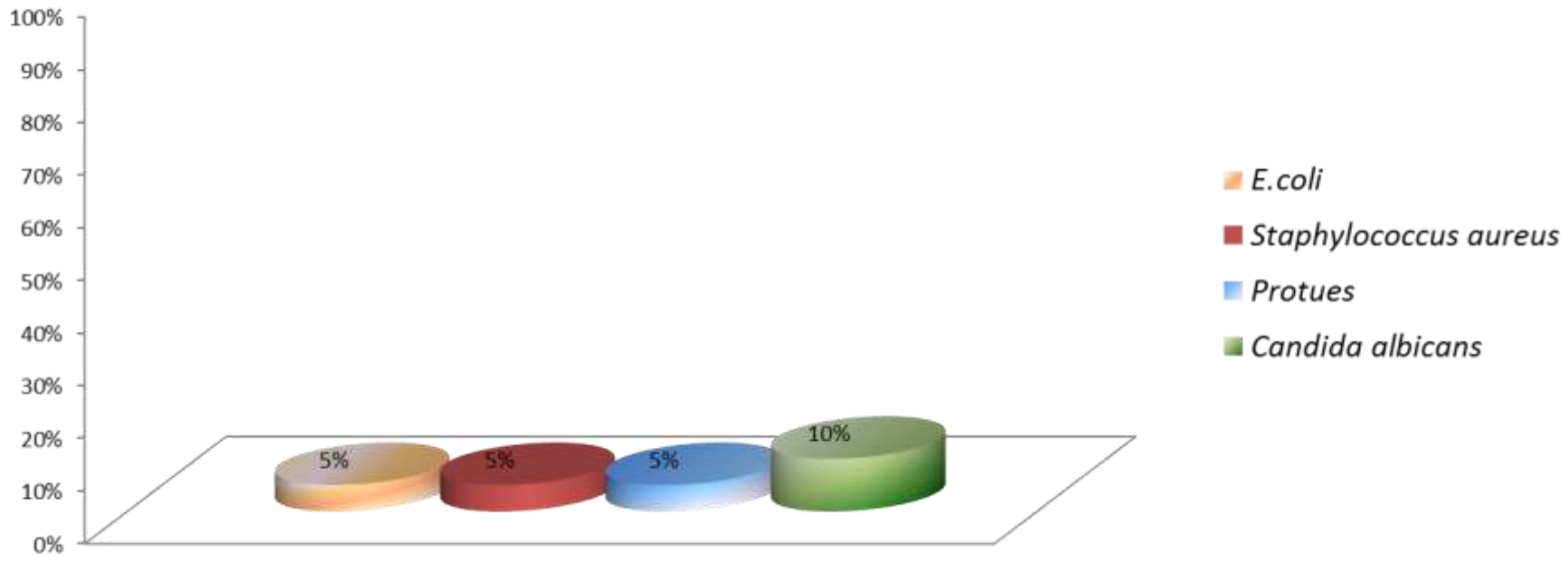
Among 40(33%) samples ,24(60%) were contaminated by Escherichia coli ,8(20%) Staphylococcus aureus, 4(10%) Proteus and 4 (10 %) Candida albicans .



The percentage of contaminated samples In dental clinics

❖In incubators :

Among 40(33%) samples ,2(5 %) were contaminated by Escherichia coli ,2(5%) Staphylococcus aureus, 2(5%) Proteus and 4 (10%) Candida albicans .



The percentage of contaminated samples In Department of incubators

Determine the sensitivity of M.O. to disinfectants (inhibition zone mm)

MICROORGANISMS	DELTA-GURAD (DENTAL CLINICS)	H2O2	ALPHA-GUARD	DELTA-GUARD (OPERATING ROOM AND INTENSIVE CARE)
E-Coli Sensitive isolate	18 mm	40	**24**	20
E-Coli Operating room (ophthalmic device)		28		17
E-Coli Intensive Care		25		20
E.coli incubators		24	**14**	
E.coli Dental clinics		26		
Proteus Sensitive isolate	**19**	23	15	19
Proteus isolate in hospital	18	**21**	12	**16**
S.aureus Sensitive isolate	18	32	19	17
S.aureus isolate in hospital I	18	**56**	15	**20**
Candida	**17**	25	19	20

❑ *Minimum inhibitory concentration (MIC) values of disinfectants:*

MIC values of Delta Guard in our study

Microorgnisms		*MIC values of Delta Guard From the perspective of manufacturers*	
	MIC values of Delta Guard in our study	*in intensive care and operating room*	*in dental clinics*
E.coli	*800 µg/ml*	*800 µg/ ml*	*400 µg/ ml*
Proteus	*800 µg/ ml*	*800 µg/ ml*	*400 µg/ ml*
Staphylococcus aureus	*800 µg/ ml*	*800 µg/ ml*	*400 µg/ ml*
Candida	*800 µg/ ml*	*800 µg/ ml*	*400 µg/ ml*

MIC values of Alpha Guard GF in our study at used in Department of incubators (ready- to use)

Microorgnisms	*MIC values of Alpha Guard GF in our study*
E.coli	*520 µg/ml*
Proteus	*520 µg/ml*
Staphylococcus aureus	*520 µg/ml*
Candida	*520 µg/ml*

Delta Guard (in dental clinics)	• was less effective in at use concentration and the dilution was incorrect.
Delta Guard (in intensive care and operating room)	• was effective in at use concentration and the dilution was correct
Alpha Guard GF (in Department of incubators)	• (ready- to use) was effective in at use concentration

❑ Determine the PH of disinfectants by PH meter

disinfectants in at use concentration	**PH** from the perspective of manufacturers	**PH** in at use concentration In hospitaL
Delta Guard (dental clinics)	6,47	**9**
Delta Guard (operating room and intensive care)	6,95	**8**
Alpha Guard GF	6,65	**6,65**

Recommendations

Recommendations

1)The concentrations of disinfectants in dental clinics must be raised to kill all the microorganisms.

2) The antiseptics and disinfectants must be changed from time to time, to prevent the development of resistance by microorganisms against them .

3) The staff of cleaning must be trained to applicate the best protocol in cleaning and use the best wipers and must pay attention to personal hygiene.

4) Special committee must be placed to conduct periodic control in all the hospitals (private and public hospitals) To ensure proper sterilization and disinfection .

5) The final PH of disinfectants must be adjuctive to be optimal PH from the perspective of manufacturers .

6) More samples must be taken from different hospitals and the hospitals must be cooperative with the working group .

Thanks For Listning