Isolation of Pathogenic Bacteria from Inanimate Surfaces and contaminated Equipments in Intensive Care Unit at Zawia Teaching Hospital, Libya

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INTRODUCTION

Hospital-acquired infection (HAI) also called a nosocomial infection is an infection which is generally supported by the environmental condition of the hospital, such as an infection that is acquired by a patient during its hospital visit or one which is developing from amongst the hospital staff [1]. Intensive care unit (ICU)-acquired infections are a major cause of morbidity and mortality worldwide [2].

The contamination may occur either by transfer of microorganisms contaminating health-workers’ hands or direct patient shedding of microorganisms in the immediate environment of a patient’s bed [3], contamination of equipment by bacteria is common, turning them into reservoirs of these microorganisms,
enabling the colonization and cross infection of patients, complicating prognosis and favoring HAI outbreaks, mostly by microorganisms multiresistant to antibiotics commonly applied in therapeutics [4], which implies severe limitations to the treatment of hospital infections, posing a great threat to public health [5].

It has been reported that HAI caused by both gram-positive and gram-negative bacteria are able to survive up to months on dry inanimate surfaces, with longer persistence under humid and low-temperature conditions [6]. Different organisms are related to contaminations in hospital environments and HAI processes [7], but the main pathogens include oxacillin-resistant Staphylococcus aureus (ORSA), vancomycin-resistant Enterococcus sp (VRE), and more recently, extended-spectrum betalactamas (ESBL) and carbapenem-resistant Acinetobacter baumannii [8,9]. Factors that may affect the transfer of microorganisms from one surface to another and cross-contamination rates are type of organisms, source and destination surfaces, humidity level, and size of inoculums [10,11]. Other factors playing a role in contamination and cross-transmission rate in the ICU may include hand hygiene compliance, nurse-staffing levels, frequency/number of colonized or infected patients, ICU structural features (e.g., single-bed or multi-bed ICU rooms) and adoption of antibiotic stewardship programs [12-14].

Another study performed in reported that the most common pathogens isolated from Device-Associated Healthcare-Associated Infections (DA-HAI) patients were Klebsiella pneumoniae (24.6%), Escherichia coli (21.9%), and Pseudomonas aeruginosa (20.2%) [16].

A local study conducted in El-khomes hospital of Libya revealed that Staphylococcus spp. (76.4%) and Bacillus cereus (27.3%) were the most commonly isolated organisms from healthcare workers and environment surfaces in ICU and operation theatre [17].

Therefore, the main aim of the study was to isolate and identify the most common pathogenic bacteria found in ICU surfaces of Zawia Teaching Hospital, and to provide an evidence of inanimate surfaces and equipment contamination, as well as to find out which bacteria may cause the nosocomial infection.

METHODS

Study settings

This study was carried out in the period from April to May 2018, by sampling equipments and machines inside the ICUs of Zawia Teaching Hospital. The visited ICUs were three ICUs equipped with twenty beds per ICU. Each bed connected with monitor, mechanical ventilation (MV), and control panel. Samples were collected from different sites including; keyboard, screen, and the arm of the MV; the screen, and power control of the monitor; patient’s bed from the right position; control panel. These sites were frequented touch by doctors and other staff workers in the ICUs.

Sample techniques

Samples were collected randomly in different days by assembling seven samples from different locations in the same bed. A total of 56 sample were collected using a sterile swab which moistened with Trypticase Soy Broth (TSB) medium to allows for microbial
growth. After that the swab were spun around their axis over the previously selected surfaces they were again stored in the medium and immediately transported to Alfa private Laboratory in Zawia city. Inside the laboratory, the samples were incubated for 48 hours at 37°C to allow microbial growth. After that, samples were cultured in blood agar, McConkey agar, and salt mantiol agar, and incubated for 24 hours at 37°C.

Suspected bacteria were growing to form colonies used the standard bacteriological procedures, in order to identify the bacterial phenotypical, and recorded the shape features of the colonies and the gram staining was done to identifying the genera and species of the bacteria isolated by microscopic examination. For culturing the Enterobacteriaceae family, the carbohydrate fermentation test was used in Triple Sugar Iron (TSI), the biochemical tests (Sulfide Indole Motility (SIM), Simmons’ citrate and Christensen’s Urea Agar growth mediums).

To identify the glucose-non fermenting gram-negative bacteria, oxidase and polymyxin B tests were used. The identification of Staphylococcus spp was done by catalase, DNase and Novobiocin tests. Streptococcus spp were identified through the characteristics of hemolytic activity on the blood agar, the use of Bile esculin agar, Brain Heart infusion (BHI) + NaCl 6.5% and optochin tests. All the biochemical tests were used to identified and isolated bacteria was carried by Alfa Laboratory staff.

**Data analysis**

Descriptive statistics were used to present the results in the form of counts and percentages, using Microsoft excel data sheath.

**RESULTS**

The results of the current study reported that, out of 56 samples taken from ICU types of equipment and machines, 7(12.5%) shows no bacterial growth, 23(41.1%) samples exhibited the presence of pathogenic bacteria (Escherichia coli, Serratia marcescens, Staphylococcus hemolytic, Pseudomonas aeruginosa, Acinetobacter baumanii, Klebsiella pneumonia, Coagulase negative Staphylococci, Serratia marcescens), and 26(46.4%) samples contaminated with non-pathogenic Diphtheria and coagulase-negative staphylococci and mixed flora. Table 1 represents the types and percentages of ICU equipments contaminated with pathogenic and non-pathogenic bacteria were as the followings; Escherichia coli 7.1%, S marcescens 6.2 %, P. aeruginosa 6.1%, A. baumanii 4.6%, K pneumonia 6.2%, S. hemolytic 3.1%, CoNS 3%.

**Table 1. Types and percentages of bacteria isolated from the visited ICUs**

<table>
<thead>
<tr>
<th>Type of bacteria</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>E coli + NPD</td>
<td>4</td>
<td>7.1 %</td>
</tr>
<tr>
<td>S marcescens</td>
<td>2</td>
<td>3.1 %</td>
</tr>
<tr>
<td>S hemolytic</td>
<td>2</td>
<td>3.1 %</td>
</tr>
<tr>
<td>P aeruginosa + NPD</td>
<td>3</td>
<td>4.6 %</td>
</tr>
<tr>
<td>A baumanii</td>
<td>3</td>
<td>4.6 %</td>
</tr>
<tr>
<td>K pneumonia + NPD</td>
<td>2</td>
<td>3.1 %</td>
</tr>
<tr>
<td>CoNS + NPD</td>
<td>1</td>
<td>1.5 %</td>
</tr>
<tr>
<td>CoNS</td>
<td>1</td>
<td>1.5 %</td>
</tr>
<tr>
<td>S marcescens + NPD</td>
<td>2</td>
<td>3.1 %</td>
</tr>
<tr>
<td>K pneumonia</td>
<td>2</td>
<td>3.1 %</td>
</tr>
<tr>
<td>P aeruginosa</td>
<td>1</td>
<td>1.5 %</td>
</tr>
<tr>
<td>NPD</td>
<td>26</td>
<td>46%</td>
</tr>
<tr>
<td>No growth</td>
<td>7</td>
<td>10.8 %</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>100%</td>
</tr>
</tbody>
</table>

NPD. Nonpathogenic Diphtheria; CoNS. Coagulase-Negative Staphylococci

Figure 1 displays the number of pathogenic and non-pathogenic bacteria reached approximately 87.5 %. This confirms the presence of bacterial
contamination in ICUs, which may lead to HAI as well as nosocomial infection.

**Figure 1.** The percentage of bacterial types compared with a total number of bacteria.

As shown in table 2, the most common contaminated sites with pathogenic bacteria were the mechanical ventilation surfaces (screen and keyboard were more contaminated than the arm of MV), followed by patient’s bed sites especially from the both bed arms. The most common type of bacteria found in different sites in the ICU were represented in figure 2.

**Table 2. ICU Equipments contaminated with bacteria**

<table>
<thead>
<tr>
<th>Equipments and Surfaces</th>
<th>Type of Contaminated Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keyboard of mechanical ventilation</td>
<td><em>K. pneumonia</em>, NPD, <em>E. coli</em> + NPD, CoNS + NPD</td>
</tr>
<tr>
<td>Screen of mechanical ventilation</td>
<td>NPD, Mix flora / CoNS, <em>K. pneumonia</em>, <em>P. aeruginosa</em> + NPD, <em>S. marcescens</em></td>
</tr>
<tr>
<td>Arm of mechanical ventilation</td>
<td>NPD</td>
</tr>
<tr>
<td>Screen of heart monitor</td>
<td>CoNS</td>
</tr>
<tr>
<td>Turn on/off of heart monitor</td>
<td>NPD, <em>A. baumanii</em></td>
</tr>
<tr>
<td>Patient’s bed</td>
<td>NPD, <em>P. aeruginosa</em>, <em>Shemolyticus</em></td>
</tr>
<tr>
<td>A plate of control touching in bed</td>
<td>NPD</td>
</tr>
</tbody>
</table>

**DISCUSSION**

This study aimed at identifying the most common pathogenic bacteria contaminates the equipment surface of ICUs at Zawia teaching hospital, and to evaluate the impact of sterilization and disinfectants that had applied on the surfaces of equipments in ICU. Sterilization and disinfection are essential for preventing the transport of infectious pathogens to patients. The results of the current study confirmed the inappropriateness of sterilization and disinfection program of these ICUs, as the pathogenic bacteria were presented on the equipments surface, which may cause the occurrence of HAI.

Earlier studies had documented the lack of compliance with the established guidelines for disinfection and sterilization [18], which was responsible on the prevalence of HAI in some developing countries ranging from 17.9% in Tunisia.
This high incidence rate can be explaining the deficiency of infection control programs in different countries. The findings of the current study reported 41.1% contamination rate of pathogenic. The highest number of isolated bacteria from equipment surface were NPD or diphtheroid isolated in pure culture and mixed with other pathogenic bacteria.

Diphtheroid is known as aerobic, non-sporulating, pleomorphic gram-positive bacilli which are more uniformly stained, lack the metachromatic granules and are arranged in a palisade manner. Although it usually commensals the skin and mucous membranes, it frequently reported in association with nosocomial infections and a vast majority of these infections are exhibiting antibiotic resistance [22]. Diphtheroid able to form a biofilm on the medical equipment, which is difficult to treat and may cause chronic infection, for catheter and prostatic infections causing recurrent infections thereby to biofilm resistant to antibiotics [23]. This biofilm matrix causing antibiotic resistance, and responsible to delayed penetration of antibiotic into the microorganisms in the biofilm, as a cause of alteration of their physiological mechanism and growth rate due to the biofilm manner of growth [24]. Diphtheroid were isolated in different sites in this study, and were co-existed with pathogenic bacteria (G-ve) and (G+ve). This environment enhances the biofilm formation and lead to antibiotic resistance. The current results also displayed that mechanical ventilation surfaces were contaminated with mixed bacteria e.g. NAD.

Inanimate surfaces considered as the source of hospital acquired infections with numerous gram-negative species, such as Acinetobacter spp., E coli, Klebsiella spp., Pseudomonas aeruginosa, Serratia marcescens, or Shigella spp., that can also survive for months on these surfaces [6]. Staphylococcus aureus is a major cause of infection in both healthcare and community settings, and it considered as one of the most common causes of HAI reported to the National Nosocomial Infections Surveillance (NNIS) system, including surgical site infection and ventilator-associated pneumonia [25]. In this study, the species of staphylococci were CoNS with mixed flora and S hemolytic. Previous local study in Elkhomes hospital also reported that the contamination rate was 76.4% S. aureus that isolated from the anterior nares of healthcare workers and one of them was MRSA [17]. A baumanii was 4.6% from isolated bacteria in our study. The genus Acinetobacter have diversity levels of nutrition and metabolic this allows Acinetobacter genus to obtain variety of substrates as carbon sources. It is active on the surfaces for days or weeks in hospital environments [8]. The existence of genus Acinetobacter in various ICU may causes clinical complications [26] and leads to acquired resistance to carbapenem antibiotics [27]. Further studies are warrant to estimate the prevalence of this bacterium on hospital equipment as and to determine the behavior of these strains in this kind of environment [8].

CONCLUSION

The findings of this study reported different types pathogenic bacteria from an inanimate surface of ICUs were present, that suggest the failure of sterilization and disinfection in these ICUs. Bacterial contamination may contribute to ICU-acquired colonization or infection; therefore, the further studies are needed to evaluate this correlation of the antibiotic-resistant bacteria, nonpathogenic diphtheria, and many others.

Disclaimer

The article has not been previously presented or published, and is not part of a thesis project.
Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

REFERENCE


