Contamination and Microbial Biofilm Formation in intensive care unit in Thi-qar province / Iraq

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ABSTRACT:

To identify the cause and the source of bacterial infection among patients of intensive care unit and to identify the ability of bacteria to produce biomass this study had been conducted in Al-Imam Al-Hussein hospital in Thi-qar province for the period from 1st September to end of December 2011 .A total of 320 swabs and samples were collected from 17 different sites of Intensive Care Unit environment and inoculated on a normal cultural media ,then incubated at 37°C for 24 hour . The growth revealed different bacterial colonies which had been tested for their morphological and biochemical characteristics. Sixty eight of pure isolates were obtained including 24 (35.29%) Gram positive bacterial isolates, 44(64.71%) of Gram negative bacterial isolates , the highest rates (19.11%) of bacterial contamination had been found on the walls and the floor . Sensitivity tests for all isolates were done using 25 types of commonly used antibiotics in Iraq , the results revealed that the genus *Enterobacter* spp. had a high resistance as a Gram negative bacteria , and *Staphylococcus* spp. had a high resistance as a Gram positive bacteria to most of the tested antibiotics, The tendency of some isolates to develop a biomass as biofilm , an important virulence determinant related to infection , was investigated in vitro using microtiter plates . The highest optical density (O.D.= 0.634 n.m.) was recorded by the isolate from pressure material by *Pseudomonas aeruginosa* and the least O.D. was recorded by medical instruments-use manual isolate (O.D. was 0.106 n.m.) by *Pantoea* spp .

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INTRODUCTION:

Cross- infection from patient to patient or from hospital personnel to patients present a constant hazards . Hospital infections are called Nosocomium, and occur in about 5% of all patients admitted. In certain clinical services , such as Intensive Care Unit (ICU) up to 10% of the patients acquire a nosocomial infection, in all there are about two million nosocomial infections each year in USA , leading directly or indirectly to 80.000 deaths [1,2] . Bacterial contamination in hospitals related directly or indirectly of incorrect uses of antibiotics by patients and when disinfectant used with concentrations lower than the recommended one for cleaning purposes in hospital leading to the appearance of new strains of resistant bacteria to the commonly used antibiotics [3,4,5] . The term 'biofilm' was coined and described in 1978 [5]. Since then, it has been well documented that biofilm-associated microbes differ from their planktonic relatives in terms of the genes that are transcribed [6]. Bacteria can develop biofilms on a number of different surfaces, such as natural aquatic and soil environments, living tissues, medical devices or industrial or potable water piping systems [6, 7]. Clusters of different microbial populations are found in almost all moist environments where nutrient flow is available and surface attachment is possible [8]. Biofilms have been found to protect the microbial community from environmental stresses [7, 9]. This is why the formation of biofilms in natural and industrial environments allows bacteria to develop resistance to bacteriophage, amoebae, chemically diverse biocides, host immune responses and antibiotics [10]. These important characteristics have resulted in biofilm science and biofilm engineering emerging as intensively developing areas of research [11] The aim of this study was to identify the types of bacterial contamination in ICU, and to study the formation of biofilm in bacterial isolates in hospitals.

Material and methods:

Study design and setting: a cross sectional study had been conducted in intensive care unit in Al-Hussein hospital at Thi-qar ,one of the southern province in Iraq for the period from 1st of September to the end of December 2011 .

1.Sampling : three hundred and twenty swabs were collected from the skin of patients , hands of medical staffs , and from different sites related to the devices and tools used in the ICU including ; medical instruments,

surgical instruments , sphygmomanometer , sets of intravenous (IV) fluid , masks of O_2 supplying apparatus, drums, and from the gowns of medical staffs, bed clothes, beside swabs were also taken from the surroundings ; floor, walls, windows and door kelons, wooden furniture, tables, cabinates, slots of cooling and heating devices , sink, beside samples from the ward air of the ICU were also taken

2. cultural media : swabs incubated with cultural media ; Blood agar, MacConkey agar and Nutrient agar ,which were prepared according to the manufacture companies , and incubated at $37^{O}C$ for (24 - 48) hours.

3. Isolation and identification : Purification of bacterial growth colonies yield pure isolates of bacteria and subsequently their cultural , morphological , microscopically and biochemical characteristics had been studied according to [12, 13,14, and 15] .

For identification of isolates the following kits were used:

- API Staph kit (BioMeriux) for staphylococci identification
- API 20E kit (BioMeriux) for Gram -ve bacilli identification
- MICEVA kit (Hi media- India) for MIC test

4. Antimicrobial Sensitivity tests : Susceptibility for the studied isolates were investigated according to [16] by using Muller - Hinton agar and the following antibiotics discs :Cefepime, Piperacillin, Cepotaxime, Gentamicin, Tetracyclin, Doxycycline, Ciprofloxacine, Ofloxacin, Levofloxacin, Nalidixic acid, Oxacillin, Vancomycin, Erythromycin, Rifampin, Clindamycin, Ampicillin, Cephalothin, Ceftazidime, Imipenem, Aztreonam, Amikacin, Chlorophinicol, Ceftriaxon, Ticarcillin - Clavulanic acid and Amoxicillin - Clavulanic acid.

5. Biofilm formation studied by microtiter plate assay we performed the microtiter plate assay described by Christensen *et al*. (1985),

Results and Discussion:

Bacterial growth had been observed in 57 cultures (17.8%) out of 320 swabs and samples which were collected from 17 sites distributed in ICU environment (Table 1) . The most evident contamination sites found in the ICU environment were the walls and floor revealed in 13 isolates (19.11%) followed by medical apparatus , 10 isolates (14.7%) of the total isolates , yet the lowest level of contamination was 1 isolate (1.47) ; at the set of IV fluid , hands of medical staff and their gowns,

and slots of cooling and heating devices , while no contamination was observed on doors and windows and wooden furniture . Table (2) shows the distribution of the pure culture according to their sites and type of genus. The pure culture were divided into two groups depending on Gram stain, accordingly 24 Gram positive isolates and 44 Gram Negative isolates were identified (Table 3). The most prevalent genus among Gram +ve bacteria was Bacillus spp. (18 isolates) found in 7 out of 17 sites , while the most prevalent genus among Gram –ve was *Enterobacter cloacae* (15 isolates) had been found . On the other hand 6 isolates of Staphylococcus spp. (25%) among Gram +ve bacteria were identified which also had been found by [17] , while *E. coli* represent only 6.8% of total Gram -ve bacteria which show inconsistency with a study that had been done in Erbil 2002 [18] where an extremely high percentage (46.21%) of contamination with this species was found , this may be due to the differences of the sites of swabs being taken from the environment of the hospital as a whole in Erbil or may be explained by the level of health awareness of both , patients and health staff in different communities [19] .The percentage of contamination with *Pseudomonas aeruginosa* was 1.4% and according to [20] this species regarded one of sources of infection in ICU, beside Greenwood *et al*. [21] mentioned that 2.3% of this species had high resistance to multiple antibiotics and disinfectants in hospital environment .Susceptibility tests for some antibiotics showed different results depending on the genus of bacteria and type of antibiotics used For *Enterobacter* spp. the resistance was statistically highly significant against 7 antibiotics, p value< 0.01 (Ampecillin , amoxicillin clavulanic acid, Cephalothin, Imipenem, Ciprofloxacin, Levofloaxin and Ofloxacin) while it was significant for 5 antibiotics with p value < 0.05 (Piperacillin, Titracillin clavulanic acid , Cefepime, Ceftriaxione and Azteronam)) , yet it was insignificant , p value > 0.05 against 7 antibiotics (Cefotaxim , Ceftazidine , Gentamycine , Amikacin , Tetracycline , Nalidixic acid and Chloramphinicol). Among Gram positive bacteria , susptibility tests conducted for *Staphylococcus* spp. showed resistance which was statistically highly significant against 6 antibiotics with p value < 0.01 (Ampicillin , Cefepime , Ceftazidine , Imipenem , Chloramphinicol and Oxacilline), while it was insignificant , p value > 0.05 against 15 antibiotics (amoxicillin clavulanic acid ,

Titracillin clavulanic acid, Cephalothin, Cefotaxim , Ceftriaxione , Gentamycine , Amikacin , Tetracycline, Ciprofloxacin , Levofloxacin , Ofloxacin , Clindamycin , Rifampin, Erythromycin, Vancomycin) .The appearance of resistance for β-lactamase antibiotics specifically amoxicillin and to a lower extent Piperacillin could be related to many causes ; production of β lactamase enzymes and its effect which lead to the breakdown of the β – lactame cycle in penicillins and cephalosporines changing it into inactive compounds [22], or may be because of the changes being occurred in the porins of the cellular membrane and ultimately it`s effect on the cell permeability [23], some Gram –ve bacteria are resistant for β –lactame antibiotic because it has an Efflux pump system which lead to pump the antibiotics from intracellular to extracellular space [24] .The gradual increase in the resistant of enterobacteriaceae against β-lactam antibiotics (1st and 2nd generation of penicillin and cephalosporines) reduce the efficacy of these antibiotics in eradicating diseases of bacterial etiology completely since these resistance will lead to continuous change in the epidemiology of these disease [25], while the effect of extended spectrum β - lactamase (ESBLs) became more evident against the 3rd generation of penicillins and cephalosporines [26] The resistant against recently introduced β - lactam antibiotic ; Aztreonam is related to many causes ; it`s sensitivity for β - lactamases enzyme produced by *Proteus mirabilis* , *Klebsiella pneumoniae* , and *E.coli*, or may be due to the weak affinity of antibiotic to the penicillin binding proteins in cell wall [27].The high sensitivity of the studied isolates for Imipenem belong to Carbapenems group and one of the recently used antibiotic, could be due to its limited use in Iraq. Although resistant was also recorded among 4.41% of these isolates, and the cause could be inferred to the development in the mechanism of bacterial resistance such as its production for Carbapenemases enzymes related to β - lactamases enzymes type D and B [28] .one of the three mechanisms that may explain the resistance of some bacteria against aminoglycosides antibiotics ; production of converted enzymes which inhibit the activity of antibiotics , changing the target of antibiotics , or through the change of the permeability for the cell barrier [29] . Biofilm formation Possible to distinguish between strains through the density of the biofilm product as stated in the (DiBonaventura *et al*., 2004) was

the isolation number (1) *Pseudomonas aeruginosa* isolated from a A device for measuring pressure most capable of forming the biofilm and through the density values photovoltaic (O.D.) recorded after 24 hours. was biofilm of the membrane which is formed isolation is the heaviest , as the value of the (O.D.) recorded her after 24 hours is equal to (0.634) nm , followed by the isolates (2, 3 and 4), respectively , as the value (O.D.) of the isolation number (4) *Enterobacter cloacae* isolated from medical instruments use manual equal to (0.136) nm (Table 4) .This may be due to the difference between isolates biomass for bio-membrane product to: - 1 - heterogeneity exists between isolates in their ability to adhesion, which is the first stage in the process of formation of membrane bio due to differing ability to produce materials constituting the portfolio, type and length of the strings material polysaccharides fatty acids (LPS) and type in the outer membrane [30] which leads to variation in the speed of adhesion and the number of adherent bacteria.

2 - The difference between isolates in their ability to produce polymers consisting of bio-membrane glycocalyx, type and quantity of what affects the thickness of the membrane formed and leads to a variation in the values of optical density between isolates [31].

3 - Mutations in the genes encoded sites of the factors to assist in the process of formation of the membrane bio-like flagella and portfolio sites or genes encoded proteins and polysaccharides consisting of the membrane [32] .

CONCLUSION:

Gradual increase in the resistant of microbes to previously and recently produced antibiotics may interfere with the tremendous effort provided by health facilities to control the spread of microbial disease in the community . this problem could be controlled to some extent by restriction of purposeless uses of antibiotics and by eliminating contamination in the environment of hospitals by applying a restricted quality standards related to hygienic manners and procedures both of patients and health staff .

Table - 1

The positive bacterial growth cultures and the pure isolates in ICU Environment:

Sites (20 swabs for each site)	Positive growth Cultures		Pure isolates (from20 swabs of this site)	
	No.	%	No.	%
Doors & windows	0	0	0	0
Bed	3	5.3	4	20
Table	2	3.5	2	10
Cabinate	6	10.6	7	35
Walls & Floor	9	15.8	13	65
Slots of cooling and Heating device	1	1.7	1	5
Wood furniture	0	0	0	0
Sink	8	14	9	45
Medical apparatus	8	14	10	50
Masks of O_2 supplying apparatus	2	3.5	3	15
Set of intravenous (IV) fluid	1	1.7	1	5
Sphygmomanometer	2	3.5	2	10
Gowns	1	1.7	1	5
Hands of medical staff (10 swabs)	1	1.7	1	5
Surgical instrument	3	5.3	3	15
Patient skin	6	10.6	6	30
Ward air (10 swabs)	4	7	5	25
TOTAL	57	100	68	

Table - 2

Distribution of pure isolates on the sites and types of Bacteria :

Genus	1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Staphylococcus Aureus														+	+		+
Staphylococcus Chromogenes													+			+	
Staphylococcus Epidermidis																+	+
Staphylococcus haemolyticus																+	
Bacillus subtilis						+											
Bacillus cereus			+	+	+	+		+	+	+							
Enterobacter Cloacae		+	+		+				+		+	+					
Enterobacter Sakazaki									+								
Bordetella spp.				+	+	+			+								
Pantoea spp.		+															
Klebsiella Pneumonia			+		+					+							
Citrobacter Freundi						+			+								
Citrobacter Yongae					+												
Escherichia hernanni					+												
Escherichia coli						+											
Pseudomonas Aeruginosa												+					
Proteus Mirabilis										+							
Rahnella Aguatilis			+														

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1- Doors & windows
2- Bed clothes
3- Table
4- Cabinate
5- Walls & Floor
6- Sink
7- Wooden furniture
8- Slots of cooling & heating device
9- Medical apparatus
10- Mask of O_2 supply
11- Set of IV fluid
12- Sphygmanometer
13- Gowns
14- Skin of palm of medical staff (only 10 samples)
15- Surgical instrument
16- Skin of patient
17- Ward air

Table - 3

Numbers and percentages of pure isolates in the studied samples :

Bacteria	No.	%	Type
Staphylococcus spp.	6	**25**	**Gram positive**
Bacillus spp.	18	**75**	**Gram positive**
Enterobacter spp.	15	34	Gram negative
Bordetella spp.	8	18.2	Gram negative
Pantoea spp.	6	13.6	Gram negative
Klebsiella pneumonia	4	9.1	Gram negative
Citrobacter spp.	4	9.1	Gram negative
Escherichia hernanni	1	2.3	Gram negative
Escherichia coli	3	6.8	Gram negative
Pseudomonas aeruginosa	1	2.3	Gram negative
Proteus mirabilis	1	2.3	Gram negative
Rahnella aguatilis	1	2.3	Gram negative
	68		

Table (3-11) optical density of the (biofilm) consisting of bacterial isolates from different sources

No. strain	Source of strain	Name of strain	Optical density of bio mass
1	A device for measuring pressure	*Pseudomonas aerogenosia*	0.634
2	Medical instruments use manual	*Bordetella* spp.	0.502
3	Cabinet	*Enterobacter cloacae*	0.236
4	medical instruments-use manual	*Enterobacter cloacae*	0.136
5	Walls & Floor	*Pantoea* spp.	0.126
6	Walls & Floor	*Bacillus cereus*	0.125
7	Ward air	*Staphylococcus epidermides*	0.113
8	Table	*Bacillus cereus*	0.112
9	medical instruments-use manual	*Pantoea* spp.	0.106

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