Tabari Biomedical Student Research Journal

Original Article

Survey of hospital environment regarding the presence of Staphylococcus aureus producing β-lactamase

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Staphylococcus aureus is a common cause of hospital-acquired infections. This bacterium is resistant to β-lactam antibiotics. The aim of this study was to evaluate the prevalence of S. aureus strains producing enzymes capable of destroying β-lactam antibiotics in a hospital environment. This descriptive and analytic study was performed in Besat Hospital, Sanandaj, Iran, 2016, on 158 swabs collected from the hospital environment. The S. aureus bacteria were identified by microbiology methods, and β-lactamase was determined using acidometric and combined disk methods, as well as Hodge test. Data analysis was conducted using Chi-squared and two-way analysis of variance in SPSS software, version 16 ($P<0.05$). Out of 201 isolated bacteria, 74.68% were S. aureus. Regarding the results of acidometric method, 22.88% of the strains was β-lactamase producing. In combined disc method, 5.08% of strains produced Metallo-β-lactamase; and according to the Hodge test, 84.50% showed carbapenemases. In places with repetitive cleaning, fewer isolates of bacteria were isolated ($P<0.05$). There was no significant difference between the results of the three methods ($P>0.05$). In this study, the β-lactamase producing strains of S. aureus isolates were observed in the hospital. Therefore, the periodic monitoring of resistance and using appropriate detergents are very important at hospitals.

Keywords: Staphylococcus aureus, Phenotypic method, Enzymic degradation of β-lactam antibiotics, Hospital environment

Introduction

Hospital surfaces such as doors, beds, ceilings, and floors are the sources of bacterial maintenance. These surfaces can be considered as the most important factors for the spread of bacteria and hospital-acquired infections. Patients, patients' family, and hospital staff can transfer the microorganisms from these surfaces to the community. Therefore, the management of hospital environment plays an important role in infection prevention [1,2]. Staphylococcus aureus is one of the most important bacterial agents that can be easily spread.

These bacteria are Gram-positive aerobic cocci that contribute to hospital-acquired infections [3,4]. Urinary tract infections, skin infections, endocarditis, and sepsis are the most important bacterial infections caused by this genus [5,6]. The β-lactam antibiotics including methicillin, penicillins, cephalosporins, and carbapenems are among the main medicines for the treatment of bacterial infections. Many bacterial isolates are resistant to these antibiotics [7,8]. According to the literature, resistant isolates produce β-lactamase, enzymes which hydrolyzes β-lactam antibiotics and disable them [9, 10]. Nowadays, there is an increase in the number of bacterial isolates resistant to β-lactam antibiotics, most of which are due to the production of an enzyme that inactivates these antibiotics [9]. Various phenotypic and molecular studies have shown the presence of these enzymes in bacterial isolates resistant to β-lactam antibiotics.

Imani Foolad et al. in 2010 used the disc diffusion method and demonstrated that out of 110 strains of Pseudomonas aeruginosa isolated from patients, 16 strains generated β-lactamase. Polymerase chain reaction (PCR) showed that

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37.5% and 12.5% of them were the carriers of sulfhydryl variable (SHV) and Temorina (TEM), respectively [11]. In 2010, Jalalpoor revealed that out of 194 bacterial isolates isolated from hospital environment, 53.7% were from the Staphylococcus genus. Based on the results of acidometric test, 79.8% of S. aureus strains and 68.55% of S. epidermidis strains were positive for β-lactamase production [12].

Abd Elrahman and Elhag in Sudan in 2015 reported that out of 23 strains of S. aureus isolated from 1,000 wound specimens, 16 strains were β-lactamase producer according to the combined disk method [13]. Regarding no paying attention to phenotypic methods for detecting β-lactamase enzymes in hospitals, this study was carried out to examine the production of this enzyme by various phenotypic methods. In addition, due to the important role that the hospital environment plays in the transmission microorganisms and resistance genes among the patients, especially the patients with hospital-acquired infection. Therefore, it is necessary to identify, prevent, and control the transmission of these bacteria. The aim of this study was to investigate the frequency of S. aureus strains producing β-lactamase enzymes isolated from the environment of Besat Hospital, Sanandaj, Iran.

Materials and Methods

This descriptive and analytic study was carried out during December, 2015 to January, 2016 in Besat Hospital, Sanandaj, Iran, with the ethics code No. 1394/327. Sampling was done randomly, and the inclusion criterion was considered as the environment of patients. The required sample size was calculated according to the following equation:

The samples were obtained from various parts of the hospital such as floor, patient mattress, patient bedside table, chairs in the departments of surgery and infectious diseases, intensive care and cardiac care units using sterile swabs. The swabs were vortexed in nutrient broth (Merck, Germany) and immediately transferred to a centrifuge tube containing the broth neutralizing medium. Thereafter, they were sent to the Microbiology Laboratory of Kurdistan University of Medical Sciences, Sanandaj, Iran.

Then, the specimens were cultured in each tube containing blood agar medium (Merck, Germany). After 24 hours of incubation at the temperature of 37°C, the purification of the isolates was carried out on blood agar medium and S. aureus was identified using Gram's staining that shows grape-like clusters. Standard biochemical microbiological tests including catalase tests (positive for S. aureus), fermentation of glucose (positive for S. aureus), motility (negative for S. aureus), bacitracin susceptibility (S. aureus is resistant), susceptibility to lysostaphin (S. aureus is sensitive), oxidase (negative for S. aureus), coagulase (positive for S. aureus), and growth in mannitol salt agar (Merck, Germany). It is worth mentioning that S. aureus produces acid by the fermentation of mannitol in mannitol salt agar medium and changes the color of medium to yellow [11].

The presence of a β-lactamase enzyme in isolates separated from S. aureus was done by acidometric method. In this method, the isolates were added to the test tubes containing 5 mg Penicillin G (Rosco, Denmark) and diluted phenol reagent. In this case, the color of the solution was violet and in the presence of β-lactamase, penicillin would break down to penicilloic acid and the color of the solution would become yellow.

To make the above solution, 0.5 ml of phenol 0.5% was added to 4.5 ml of distilled sterilized water; and then a small amount of 5 million units penicillin G powder was added to the solution. When penicillin G was dissolved, sodium hydroxide solution 1 M was continuously dropped to the vial until the solution was purple. The pH at this time should be 8.5. Thereafter, 0.2-1 mm of the tubule was inserted into the vial.

After the rise of the solution in the tube, it was immediately drawn onto the colonies of the bacteria. The end of the tube was completely blocked by the bacteria. The final result (color change) was read by eye after 5-15 minutes [12]. According to the Clinical and Laboratory Standards Institute's for the determination of the production of broad-spectrum β-lactamase enzymes, a combined disk method was applied.

A 0.5-McFarland standard suspension was prepared from the samples and was cultured on Mueller Hinton Agar medium (Merck, Germany). Then, an imipenem disk (10 µg), as well as a disk of the combination of ethylene diamine tetraacetic acid (EDTA) (0.5 M) and an imipenem (10 µg) were utilized. The discs were placed at a distance of about 25 mm from each other and then incubated at the temperature of 37 °C for 24 hours.

After incubation, the bacterium was considered as a Metallo-β-lactamase producer if the diameter of the non-growth zone of the bacteria around the combined disk was 7 mm or more than the diameter of the inhibition zone

around the imipenem disk [14]. Regarding the fact that imipenem is capable of removing bacteria up to the diameter of 7 mm, the difference in halo to 7 mm or greater indicates the presence of Metallo-β-lactamase and its elimination by EDTA.

In accordance to Clinical and Laboratory Standards Institute's Hodge test was performed to separate carbapenemase-producing strains. Initially, the 0.5-MacFarland standard suspension was prepared from a strain of S. aureus sensitive to imipenem (lacking mecA gene) (ATCC® 25923TM). Then, the dilution of 1/10 was provided in Muller-Hinton medium (Merck, Germany). A plate was allocated to each strain for culturing [11, 15].

The plates were incubated for 5 minutes at room temperature, and then an ertapenem disk (10 µg) was placed in the center of the plate. In the next step, three separate strains of S. aureus were cultured with a swab on a medium from the center, slightly close to the ertapenem disk, to the edge on each plate. The plates were incubated for 24 hours at the temperature of 37˚C.

The cloverleaf-shaped inhibition zone around the ertapenem disc was interpreted and evaluated. In this test, S. aureus strains were cultured on a sensitive strain of S. aureus. Therefore, after incubation, the growth restriction region was observed around the ertapenem disk. Resistant S. aureus grows near the ertapenem disk and transmits resistance genes to sensitive E. coli.

As a result, the growth of standard strains of S. aureus near those isolated from the environment can be seen as a cloverleaf-shaped zone. However, sensitive S. aureus did not grow around the ertapenem disk and the cloverleaf-shaped region was not observed [15]. Data analysis was performed using Chi-squared and two-way analysis of variance with the help of SPSS software, version 16. In this study, p-value less than 0.05 was considered statistically significant.

Results

Out of 158 cultivated swab specimens on blood Agar medium, 201 isolates were separated, and 118 (74.68%) isolates were S. aureus. The results of the acidometric method showed that 27 (22.88%) strains were β-lactamase producing S. aureus (Figure 1). The separation of Metallo-β-lactamase producer strains using combined disk method revealed that 6 strains (5.08%) of them were a Metallo-β-lactamase producer (Figure 2).

Additionally, the results obtained from Hodge test demonstrated that 60 strains (84.50%) were carbapenem-resistant (Figure 3). There was a significant relationship between the ongoing cleaning of various surfaces and separated bacterial isolates ($P<0.05$). However, there was no significant difference between the three phenotypic methods of enzyme production ($P>0.05$).

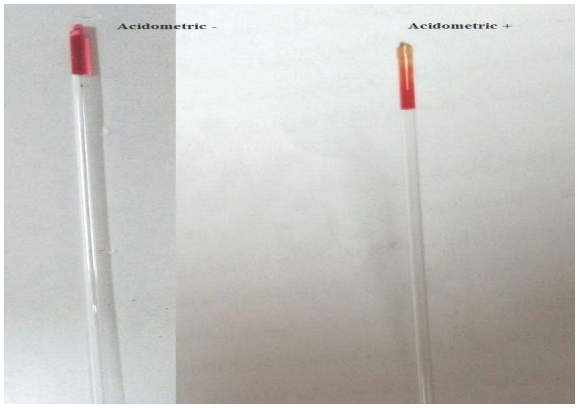

Figure 1. Results of acidometric method; penicillin would break down to penicilloic acid in the presence of β-lactamase and the color would change to yellow.

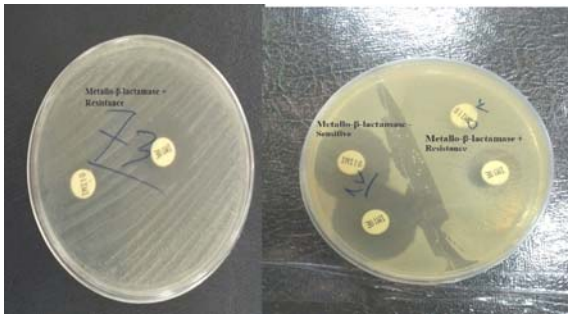

Figure 2. Results of combined disk method; the diameter of growth restriction around the combined disk would be 7 mm or more in the presence of metallo-β-lactamase.

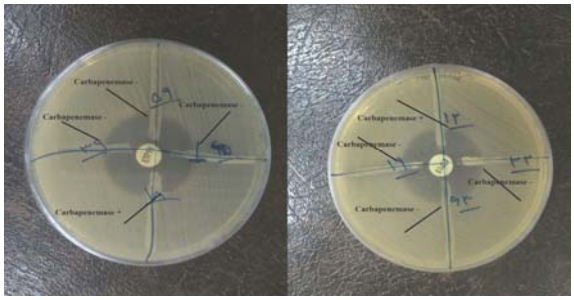

Figure 3. Results of Hodge test; the cloverleaf-shaped inhibition zone would be observed in the presence of carbapenemase

Discussion

Hospitals play an important role in providing health and education services to people in the society. Nevertheless, hospital-acquired infections have a significant outbreak, which can increase mortality and morbidity and impose additional treatment costs. In the case of these infections, three factors of the environment, host, and pathogens are important [16]. The environment provides a condition for host-pathogen ecology. Therefore, an important factor that can break the transmission chain of these infections and prevent them is to observe environmental health standards [17]. The results obtained from the present study showed the presence of S. aureus, which produces enzymes that inactivate β-lactam antibiotics in the hospital environment. Therefore, the hospital environment is a factor in the transmission and spread of bacteria [12].

Kabiri et al. in Iran, 2015, showed that out of 216 bacterial isolates separated from the delivery room, 3.2% of the strains were Enterococcus. The disk diffusion method confirmed that 57% of Enterococcus strains were multidrug resistant [18]. In this study, 201 isolates were separated that 74.78% of them were S. aureus strains. Heeding with environmental health standards is different in various parts of the hospital.

Therefore, the amount of isolation and different types of bacterial strains and sometimes resistance is also different. Moreover, a low percentage of bacteria in the delivery room is due to elective surgical procedures and sufficient opportunity to eliminate the contamination [16]. In a study conducted by Jalalpoor et al. in Iran, 2011, the results of acidometric and disk diffusion methods revealed that out of 80 bacterial isolates separated from the hands of the hospital staff, 61.85% of them had β-lactamase.

The frequencies of this enzyme between Staphylococcus, Bacillus, and Enterobacteriaceae were 70.83%, 72.74%, and 70%, respectively. Furthermore, the highest and lowest resistances of bacteria were against penicillin and vancomycin, respectively [19]. In this study, the results of the acidometric method showed that 22.88% of strains of S. aureus had β-lactamase, which was less than the amount obtained by Jalalpoor et al., (2011) [19].

Bacteria are usually transmitted to staffs' hands by direct contact with patients or environmental contaminants. Various factors such as unfavorable environmental health conditions, worn building, inadequate room ventilation, insufficient sanitary facilities, frequent sewer blockage, inappropriate sanitary conditions of patients' bedside, and the communal air channels are major contributors to bacterial transmissions in hospitals [16,19].

Limitations for unnecessary traffic in hospital, the use of personal protective equipment to control the transmission of bacteria such as socks, shoes, masks, and disposable clothing, the use of standard and efficient hand and foot disposables for the proper disinfection and administration of antibiotics can reduce the amount of these contaminants. The bacterial and antibiotic resistance levels will be lower in places, where these factors are observed [19].

In another study carried out by Jalalpoor in Iran, 2011 using a phenotypic combined disk method, out of 378 Klebsiella pneumonia isolates separated from patients, 64% and 23% were Metallo-β-lactamase producers [20]. In our study, the isolation of Metallo-β-lactamase producing strains was performed by a combined disk test. The results of this method showed that 5.8% of S. aureus strains were Metallo-β-lactamase producer.

The Metallo-β-lactamase genes can be spread through chromosomes and plasmids across bacterial strains. Therefore, the phenotypic identification of the strains producing these enzymes plays an important role in epidemiological studies and is important in controlling and preventing the distribution of these bacteria [21]. Robles et al. in 2014, Brazil, reported that 79% and 69.6% of 100 S. aureus isolates separated from livestock with mastitis were resistant to oxacillin sodium according to Hodge test and polymerase chain reaction, respectively [22].

In this study, Hodge test demonstrated that carbapenemase production was positive in 60 strains (84.5%), which were resistant to ertapenem. Carbapenems such as imipenem, ertapenem, and meropenem are used against both Gram-positive and -negative bacteria. Ertapenem is effective on methicillin-sensitive Staphylococci, however, it does not affect a variety of methicillin-resistant strains [23].

In addition, the production of carbapenemase by bacteria has become a major global issue. Therefore, fast and inexpensive laboratory methods are needed to detect strains resistant to carbapenems and the carbapenemase producers [15,24]. Hospital surfaces are important in the transmission of bacterial hospital-acquired infections including S. aureus resistant to antimicrobial agents.

Beta-lactamase genes produced by bacterial agents are usually transmitted by genetically engineered elements such as plasmids and expanded rapidly in the community. These

plasmids can carry resistance genes to other antibiotics and increase the prevalence of multidrug resistance isolates [12, 25]. The results of this study indicated the presence of almost a wide range of strains of S. aureus producing monohydrate β-lactamase.

In this study, it was confirmed that bacterial isolates were less frequent in places that were regularly cleaned. Therefore, disinfection with proper, effective, and safe material with the least damage to the equipment and personnel is important in controlling the nosocomial infections and preventing their spreading [26]. In the present study, β-lactamase production methods were compared and demonstrated.

One of the strengths of the current study was the large sample size, reliable criteria for detecting bacteria producing β-lactamase taken from the Clinical and Laboratory Standards Institute. In addition, being inexpensive, repeatable, and easy to implement the methods in a short time were among the other strengths of this study. Hospital environments are constantly exposed to contamination, and it is impossible to sample from all parts of the hospital. Therefore, the sample collection should be done more accurately.

Additionally, there is the possibility of contamination of samples in the laboratory environment during the study, which should be addressed. This issue could be considered as one of the limitations of the present study. Consequently, monitoring and periodic review of the environment and the microbial infection in the environment, appropriate selection of disinfectants, and personnel training are necessary in order to minimize the environmental transmission of nosocomial infections.

Acknowledgments

This paper is a part of the dissertation of medical student Massoud Heidarifar. The authors thank the Deputy of Research and Technology of Kurdistan University of Medical Sciences, Sanandaj, Iran, for financial support.

Conflicts of interest

The authors declare no conflicts of interest.

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