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Recent Bacterial Contaminants of Hospital Intensive Care Unit (ICU) and the Effectiveness of Most Used Antibiotics in Al-Bayda Medical Center

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ABSTRACT

Infections caused by drug-resistant bacteria are among the most common causes of death in the intensive care unit, and this study is conducted to investigate the contaminants of the intensive care unit (ICU) in the target hospital and to evaluate the efficacy of the most frequently used antibiotics. Using sterile swabs, twenty samples were taken from different places within the intensive care unit. Basic biochemical reactions were used to identify the isolated bacteria and the Phoenix 100 BD digital system technique was used to confirm the identification and to assess the sensitivity of the isolated bacteria against sixteen standard antibiotics belonging to different classes. Three different genera of the Gram-negative bacteria and one genus of the Gram-positive bacteria were found as contaminants inside the examined ICU, *Enterobacter cloacae, Pasturella multocida, Acinetobacter baumannii*, and *Bacillus cereus*. A diverse susceptibility of *Enterobacter cloacae, Pasturella multocida* has been shown in this study against tested antibiotics. However, *Acinetobacter baumannii* showed resistance to all tested antibiotics except for Colistin while *Bacillus cereus* bacteria showed the opposite as it was sensitive to all tested antibiotics except for Colistin. Conclusion: The targeted intensive care unit was contaminated with 4 types of pathogenic bacteria, including multi and pan-drug-resistant types, the issue which is considered a life-threatening risk factor for patients. The hospital management has been reported the results and the instructions were applied.

الملوثات البكتيرية الحديثة لوحدة العناية المركزة بالمستشفى وفعالية المضادات الحيوية الأكثر استخداماً في مركز البيضاء الطبي

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الكلمات المفتاحية:
الملوثات البكتيرية
وحدة العناية المركزة
مقاومة الأدوية المتعددة
مقاومة عموم الأدوية

الملخص

تعد العدوى التي تسببها البكتيريا المقاومة للأدوية من أكثر أسباب الوفاة شيوعًا في وحدة العناية المركزة ، وتجرى هذه الدراسة للتحقق من ملوثات وحدة العناية المركزة (ICU) في المستشفى المستهدف وتقييم فعالية المضادات الحيوية الأكثر إستخداما. باستخدام المسحات المعقمة ، تم أخذ عشرين عينة من أماكن مختلفة داخل وحدة العناية المركزة. تم استخدام اختبارات التفاعلات الكيميائية الحيوية الأساسية لتعريف البكتيريا المعزولة وتم استخدام تقنية النظام الرقمي Phoenix 100 BD لتأكيد التعريف ولتقييم حساسية البكتيريا المعزولة ضد ستة عشر مضادًا حيويًا قياسيًا ينتمون إلى فئات مختلفة. تم العثور على ثلاثة أجناس مختلفة من البكتيريا سالبة الجرام وجنس واحد من البكتيريا موجبة الجرام كمواد ملوثة داخل وحدة العناية المركزة المفحوصة ، Enterobacter cloacae، Pasturella multocida، Acinetobacter

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baumannii، وBacillus cereus و Enterobacter cloacae ، وقد تم حساسية متنوعة ضج المضادات الحيوية المختبرة ظهرت في هذه الدراسة من قبل بكتيريا Enterobacter cloacae و Pasturella multocida . ومع ذلك ، أظهرت Acinetobacter baumannii مقاومة لجميع المضادات الحيوية المختبرة باستثناء Colistin بينما أظهرت بكتيريا Bacillus cereus عكس ذلك حيث كانت حساسة لجميع المضادات الحيوية المختبرة باستثناء Colistin. الخلاصة: تلوثت وحدة العناية المركزة المستهدفة بأربعة أنواع من البكتيريا المسببة للأمراض ، بما في ذلك الأنواع المتعددة والمقاومة للأدوية ، وهي القضية التي تعتبر عامل خطر يهدد حياة المرضى. أبلغت إدارة المستشفى عن النتائج وتم تطبيق التوصيات.

Introduction

The survival of patients with life-threatening conditions such as shock states and trauma is widely helped by the intensive care units (ICUs), but on the other hand, these units are at increased risk of hospital-acquired infection named nosocomial infection. Patients who stay for more than a week in the intensive care unit are five times more likely to acquire nosocomial infections compared to those who are hospitalized but do not require the intensive care unit. The infection acquired inside the intensive care room is a major and common cause of death for patients who entered the intensive care room as a result of other serious diseases such as trauma or full-thickness burns and survived them.

Organs of the respiratory tract and urinary tract systems are the most affected by nosocomial infection. This infection has a significant effect on the rate of disease and death, where it is reported that more than 2 million patients in U.S. hospital ICUs are affected by nosocomial infection, annually. Also, it has been documented that hospital-acquired infections are responsible for death inside hospitals reach to more than 80,000 and resulting in billions of excess in health care costs [1, 2]. This infection becomes the worst and most cause of death inside ICU if the cause is drug-resistant bacteria, especially with infection caused via a venous catheter or ventilator-associated infection. Many factors are responsible for nosocomial infection transmissions such as ventilating and aeration systems and hospital staff. Usually, bacterial infections are treated empirically and depend on the clinical picture of the patients. Thus, it is necessary to identify all possible types of bacteria responsible for nosocomial infection. If there is no enough data about the bacterial prevalence inside the ICUs and if their pattern of antimicrobial-resistant is not clear, several antibiotics will be prescribed, the point which leads to miss use of antibiotics, the issue which does not only have any benefit in the way of treatment but also make some complication for patients. It is well known that miss use of antibiotics motivates the Clostridium difficile bacterium to induce diarrhea. Understanding the pattern of bacterial prevalence and drug resistance inside the ICU room is considered a first step to treat nosocomial infections [3, 4].

Despite the number of beds inside the ICU units being usually limited, the rate of incidence of hospital-acquired infection is high. Today, managing risks that cause nosocomial infections becomes a priority [5]. Considering that infections acquired by patients inside ICU form about half of all infections, controlling and minimizing the incidence of infections acquired within these units will lead to substantial economic benefits. In terms of the above introduction, this study aimed to investigate the contaminants of the abiotic surfaces inside the intensive care unit (ICU) in the target hospital and to evaluate the efficacy of the most frequently used antibiotics.

Material and Methods:

Preparation of culture media:

Different media; Nutrient agar, MacConkey agar and Mannitol salt agar were prepared as manufactures instruction written on the media bottles at laboratory of Microbiology and immunology at Faculty of Pharmacy University of Omar Al-Mukhtar, Al-Bayda city, North east of Libya. Sensitive balance was used for weighing, sterilized distilled water and Autoclave device were used for preparation of media.

Collection and Culture of Microbial Isolates:

With use of sterile wet swabs, 20 samples were taken from most of abiotic things inside the intensive care unit of the targeted hospital. Samples were taken from surfaces of electronic medical devices, Doctors table, patient's food tables, patient's beds, refrigerator door, computer keyboard, door and windows of the targeted intensive care unit. Each sampled swab was streaked on a separate labelled Nutrient agar petri dish and incubated at 37 C0 for 24 hours. Overnight sub-cultures were done in same condition in purpose of purification.

Identification of Isolated Bacteria:

All purified isolates were subjected to Gram stain in order to distinguish Gram negative bacterial isolates from Gram positive ones. For identification purpose and according to Cheesbrough, 2000[6]., first, all isolates were sub-cultured on differential media and subjected to different biochemical tests; catalase, citrate, urease, oxidase and triple sugar iron tests. All isolates were re-identified and tested for their sensitivity against the tested standard antibiotics with use of the accurate Phoenix 100 BD digital system technique at the central laboratory of chest infection hospital, Benghazi, Libya. The samples were processed according the instructions supplied with the Phoenix 100 BD digital system technique.

Tested Standard Antibiotics:

A panel of sixteen antibiotics belong to different antibiotic classes were tested in this study for their effectiveness towards the isolated bacteria; Augmentin 30mcg, Ampicillin/cloxacillin 25mg/5 mcg, Cephalexin 30 mcg, Ceftazidime 30 mcg, Ceftazolin 30 mcg, Ceftriaxone 30 mcg, Cefixime 30 mcg, Imipenem 10mcg, Tetracycline 30 mcg, Gentamicin 10 mcg, Azithromycin 15 mcg, Amikacin 30 mcg, Tigecycline 15 mcg, Levofloxacin 5 mcg, Ciprofloxacin 5 mcg and Colistin 10 mcg.

Result:

This study proved that the investigated ICU was contaminated with four pathogenic bacterial genera (Figure 1), 85% were belonging to Gram-positive bacteria and 15% belong to Gram-positive bacteria. Three of the four detected genera belong to Gram-negative bacteria; *Enterobacter cloacae, Acinetobacter baumannii,* and *Pasturella multocida* with the prevalence of 35%, 15%, and 35% of the total, respectively. The fourth genus detected in this study belonged to the Gram-positive bacteria; *Bacillus cereus* which constituted a prevalence of 15% of the total. With the manual biochemical tests and among the isolated Gram-negative bacteria, Enterobacter cloacae showed pink colonies growth on MacConkey agar medium, catalase-positive, citrate positive, and gave Yellow slant/Yellow bottom and Gas production on TSI agar medium. *Pasturella multocida* showed orang colonies growth on MacConkey agar medium, catalase, and oxidase-positive, while *Acinetobacter baumannii* gave Pale pink colonies growth on MacConky agar medium, Mucoid grey whitish colonies growth on Blood agar media, and showed urease, catalase, and oxidase-positive tests.

However, the Gram-positive *Bacillus cereus* gave Gray rough colonies on Blood agar and showed citrate, urease, catalase, and oxidase-positive tests. Out of the all investigated samples. When the identified bacterial isolates were tested for their susceptibility towards the sixteen tested antibiotics, this study showed varied results.

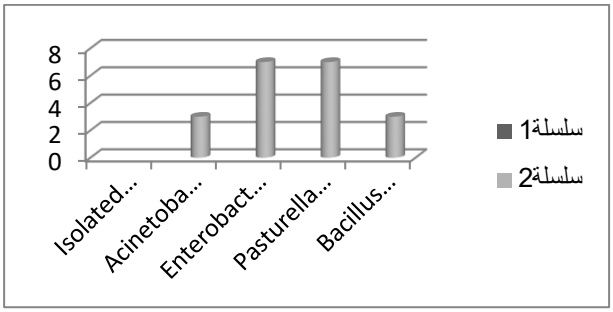

Figure (1): Bacterial genera isolates contaminated the investigated ICU

Half of the tested antibiotics were effective and inhibited the growth of Enterobacter cloacae, in time only three showed intermediate growth inhibition activity, while the rest five antibiotics; Augmentin 30mcg, Ampicillin/Cloxacillin 25mg/5 mcg, Ceftazidime 30 mcg, Ceftazolin 30 mcg, and Colistin 10 mcg were resisted by this bacteria (Figure 2-A & 2-B). In this study, all tested antibiotics showed growth inhibition activity against the isolated *Pasturella multocida* except Tetracycline 30 mcg and Colistion 10mcg, where both have been resisted (Figure 3-A & 3-B). In addition, the results proved that the growth of the isolated *Acinetobacter baumanii* was inhibited only by Colistin 10 mcg and resisted all other fifteen used antibiotics (Figure 4-A & 4-B), while Colistin 10 mcg showed no activity against Bacillus cereus which was inhibited by all other used antibiotics (Figure 5-A & 5-B).

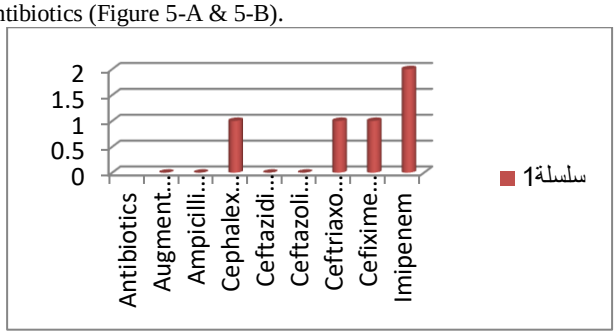

Figure (2-A): Activity of Tested Antibiotics against *Enterobacter cloacae*

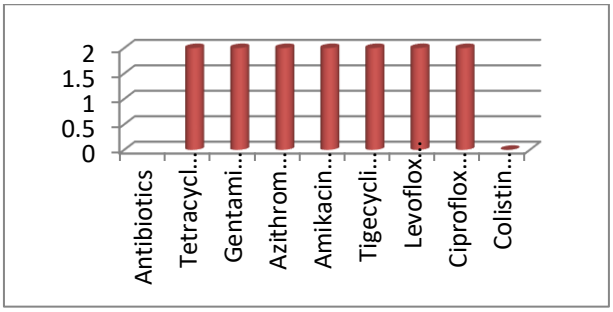

Figure (2-B): Activity of Tested Antibiotics against *Enterobacter cloacae*

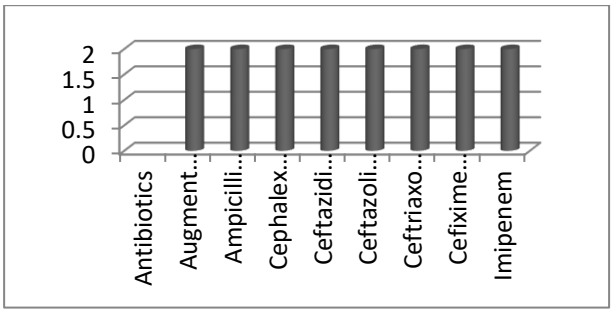

Figure (3-A): Activity of Tested Antibiotics against *Pasturella multocida*

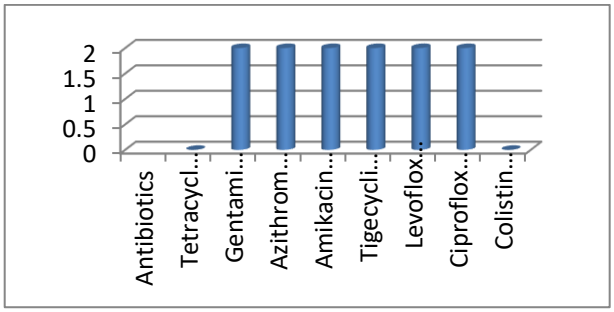

Figure (3-B): Activity of Tested Antibiotics against *Pasturella multocida*

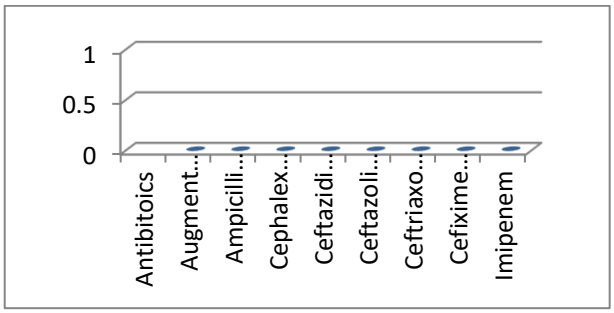

Figure (4-A): Activity of Tested Antibiotics against *Acinetobacter baumannii*

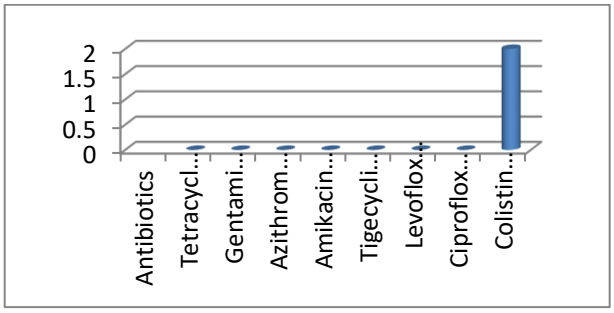

Figure (4-B): Activity of Tested Antibiotics against *Acinetobacter baumannii*

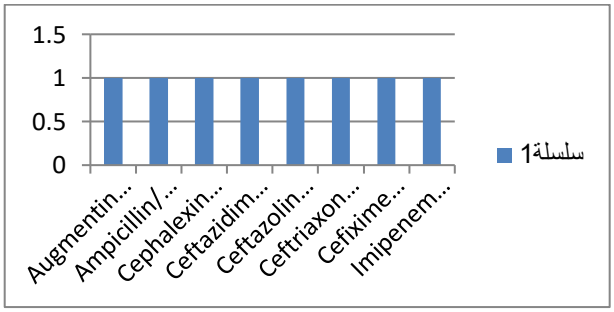

Figure (5-A): Activity of Tested Antibiotics against *Bacillus cereus*

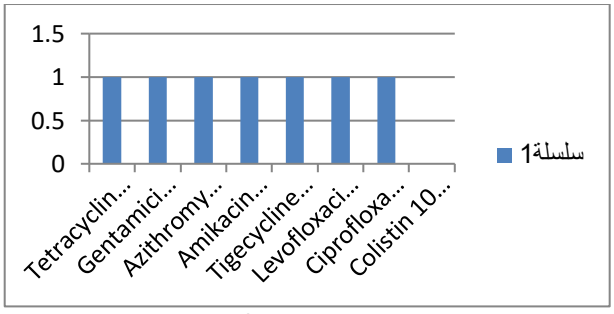

Figure (5-B): Activity of Tested Antibiotics against *Bacillus cereus*

Discussion:

It has been well documented that the infection acquired inside the hospital's ICUs is caused by the ICUs' microbial contaminants in general and bacterial contaminants in specific. Such infection is considered a worldwide challenging health problem specifically if the cause is a multi-drug resistant pathogen, and most of the recent research is directed to study the profile of the ICU suspected microbial contaminants that be considered a health risk factor. In this concept, this study has been carried out to help in covering this profile. The results of this study revealed that the targeted ICU was contaminated with four different bacterial genera; two of them; Enterobacter cloacae and Pasturella multocida which were appeared in higher and equal in prevalence.

Enterobacter cloacae bacteria is known to be primarily associated with healthcare-related infections and is the cause of many diseases such as endocarditis, respiratory infections, osteomyelitis, urinary tract infections, and soft tissue infections [7]. This pathogen infection can be disseminated by a prolonged hospital stay, use of intravenous catheters, intravenous feeding, poor hand hygiene, and also by the use of extended-spectrum antibiotics [8]. In this study, the Enterobacter bacteria were isolated from the above surface of the doctor's table located inside the investigated ICU, away from the patient's beds, but there is a big probability to transmit through the doctor's and nurse's hands and infect the ICU inpatient, and this is a risk factor threats the patients' health, specifically with the result showed that the isolated Enterobacter bacteria was resisted four of the tested beta-lactam antibiotics; moderately affected by three and inhibited only by the Imipenem antibiotics. The prevalence of this bacterium shown in this study is in agreement with Rahbar study which reported that Enterobacter cloacae appeared with a high prevalence of 33% which is near to the 35% shown in this study and showed a high prevalence of resistance to broad-spectrum beta-lactam antibiotics [9].

Despite the zoonotic infectious organism *Pasturella multocida* is not commonly reported inside the ICUs, it also appeared as a contaminant in the targeted ICU in this study and showed the same high prevalence of *Enterobacter cloacae* 35%. *Pasteurella multocida* is often isolated from patients after an animal scratch or bite, where this bacterium known as part of the nasopharynx normal flora in many domestic animals especially dogs and cats[10, 11, 12]. In this study, this bacterium is not isolated from patients bitten or scratched by animals but instead is isolated from inanimate surfaces; legs, and edges of the Patient's beds and instrument bases. *Pasturella multocida* is considered very rare to habitat ICUs, but in agreement with this study a case report study carried out reported a case of *Pasturella multocida* meningitis with a complicated outcome [10].

During this study, we were surprised by the presence of these bacteria on the equipment inside the targeted intensive care unit, and we attribute its presence to its entry loaded with dust with the windows of the room. Cautions should be taken to prevent the arrival of this bacteria to the patients where this bacterium could cause soft tissue infection and other human invasive infections such as pneumonia, bacteremia, meningitis, and endocarditis, especially in immunocompromised patients. The good issue is that most of the commonly used antibiotics tested in this study showed powerful activity against the isolated *Pasturella multocida.*

In this study, in addition to the occurrence of *Enterobacter cloacae* and *Pasturella multocida, Acinetobacter baumannii* bacterium was also identified inside the targeted ICU room where it was found to contaminate the refrigerator door handle, and the risk of its transmission to the patients via nurses' hands is very probable. This bacterium in this study was the worst isolated pathogen since it resisted all tested antibiotics except for Colistin, and the presence of such condition form a very risk factor in patients' life occupying the targeted ICU room. This result makes the ICU a source of hospital-acquired infections caused by this microorganism. Raro and his team in their study were in agreement with this in that *Acinetobacter baumannii* are major pathogen causing infections in intensive care units [13]. In addition, Choi and his team in their study also found *Acinetobacter baumannii* habitat in the ICU room they investigated in a bit higher prevalence; 17.9% compared with 15% prevalence found in this study[14].. It is well reported that this pathogen is commonly found to contaminate the ICU rooms. Munoz-Price team in their study isolated *Acinetobacter baumannii* from the environment of 39% of the tested ICU rooms occupied by patients with *Acinetobacter baumannii* positive infection, and they isolated *Acinetobacter baumannii* from the environment of 10% of the tested ICU rooms occupied by patients with *Acinetobacter baumannii* negative infection. This pathogen is responsible for a number of nosocomial infections such as urinary tract infection, skin and soft tissue infection, bacteremia, meningitis, and pneumonia which is being the most frequently reported infection [15].

In addition to the Gram-negative bacteria appearing contaminated in the tested ICU room, the Gram-positive Bacillus cereus bacteria were also isolated with a prevalence same to that of the isolated Acinetobacter baumannii, but showed a different sensitivity profile against the sixteen tested antibiotics. All tested antibiotics effectively inhibited the growth of Bacillus cereus except Colistin. This is an expected result in return to the mode of action of Colistin, which acts by binding to lipopolysaccharides and phospholipids in the outer cell membrane of Gram-negative bacteria, leads to disruption of the cell membrane, leakage of intracellular contents, and then the death of bacteria. The unusual finding in this study is that this bacteria; Bacillus cereus is isolated from the computer keyboard, which means that it comes via the hands of people who work on that keyboard, the issue which forms a source for transmission of this bacteria within the ICU room and become a risk factor threats patients life. Bacillus cereus is the 2nd most frequent bacterial agent responsible for food-borne outbreaks in France and the 3rd in Europe [16, 17], and accordingly, this study assumes that the bacteria came to the keyboard from hands contaminated by it through food consumption.

Conclusion:

This study conclude that the investigated ICU room was contaminated with four bacterial types. *Enterobacter cloacae* and *Pasturella multocida*, showed equal prevalence with varied susceptibility towards tested antibiotics. The other two bacterial contaminants were *Acinetobacter baumannii* and *Bacillus cereus*, which occurred with the same prevalence and Colistin was the only effective one and the only resisted one, respectively.

Recommendations:

This study recommends the use of the closed conditioned system instead of the windows designed system inside the intensive care units to prevent the entrance of dust which usually carries some problematic bacteria. Furthermore, Professional deep cleaning and the sterilizing system must be adapted for the intensive care units.

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