Prevalence of metallo-β-lactamase producing bacteria in intensive care unit in Thi-qar province / Iraq

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Abstract

To identify the bacteria that producing the enzyme of metallo-β-lactamases (MBLs) with (E test) method 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 37OC for 24 hour . The growth revealed different bacterial colonies which had been tested for their morphological and biochemical characteristics. The final diagnosis by using API20 E was used for the gram negative bacteria and API staph. Used for staphylococci. Sixty eight of pure isolates were obtained including 24 (35.29%) Gram positive bacterial isolates, 44(64.71%) of Gram negative bacterial isolates. 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 ability of bacteria isolates to produce Metallo ß-lactamase using progressive concentration stripes method(E test) were studied The results showed (1.4 %) of these isolates gave positive results for each Pseudomonas aeruginosa , Enterobacter cloacae and Proteus mirabilis.

Introduction

The emergence of acquired metallo-β-lactamases (MBLs) in Gram-negative bacilli is becoming a therapeutic challenge, as these enzymes usually possess a broad hydrolysis profile that includes carbapenems and extended-spectrum β-lactams (ESBL) with the exception of monobactams. Because most of these carbapenemases confer only reduced susceptibility to carbapenems in Enterobacteriaceae, the presence of MBLs may remain underestimated. This, together with the mobile nature of the gene cassettes carrying the IPM- and VIM-type enzymes, may enhance their spread and compromise the future usefulness of carbapenems for the treatment of serious infections caused by Gram-negative bacilli, therefore requiring more attention than ever before. These facts highlight the challenge for clinical microbiologists and infectious disease specialists to accurately detect MBL-producing isolates, to implement prompt infection control, and to treat infections caused by MBL or MBL+ESBL producers appropriately(1,2). There are an increasing number of studies reporting on the emergence of Enterobacteriaceae that produce MBLs, which invariably hydrolyze carbapenems. Outbreaks with VIM-type-producing isolates have been reported mostly with *Pseudomonas aeruginosa*, but also with *Klebsiella pneumoniae* in Greece (3).

The aim of this study was to identify the types of bacterial producing Metallo Beta Lactmase (MBLs), 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 O2 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 37OC 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 (4, 5,6, and 7) . 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 E test. 4. Antimicrobial Sensitivity tests : Susceptibility for the studied isolates were investigated according to (8) 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- The Metallo B Lactmase (MBLs) production was measured by using progressive concentration stripes method (E test) were studied the results.on Muller - Hinton agar , then results were recorded according to The manufacturer's company When the ratio between (IPM) on (IPM + EDTA) greater than 8 is any positive isolation enzyme-producing MBL. Or Where there is an inhibition of the coated side with (IPM + EDTA) and no inhibition on the other side of any (IPM) only positive longer any isolation enzyme-producing MBL. When the ratio between (IPM) on (IPM + EDTA) less than or equal to 8 negative situation is any isolation enzyme-producing MBL. When no inhibition zone on both sides (IPM) and (IPM EDTA) in such cases are due to acquire resistance other mechanics isolation instead of mechanical production of an enzyme Metallo B Lactmasae Or When there is a complete inhibition of the bar on both sides which is the side containing IPM greater than 4 and the other side containing IPM EDTA greater than 64 such cases are due to acquire resistance other mechanics isolation instead of mechanical production of an enzyme Metallo B Lactmasae

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. 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)) .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 [9], or may be because of the changes being occurred in the porins of the cellular membrane and ultimately its effect on the cell permeability [10], 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 [11].

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

References : Irene Galani, Panagiota Danai Rekatsina, Despina Hatzaki, Diamantis Plachouras, Maria Souli and Helen Giamarellou(2008). Evaluation of different laboratory tests for the detection of metallo-B-lactamase production in Enterobacteriaceae . J. Antimicrob. Chemother. 61(3): 548–553.