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Bacterial contamination and its Susceptibility 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 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^{O}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, MIC tests for 5 types of antibiotics were applied on the most resistant and the most sensitive isolates which reflect that all isolates have a low rate of MIC against Ciprofloxacin .

Key words: ICU, bacterial contamination, MIC.

الخــلاصة

تم التحري عن التلوث البكتيري في مستشفى الأمام الحسين(ع) التعليمي في محافظة ذي قار خلال الفترة الأول من شهر أيلول ولغاية نهاية شهر كانون الأول عام ٢٠١١ ، إذ جمع ٣٢٠ مسحة وعينة من ١٧ موقع مختلف في بيئة وحدة العناية المركزة . زرعت العينات على أوساط زرعيه وحضنت في درجة حرارة ٣٧ ^{O}م ولمدة ٢٤ ساعة. اظهر نمو المستعمرات البكتيرية وجود اجناسا بكتيرية مختلفة وذلك بالتحري عن خواصها المظهرية والكيموحيوية لذا عزلت وشخصت ٦٨ عزلة بكتيرية نقية منها ٢٤ (٣٥،٢٩%) موجبة لصبغة كرام و٤٤ (٦٢،٧١%) منها سالبة لصبغة كرام . بلغ أعلى نسبة تلوث بكتيري (١٩،١١%) وجدت في مسحات الجدران والأرضية , اختبرت حساسية العزلات لـ ٢٥ من المضادات الحيوية. أظهرت النتائج أن *Enterobacter spp.* كانت لها أعلى نسبة مقاومة من البكتريا السالبة لصبغة كرام, بينما *Staphylococcus spp.* كانت لها أعلى نسبة مقاومة من البكتريا الموجبة لصبغة كرام . كذلك تم اختبار MIC لخمسة أنواع من المضادات الحيوية الأكثر مقاومة أو الأكثر حساسية لها من قبل العزلات المدروسة , وتبين أن جميع العزلات البكتيرية لها نسبة واطئة من قيمة MIC تجاه Ciprofloxacin .استنتج البحث إن التلوث البكتيري في وحدة العناية المركزة لاتزال تشكل عقبة كبيرة وهي نتيجة طبيعية للخلل الموجود في التطبيقات الدقيقة لشروط التعقيم فيها .

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 . Ultimately the patients will need additional treatment and for long periods of admission in hospital to be recovered, and this may lead to severe side effects [3,4,5] .

The aim of this study was to identify the types of bacterial contamination in ICU, and to study the sensitivity of bacterial isolates to commonly used antibiotics 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 , sphygmomano- meter , 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 [6, 7,8, and 9) .

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 [10] 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.

The MIC was measured by using Ceftriaxone and Meropenem powder utilized using two fold dilution method on Muller - Hinton agar [10], then results were recorded according to[11]

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 [12] , 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 [13] 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 [14] .

The percentage of contamination with *Pseudomonas aeruginosa* was 1.4% and according to [15] this species regarded one of sources of infection in ICU, beside Greenwood *et al*. [16] 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 it`s effect which lead to the break down of the β – lactame cycle in penicillins and cephalosporines changing it into inactive compounds [17], 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 [18, 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 [19] .

The gradual increase in the resistant of enterobacteriaceae against β-lactam antibiotics (1st and 2nd generation of penicillins 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 [20], while the effect of extended

spectrum β - lactamase (ESBLs) became more evident against the 3rd generation of penicillins and cephalosporines [21]

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 [22].

The high sensitivity of the studied isolates for Imipenem belong to Carbapenems group and one of the recently used antibiotic, could be due to it`s 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 it`s production for Carbapenemases enzymes related to β - lactamases enzymes type D and B [23] .

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 [24] .

The test for MIC was applied to detect the lowest concentration of

a specific drug that prevent the growth of an organism in vitro . Inter-preting the significance of a given MIC requires knowledge of the level of the drug that can be reached in the patient . On the other hand MIC can also used to determine whether the resistance of a microorganism increased against aspecific antibiotic and if it is sufficiently susceptible or not that is to achieve successful treatment [2] .

The results of MIC tests showed that the lowest concentration of Ciprofloxacin was 0.016 µm/ml (Table 4), to exert an effect on *Enterobacter spp.* , *E. coli* , *Citrobacter spp.* and *Pantoea spp.* The lowest concentration of Piperacillin tazobactum was 0.25 µm/ml against *Enterobacter spp.*, and Amikacin against *Bacillus spp.*, while the lowest concentration of Ceftriaxone was 1 µm/ml against *Bordetella spp.*, for mropenem was 0.05 against *Bordetella spp.*, *Pantoea spp.*, Staphylococcus *spp.*, and Bacillus *spp*.

Conclusions

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			+														

*

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 – 4: MIC test of five antibiotics with the studied isolates.

Bacteria	Ciproflo-Xacin	Piperacillin-tazobactum	Amikacin	Ceftriaxone	Meropenem
Enterobacter spp.	3 - 0.016	96 – 0.25	64 – 0.5	1024 – 2	16 – 0.5
Klebsiella pneumonia	3 – 0,125	48 – 16	4 – 0.75	1024 – 8	1
Escherichia coli	3 - 0.016	48 – 16	256 – 1.5	256 – 4	2 – 1
Citrobacter spp.	2 - 0.016	256 – 4	64 – 0.38	1024 – 8	2 – 1
Bordetella spp.	0.25-0.023	192 – 16	16 – 0.75	1024 – 1	4 – 0.5
Pantoea spp.	0.094- 0.016	321 – 12	1.5 - 0.5	1024 – 4	4 – 0.5
Proteus mirabilis	1	1.5	32	2	16
Pseudomonas aeruginosa	0.19	12	3	1024	32
Escherichia hernanni	0.125	128	96	32	2
Rahnella aguatilis	1	8	2	512	4
Staphylococcus spp.	1 – 0.25	48 – 8	4 – 0.75	1024 – 4	4 – 0.5
Bacillus spp.	1 – 0.094	256 – 2	4 - 0.016	1024 - 16	4 – 0.5

References

1-Michael T. Madigan; John M. Martinko and Jack Parker (2000): Biology of microorganisms , 9th ed. South Illinois Univ. Carbondale, Prentice Hall , pp. 906 .

2-Eugene W. Nester; Denise G. Anderson ; Evans Roberts Jr. ; Nancy N. Pearsall and Martha T. Nester (2004) : Microbiology, A human perspective , 4th ed. McGraw Hill , pp.499 – 502 .

3-Deep A.; Childiyal R.; Kandian S. and Shinker N. (2004): Clinical and microbiology profile of Nosocomial infection in the Pediatric Intensive Care Unit (PICU), Indian pediatrics , 41 : 1238 – 44 .

4-Dorchis F. (2005): Nosocomial infections and air filtration in operating suites – application of French Standard , NFS 90 – 351 .

5-Hotchkiss J.R.; Holley P. and Crooke P.S. (2007): Analyzing pathogen transmission in the Dialysis Unit Clin. J. Am. Soc. Nephrol 2 :1176 – 85 .

6-Retty A.F. ; Danil F.S. and Aice S.W. (2007) : Balley and Scott's of Diagnostic Microbiology , 12th ed. Press , Houston , Texas .

7-Steve K. Alexander and Dennis Strete (2001) : Microbiology A photo- Graphic Atlas for Laboratory , 1st ed. Inc. San Francisco .

8-Finegold S.M. and Martin W.J. (1982): Diagnostic Microbiology, 6th ed. Mosby com.

9-Koneman E.W. ; Allen S.D. ; Jawa W.M. and Sachreckeber P.C. (1992): Color Atlas and textbook of diagnostic microbiology, 4th ed. J.B. Lippincott com. Philadephia .

10-Stocks E.J. and Ridgway G. (1987): Handaling clinical speciments for microbiology studies, 5th ed. , Churchill Livingston , Edinburgh, 173 – 201 .

11- (CLSI) Clinical and Laboratory Standard Institute (2007): Perfor- mance standards for Antimicrobial susceptibility testing , 17th informational Supplement Vol. 27 No. 1 , Pennsylvania USA .

12-Amee R. ; James R. Manges M.P.H. ; Timothy T. and Johnson A.D. (2001): Widespread distribution of urinary tract infection caused by a multidrug resistance *Escherichia coli* clonal group. J. Medical Science, Vol. 345 No. 14.

13-Muhammed S.A. (2002) : A bacteriological study on the incidence urinary tract infection in Rizgari Teaching Hospital in Erbil City, Msc. thesis , college of scince , Univ. of Salahaddin .

14-Delzell J.E. and Lefevre M.L. (2000): Urinary tract infection during pregnancy, Am. Academy of family physicians , 35(3) : 40-66.

15-Levin M.H. ; Olson B. ; Nathan C. ; Kabins S.A.and Weinstein R.H. (1984): Pseudomonads in skin in an Intensive care unit relation to patients , J. Clin. Path. 73 : 424 – 27 .

16-Greenwood D. ; Slack R.; Peutherer J. and Barer M. (2007): Medical Microbiology , 17th ed. , Churchill Livingstone Elsevier .

17-Pfeillf D.; Janes E. and Weideman B. (2000) : Role of Penicillin binding protein , the initiation of the ampc β- Lactamas Expression in *Enterobacter cloacae* , J. Antimicrob. Agent Chemother. 44 : (1) 169 – 72 .

18-Mims C.; Playfair J. ; Roitt I.; Wakelin D.; Williams R. and Anderson R.M.(1993): Medical Microbiology 1st ed. year book Euro Lim. Mosby com. USA .

19-Schweizer H.P. (2003): Efflux as a mechanism of resistance to anti- Microbial in *Pseudomonas aeruginosa* and related bacteria : Unanswered questions. J. Genetic and molecular research 2(1):48 – 62 .

20-Sahm F.D. ; Thornsberry C. ; Mayfield C.D. ; Jones E.M. and Karlo- wsky A.J. (2001): Multidrug – resistant urinary tract iso lates of *Escherichia coli* , Prevalence and patient Demographics in the united state in 2000, Antimicrobial agent and Chemotherapy , 45 : 1402 – 6 .

21-Woodford N.; Ward E. and Kaufmann M.E. (2006): Molecular Characterization of *Escherichia coli* isolates producing CTX – M – 15 extended – spectrum β –lactamase (ESBL) In Health Protection Agency, U.K.

22-Brown S. and Amyes S. (2006): O_XA β – lactamases in *Acinetobacter* The story so far, J. antimicrob. chemother. 57 (1): 1-3.

23-Laclero M.; Glupcynski Y. and Tulkens P. (1999): Aminoglycosides Activity and resistance, J. Med. Microb. 43 (4): 727 – 37.

24-Levinson W. and Jawetz E. (2004): Medical Microbiology and Imm- unology examination and board review, 8th ed. Lange Medical books, McGraw – Hill, New York.